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Mimotopes of the Vi Antigen of *Salmonella enterica* Serovar Typhi Identified from Phage Display Peptide Library

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The capsular polysaccharide Vi antigen (ViCPS) is an essential virulence factor and also a protective antigen of *Salmonella enterica* serovar Typhi. A random 12-mer phage-displayed peptide library was used to identify mimotopes (epitope analogues) of this antigen by panning against a ViCPS-specific monoclonal antibody (MAb) ATVi. Approximately 75% of the phage clones selected in the fourth round carried the peptide sequence TSHHDSHGLHRV, and the rest of the clones harbored ENHSPVNIAHKL and other related sequences. These two sequences were also obtained in a similar panning process by using pooled sera from patients with a confirmed diagnosis of typhoid fever, suggesting they mimic immunodominant epitopes of ViCPS antigens. Binding of MAb ATVi to the mimotopes was specifically blocked by ViCPS, indicating that they interact with the same binding site (paratope) of the MAb. Data and reagents generated in this study have important implications for the development of peptide-base diagnostic tests and peptide vaccines and may also provide a better understanding of the pathogenesis of typhoid fever.

Typhoid fever, a disease caused by *Salmonella enterica* serovar Typhi, remains an important infectious disease problem in many developing countries around the world. The global annual incidence is approximately 17 million cases per year, with approximately 600,000 deaths (8, 12). The problem was recently exacerbated by the appearance of antibiotic-resistant strains and increased urbanization. Although typhoid fever has been known for over two centuries and the causative agent was discovered in 1884, the pathogenesis and roles of various components of human immune response to *S. enterica* serovar Typhi have not been completely understood.

Phage display technology represents an important advance in the capability to rapidly identify antigenic epitopes of pathogenic microorganisms (2–6, 16, 18, 24, 25). With this approach, peptide or protein is expressed as a fusion entity with a coat protein of bacteriophages, resulting in display of the fusion polypeptide on the surface of the virion, while the DNA encoding the fusion polypeptide resides within the virion. Phages displaying peptides are then allowed to interact with antibodies immobilized on a solid support, and the binding phages are then eluted and can be specifically enriched by several cycles of affinity selection. The identity of the fusion peptide can then be determined by sequencing the inserts present in the genome of the recombinant phage (13, 20, 21).

Carbohydrate antigens are immune targets associated with a variety of infectious pathogens (10, 11). One of the problems in developing carbohydrate-based therapeutics is the difficulty involved in synthesizing complex carbohydrate ligands. A possible alternative to the use of carbohydrate would be the development of protein or peptide mimics that could serve the same function. With the development of large random peptide libraries displayed on the surface of filamentous phage (18), it became possible to identify small peptides that could mimic a carbohydrate structure. Although it is not intrinsically obvious that peptides can mimic nonpeptide structures, there are naturally occurring compounds that do so. For example, the protein tendamistat (Hoe-467) binds to the enzyme α-amylase, with the tripeptide WRY occupying the carbohydrate-binding site of the enzyme (22). There have been many successful examples of identification of peptide mimotopes of carbohydrates from phage display peptide libraries (1, 7, 9, 14, 19, 26).

In this paper, we describe the isolation of peptide mimotopes of complex carbohydrates in *Salmonella enterica* serovar Typhi that react with both carbohydrate-specific monoclonal antibody (MAb) and polyclonal antibody (PAb)-containing sera from typhoid patients. To our knowledge, this is the first demonstration of Vi polysaccharide mimotope identification using pooled sera from patients with a confirmed diagnosis of typhoid fever.

MATERIALS AND METHODS

Bacterial strains and reagents. *Escherichia coli* strain ER2738 [(F′ *lacI*^{q} Δ(*lacZ*)M15 *proA*$^+$ *B*$^+$ *zzf*::Tn*10* (Tet^r)/*fhuA2 supE thi* Δ(*lac-proAB*) Δ(*hsdMS-mcrB)5*(r_k^- m_k^- McrBC$^-$)] was obtained from New England Biolabs. Mouse immunoglobulin G (IgG), and goat anti-mouse IgG horseradish peroxidase (HRP) conjugate were purchased from Sigma Chemical Co. HRP-conjugated anti-M13 MAb was purchased from Amersham Pharmacia Biotech, Little Chalfont, United Kingdom. All the inorganic chemicals and organic solvents used were of molecular biology grade.

Antibodies. The PAbs used in this study were pooled typhoid patient sera (PTS [sera from 10 individual patients with confirmed typhoid fever]). Medical diag-

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nosis of these patients was confirmed by high antibody titers (>1:640) for one or both O and H antigens, as determined by Widal test. The anti-Vi MAb ATVi was a kind gift from Suttipant Sarasombath, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. Production of MAb ATVi from a hybridoma cell line was conducted as described by Pongsunk et al. (15).

Selection of mimotopes by panning. A 12-mer random peptide library (from New England BioLabs) was used in this study. Two independent affinity selections were carried out with MAb ATVi and PAb PTS, respectively. Microtiter plate wells were separately coated with these antibodies (100 μg/ml for MAb ATVi and a 1:1,000 dilution for PAb PTS in 0.1 M $NaHCO_3$ [pH 8.6]) overnight at 4°C. Nonspecific binding was blocked by incubating with 200 μl of blocking buffer (0.1 M $NaHCO_3$ [pH 8.6], 5-mg/ml bovine serum albumin [BSA], 0.02% NaN_3) in each well for 1 h, and then the wells were washed six times with 110 μl of TBS (50 mM Tris-HCl [pH 7.5], 150 mM NaCl). An aliquot (10 μl; 1.5×10^{11} PFU) of phage solution was added to each coated well. The mixtures were incubated at 37°C for 1 h, and the wells were washed extensively 10 times (5-min interval) with TBST (TBS plus 0.1% [vol/vol] Tween 20). Bound phage was eluted with 100 μl of glycine-HCl (0.2 M [pH 2.2], 1-mg/ml BSA) by rocking gently for 7 to 8 min and neutralized with 1 M Tris-HCl (15 μl [pH 9.1]) to pH 7 immediately. The output PFU was determined by titrating the eluate (10 μl), while the remaining eluate was amplified in *E. coli* strain ER2738 at 37°C for 5 h. The panning procedure described above was repeated for another three rounds, but the Tween concentration in the washing steps was raised to 0.5% (vol/vol). After four rounds of selection, individual plaques were picked and used to infect *E. coli* ER2738 cells for amplification. Streptavidin and BSA were used as positive and negative controls, respectively.

Preparation, amplification, and titration of the selected phages. The selected phage clones were amplified to a high titer and purified twice by precipitation with 20% (wt/vol) polyethylene glycol 8000 (PEG 8000)–2.5 M NaCl according to the method described by Wang et al. (23). The phage titration method was adapted from Sambrook et al. (17).

Plaque amplification. Individual blue plaques from the third and fourth rounds of panning were randomly picked from Luria-Bertani (LB) agar plates (used in output titration) and used to infect *E. coli* ER2738 cells. The culture was grown in LB broth supplemented with tetracycline (20 μg/ml) at 37°C for 4.5 to 5 h before being centrifuged at 15,000 × *g* for 30 s. The supernatant was transferred to a fresh tube and respun as described above. The upper 80% of phage-containing supernatant was collected and stored in 4°C.

Phage ssDNA extraction. Phage single-stranded DNA (ssDNA) was extracted as follows. Selected amplified phage (500 μl) from individual clones was transferred to a fresh microcentrifuge tube. PEG-NaCl (200 μl) was added and incubated at room temperature for 10 min. After spinning at 15,000 × *g* for 10 min, the supernatant was discarded, and the pellet was suspended thoroughly in 100 μl of iodide buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA, 4 M NaI). Then 250 μl of ethanol was added. The mixtures were incubated for 10 min at room temperature. The mixtures were then spun at 15,000 × *g* for 10 min, and the supernatant was discarded. The pellet was washed with 70% ethanol, dried, and suspended in 30 μl of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA).

DNA sequencing. The ssDNA (10 μl) was sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction kit and an automated DNA sequencer (ABI PRISM 377′; Perkin-Elmer). The primer used was 5′-CCCTCA TAGTTAGCGTAACG-3′.

Sandwich ELISA. Sandwich enzyme-linked immunosorbent assay (ELISA) to determine the ability of the phages to bind to MAb was performed as follows. Microtiter plates (Nunc, Inter Med, Roskilde, Denmark) were coated with 5.0 μg per well of unpurified tissue culture supernatant proteins containing MAb ATVi and blocked with TBS–0.5% BSA for 1 to 2 h at 4°C. Various concentrations of the phages (100 μl) were added to wells and incubated for 2 h at room temperature. Plates were washed with TBS–0.5% Tween 20 and HRP-conjugated anti-M13 antibody (1:5,000 in blocking buffer) was added. Following an incubation for 1 h, the plates were washed and substrate ABTS [2, 2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic-acid); Sigma] was added. The A_{414} was measured with an EL-320 microplate reader (Titertek Multiskan II; Flow Labs, Mt. Waverley, Victoria, Australia).

Indirect ELISA. Microtiter plates (Nunc, Inter Med) were directly coated with purified phage and ViCPS antigen (from Typhim Vi, Pasteur Mérieux Connaught, France) at 4°C for 18 h. Plates were blocked with 1% BSA. Primary antibodies (MAb ATVi) were added and the mixture was incubated for 1 to 2 h at 4°C. The plates were washed and incubated with HRP-conjugated antimouse antibody for 2 h, and the rest of the steps were the same as those described above for the sandwich ELISA.

TABLE 1. Peptide ligands of MAb ATVi obtained in the third and fourth rounds of panning

Phage clone	Sequence[a]	Frequency	%	Total %
3rd round				
NM3.04	**ENHSPVNIAHKL**	2×	20	60
NM3.06	ENHSPVNIAHKL			
NM3.10	ENHSPVNIAHKV	1×	10	
NM3.01	ENHSPVNIDHKL	2×	20	
NM3.18	ENHSPVNIDHKL			
NM3.17	EDHSPVNIDHKL	1×	10	
NM3.14	ENHYPLHAAHRI	1×	10	20
NM3.05	ESHQHVHDLVFL	1×	10	
NM3.02	**TSHHDSHGDHHV**	1×	10	20
NM3.13	PGHHDFVGLHHL	1×	10	
4th round				
NM4.03	**TSHHDSHGLHRV**			
NM4.04	TSHHDSHGLHRV			
NM4.06	TSHHDSHGLHRV			
NM4.07	TSHHDSHGLHRV			
NM4.09	TSHHDSHGLHRV			
NM4.11	TSHHDSHGLHRV	11×	55	
NM4.12	TSHHDSHGLHRV			
NM4.14	TSHHDSHGLHRV			75
NM4.15	TSHHDSHGLHRV			
NM4.19	TSHHDSHGLHRV			
NM4.20	TSHHDSHGLHRV			
NM4.05	TSHHDSHGVHRV	2×	10	
NM4.16	TSHHDSHGVHRV			
NM4.08	TSHHDSHDLHRV	1×	5	
NM4.18	TSHHDYHGLHRV	1×	5	
NM4.01	**ENHSPVNIAHKL**	2×	10	
NM4.13	ENHSPVNIAHKL			
NM4.10	ENHYPVNIAHKL	1×	5	25
NM4.02	DNHSPVNIAHKL	2×	10	
NM4.17	DNHSPVNIAHKL			

[a] Consensus sequences are shown in boldface, whereas amino acid residues different from the consensus are underlined.

CB-ELISA. Checkerboard ELISA (CB-ELISA) was performed by coating microtiter wells with various concentrations of MAb ATVi at 5 to 20 μg per well for 18 h at 4°C and then blocked with 0.5% BSA. Various concentrations of phage (dilution from 10^{10} PFU to 10^7 PFU) were then incubated with MAb for 2 h at 4°C. Phages bound to antibodies were detected with HRP-conjugated anti-M13 antibody as described above.

Competitive ELISA. Microtiter wells were coated with MAb ATVi and blocked as described above. Various concentrations of Vi polysaccharide (0 to 10,000 ng/ml in phosphate-buffered saline [PBS]) were mixed with equal volumes of purified phage solution (diluted 1:100 in PBS). The mixtures were added to MAb ATVi-coated microtiter wells (100 μl/well) and incubated at 37°C for 1 h. After the wells were washed six times with PBS-Tween (200 μl/well), the amounts of bound recombinant phage were determined with anti-M13 antibody-HRP conjugate as described above.

RESULTS

Sequence analysis of phage clones selected from panning with MAb. Ten and 20 individual clones were randomly selected from the third and fourth rounds of panning, respectively. The encoded peptide sequence was deduced by sequencing the insert in each of the selected phage clones. The results are summarized in Table 1.

In the third round of panning, eight classes of phage clones

were found and were grouped into three populations (Table 1). The first four classes were grouped into the major population; the latter two classes (NM3.14 and NM3.05) were grouped into the intermediate population; meanwhile, the last two classes (NM3.02 and NM3.13) were grouped into the minor population. The consensus sequence displayed by the major population of phage consists of approximately 60% of the clones screened. Within this population, the sequences of each class of phage clone were almost identical (different only by 1 or 2 amino acid residues) (Table 1). The 12-amino-acid sequences displayed by the minor population of phage were TSHHDSHGDHHV (10%) and PGHHDFVGLHHL (10%) (amino acid residues different from the consensus are underlined). Interestingly, 10 of the amino acid residues in peptide sequence TSHHDSHGDHHV were also found in the dominant sequence TSHHDSHGLHRV, identified in the subsequent round of panning, except for a histidine (H) residue in the C-terminal penultimate position and an aspartate (D) residue located on the N-terminal side of histidine (H).

The most consensus sequences screened from the fourth round of panning were TSHHDSHGLHRV (55%), followed by TSHHDSHGVHRV (10%), ENHSPVNIAHKL (10%), and DNHSPVNIAHKL (10%). Another two additional sequences, which were related to the major consensus sequence TSHHDSHGLHRV, also appeared in the fourth round of panning. The peptide sequences TSHHDSHDLHRV and TSHHDYHGLHRV were almost identical with TSHHDSHGLHRV (Table 1).

In summary, seven classes of phage clones were found in the fourth round of panning. We grouped them into two populations. The first four classes were grouped into the major population (that composed approximately 75% of the phages screened); the latter three classes were grouped into the minor population (25%). Within each population, the sequence of each clone was almost identical (different by only 1 residue amino acid, which is underlined). However, two very different sequences were seen in these two populations of phage. The 12-amino-acid consensus sequence displayed by the major population of phage was TSHHDSHGLHRV, and that displayed by the minor population of phage was ENHSPVNIAHKL.

Sequence analysis of phage clones selected from panning with pooled patient sera. Ten and 18 individual clones were randomly selected from third and fourth rounds of panning, respectively. The deduced amino acid sequences of the selected mimotopes are presented in Table 2.

In the third round of panning, 30% of phages screened carried the peptide sequence ENHSPVNIAHKL, and other sequences composed approximately 70%. Another three related peptide sequences (NP3.24, NP3.19, and NP3.06; Table 2) were also detected in the third round of panning. However, three of these peptide sequences were not found in the fourth round of panning.

There were three seemingly unrelated peptide sequences (NP3.05, NP3.18, and NP3.15) found in third round phage pools that shared only 1 to 3 amino acid residues with ENHSPVNIAHKL (Table 2). Phage bearing the sequence TNHLGLQSSHRF (NP3.13) had 4 amino acids in common with the peptide sequence displayed by the fourth round phage clone in the major population, TSHHDSHGLHRV (89% of the phages screened). However, only 1 out of 10 phage clones screened in the third round carried TNHLGLQSSHRF.

TABLE 2. Peptide ligands of PAb PTS obtained in the third and fourth rounds of panning

Phage clone	Sequence[a]	Frequency	%	Total %
3rd round				
NP3.03	**ENHSPVNIAHKL**			
NP3.09	ENHSPVNIAHKL	3×	30	
NP3.10	ENHSPVNIAHKL			60
NP3.24	DNHSPVNIAHKL	1×	10	
NP3.19	QNHSPVYIAHKL	1×	10	
NP3.06	QNHSPVNIAHKI	1×	10	
NP3.05	FPIAALNNETSF	1×	10	10
NP3.18	QNTDQATPHRML	1×	10	10
NP3.15	QITDQVNVHHML	1×	10	10
NP3.13	**TNHLGLOSSHRF**	1×	10	10
4th round				
NP4.01	**TSHHDSHGLHRV**			
NP4.02	TSHHDSHGLHRV			
NP4.03	TSHHDSHGLHRV			
NP4.05	TSHHDSHGLHRV			
NP4.06	TSHHDSHGLHRV			
NP4.07	TSHHDSHGLHRV			
NP4.08	TSHHDSHGLHRV			
NP4.09	TSHHDSHGLHRV	16×		89
NP4.10	TSHHDSHGLHRV			
NP4.11	TSHHDSHGLHRV			
NP4.13	TSHHDSHGLHRV			
NP4.14	TSHHDSHGLHRV			
NP4.15	TSHHDSHGLHRV			
NP4.17	TSHHDSHGLHRV			
NP4.19	TSHHDSHGLHRV			
NP4.20	TSHHDSHGLHRV			
NP4.16	**ENHSPVNIAHKL**	2×		11
NP4.18	ENHSPVNIAHKL			

[a] Consensus sequences are shown in boldface, whereas amino acid residues different from the consensus are underlined.

Surprisingly, only two populations of phage clones bearing the predominant sequence motifs were observed in the fourth round of PAb-selected phage pool. Within each population, the sequences of each clone were identical, but two very different sequences were seen in these two populations of phage. The 12-amino-acid sequence displayed by the major population of phage was TSHHDSHGLHRV, which was present in 89% of the phage clones screened. The 12-amino-acid sequence displayed by the minor population of phage was ENHSPVNIAHKL. The frequency of phage carrying this peptide in fourth round of panning was reduced to 11% as compared to the third round of panning (30%).

Analysis of binding to MAb ATVi by two different type of phage clones. Two different groups of MAb ATVi-selected phage clones (NM4.04 and NM4.13) were compared in a sandwich ELISA to determine their relative avidities for binding to the selecting MAb used in panning. Phages NM4.04 and NM4.13 represent the two major populations of phage clones that carried the consensus peptide sequences of TSHHDSHGLHRV and ENHSPVNIAHKL, respectively. The results are shown in Fig. 1. The binding of MAb ATVi by NM4.04 was

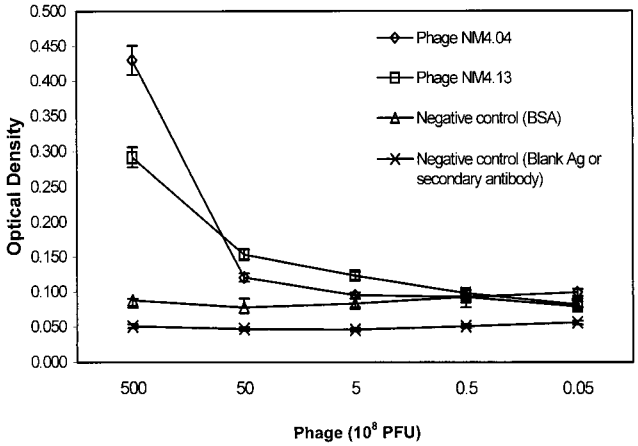

FIG. 1. Analysis of phage binding by ELISA. A microtiter plate was coated with the MAb ATVi at 5 μg per well for 18 h at 4°C and then blocked with 0.5% BSA. A 10-fold dilution of phage concentration (from 5×10^{10} PFU to 10^6 PFU) was then incubated with the MAb. Bound phage was detected with HRP-conjugated anti-M13 antibody. The optical density (A_{414}) values shown are the mean of triplicate samples. Ag, antigen.

considerably greater than that by NM4.13. Levels of binding by negative controls (BSA with no antigen or no conjugate) were all significantly lower.

Phage clone NM4.04 was chosen for further analysis by a CB-ELISA. The titration curves presented in Fig. 2 demonstrated that the binding is specific, and a decrease in coating antibody and/or phage concentration resulted in the decrease in binding of this phage clone.

Specificity of binding to MAb ATVi by selected phage clones. Two different approaches were used to assess the specificity of the selected mimotopes. First, binding of phage clone NM4.04 was directly compared with that of an irrelevant clone, NS3.08, which was isolated by panning against streptavidin and carried the peptide sequence RVSYNHDQTTFS. As shown in Fig. 3, NM4.04 has a higher binding activity than NS3.08. Second, the specificity of binding of clone NM4.04 was tested with a Vi mimotope peptide.

Use of Vi mimotope peptide in competitive ELISA. To determine whether the positive recombinant phage peptides actually mimicked the epitope recognized by MAb ATVi or just bound nonspecifically to the surface of the antibody molecule outside the antigen-binding site, the selected positive phage clone was tested for binding to MAb ATVi by performing a competitive ELISA (Table 3). Phages binding to antibody were detected with HRP-conjugated anti-M13 antibody and then ABTS substrate. The optical densities (A_{414}) are the mean of triplicate samples. Free purified Vi capsular polysaccharide (ViCPS) and intact bacteria ViCPS competitively inhibited the binding of phage clone NM4.04 to immobilized MAb (Fig. 4). This strongly suggested that the phage-displayed peptide mimotope bound to the antigen-binding site of the MAb, mimicking, in part, the structural epitope of Vi antigen of *S. enterica* serovar Typhi.

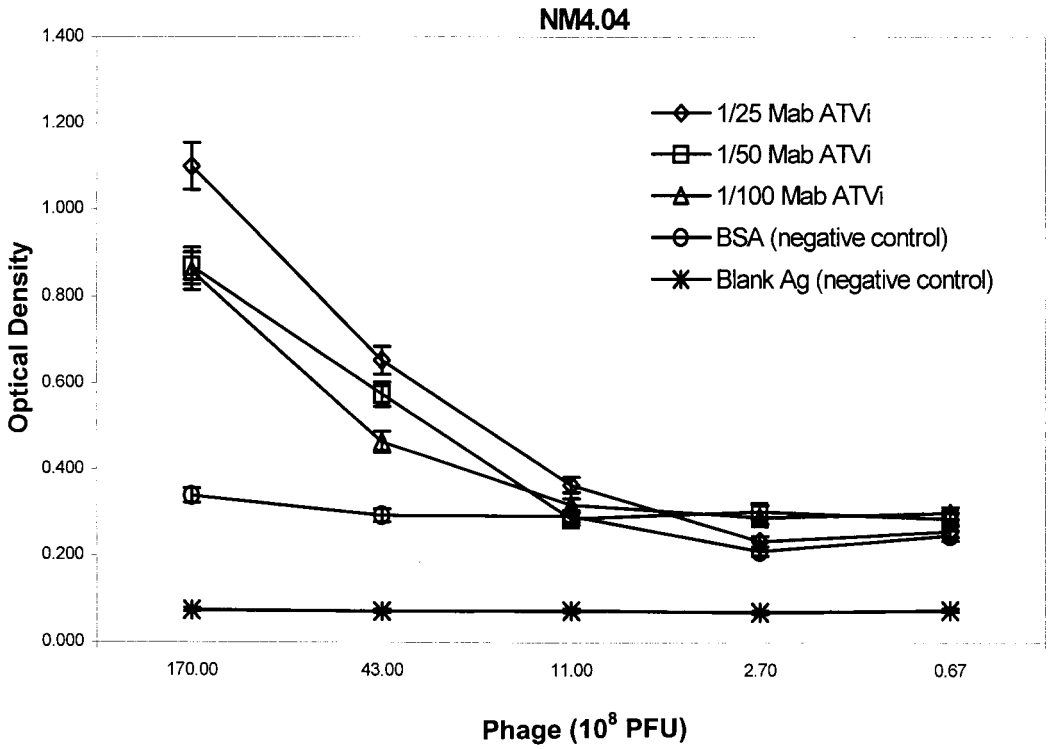

FIG. 2. Binding analysis by CB-ELISA. Microtiter wells were coated with various concentrations (dilutions of 1/25, 1/50, and 1/100) of MAb ATVi and then blocked with 0.5% BSA. Various concentrations of phage (NM4.04) were then incubated with MAb (fourfold serial dilution, from 10^{10} to 10^7 PFU). Bound phage was detected with HRP-conjugated anti-M13 antibody. The optical density values represent A_{414}. Assays were performed twice in triplicate, and the error bars represent the standard deviation of the mean of two sets of experiment. Ag, antigen.

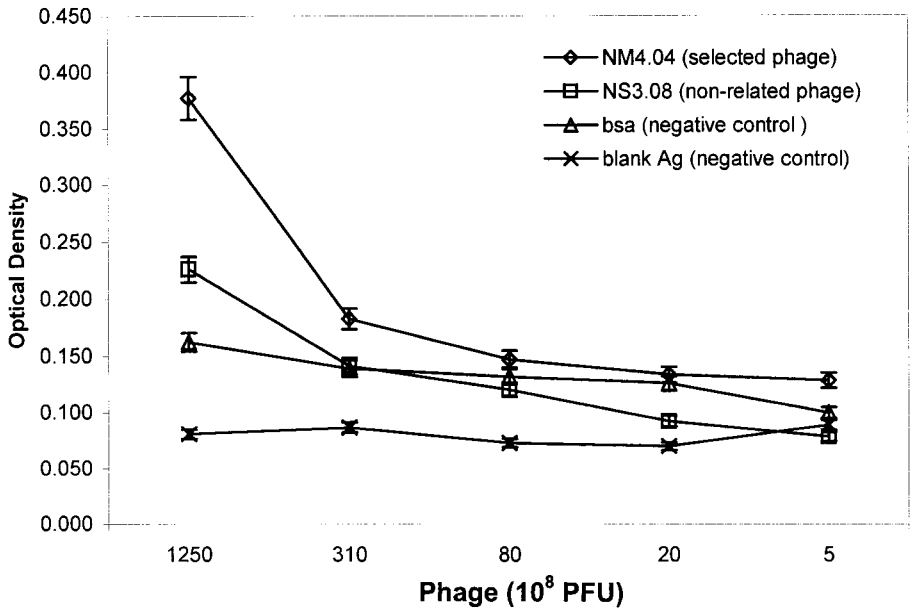

FIG. 3. Comparison of binding by selected and nonrelated phage clones using a sandwich ELISA. After being coated with MAb ATVi, different phage clones were serially diluted, and bound phage was measured by HRP-conjugated anti-M13 antibody. The optical density (A_{414}) values are the mean of triplicate samples, and the error bars represent the deviation of the mean. Ag, antigen.

TABLE 3. Mimotopes obtained in the third and fourth rounds of panning using Vi antigen-specific MAb ATVi and PAb sera from patients with confirmed typhoid fever

Round of panning	Peptide sequence[a]	% Frequency of sequences[b]	
		MAb	PAb
3rd	**ENHSPVNIAHKL**	20 (2)	30 (3)
	ENHSPVNIDHKL	20 (2)	
	ENHSPVNIAHKV	10 (1)	
	EDHSPVNIDHKL	10 (1)	
	ENHYPLHAAHRI	10 (1)	
	ESHQHVHDLVFL	10 (1)	
	TSHHDSHGDHHV	10 (1)	
	PGHHDFVGLHHL	10 (1)	
	DNHSPVNIAHKL		10 (1)
	QNHSPVYIAHKL		10 (1)
	QNHSPVNIAHKI		10 (1)
	FPIAALNNETSF		10 (1)
	QNTDQATPHRML		10 (1)
	QITDQVNVHHML		10 (1)
	TNHLGLQSSHRF		10 (1)
4th	**TSHHDSHGLHRV**	55 (11)	89 (16)
	TSHHDSHGVHRV	10 (2)	
	TSHHDSHDLHRV	05 (1)	
	TSHHDYHGLHRV	05 (1)	
	ENHSPVNIAHKL	10 (2)	11 (2)
	ENHYPVNIAHKL	05 (1)	
	DNHSPVNIAHKL	10 (1)	

[a] Consensus sequences are shown in boldface, whereas amino acid residues different from the consensus are underlined.

[b] Numbers in parentheses represent the number of isolated phage clones bearing the same peptide sequence.

DISCUSSION

In this communication, we have presented a comparative study on mimotope identification using either pooled patient sera or MAb against Vi antigen to assess the diversity of mimotopes that could be detected and to determine whether it is possible to isolate common mimotopes recognized by both antibodies.

Selection using PAb sera generated a greater number of different 12-mer sequence motifs than those obtained with the MAb. With MAb ATVi screening, three different groups of sequences were identified, while the PAb screening generated five different groups of 12-mer amino acid sequences. This is not surprising, because pooled typhoid fever patient sera would most likely contain many subpopulations of antibodies directed toward various *S. enterica* serovar Typhi epitopes (10, 11). What is surprising is the fact that among the different sequence motifs selected by two different approaches, there are at least two common motifs identified from both pannings. To our knowledge, this is the first example of selection of immunodominant mimotopes of a carbohydrate antigen from phage display peptide library by using patient sera that are also recognized by a known MAb.

The other interesting observation is the different dominating motifs isolated from different rounds of the panning process. From the third round of panning, the common motif isolated with both patient sera and MAb was the 12-mer peptide ENHSPVNIAHKL (termed mimotope Vi12B), representing 20% clones for the MAb selection and 30% clones from patient sera. However, from the fourth round of panning, a new common motif was identified that carries the sequence TSHHDSHGLHRV (mimotope Vi12A). We also found out that epitope Vi12A appeared to be the most common sequence obtained with both screenings in the fourth round of

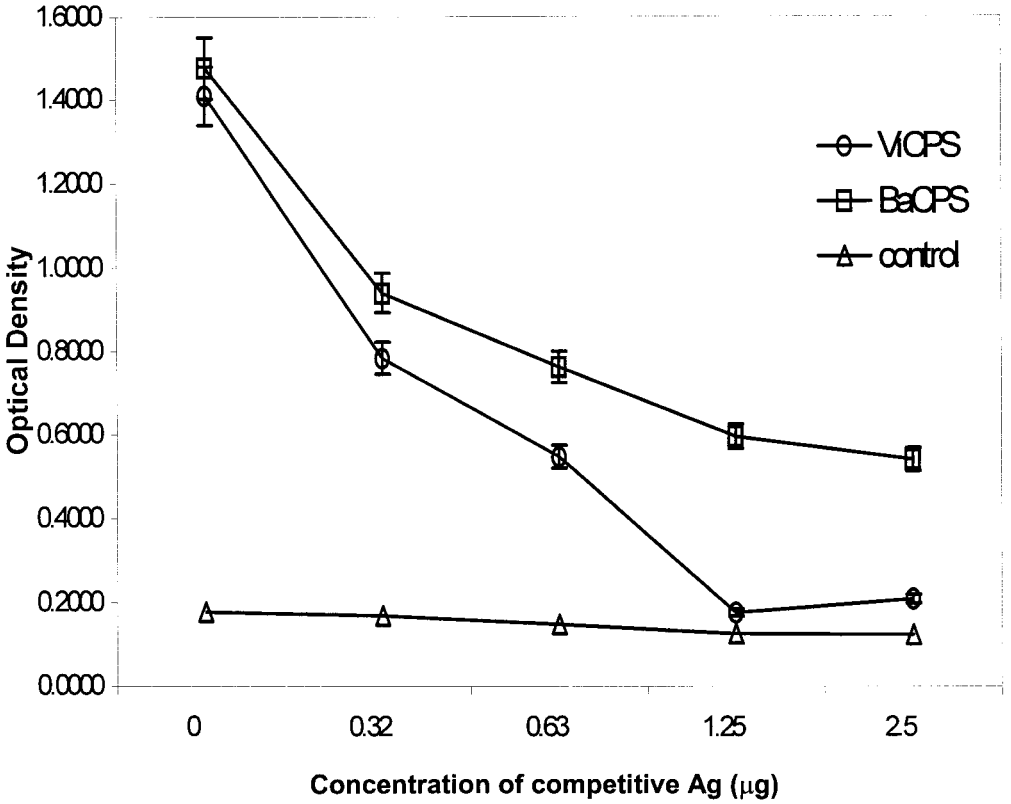

FIG. 4. Binding specificity analysis using competitive ELISA. For this assay, 10^9 phage particles were mixed with twofold increasing amounts of competitive antigen (Ag) before being transferred to the MAb-coated plate. Bound phage was detected with HRP-conjugated anti-M13 antibody. The optical density values are the mean of triplicate samples. The assays were performed twice, and the error bars represent the standard deviation of the mean of two sets of experiment. BaCPS, Vi-containing bacteria; control, negative control phage M13K07.

panning. The same clone was isolated 11 times (55%) in the MAb panning, whereas in the PAb panning, it was easily the most common sequence obtained, found in 89% of the phages selected. Mimotope Vi12B appeared to be the second highest frequency obtained (10% with MAb panning and 11% with PAb panning, respectively). Although these two sequence motifs were different, there are considerable similarities among them: both contain polar, acidic, and hydrophilic residues.

Peptides that mimic carbohydrate epitopes have been isolated previously by using phage display libraries. Peptides that bind the lectin concanavalin A (ConA) have been reported (9). It was demonstrated that peptides containing the consensus sequence (YPY) bind ConA with an affinity comparable to that of the natural ligand (methyl-α-D-mannopyranoside). Phage and synthetic peptides with the motif blocked the binding of ConA to its carbohydrate ligand. Hoess et al. identified a peptide motif (PWLY) that mimics the Ley carbohydrate antigen on the surface of tumor cells and is capable of blocking the binding of antibody to tumor cells and to purified carbohydrate (7).

The peptides identified by both groups, YPY and PWLY, are similar in that they both possess aromatic residues as well as hydrophobic residues. The presence of aromatic residues is characteristically seen in peptides mimicking carbohydrates (7, 9, 14, 19, 26). However, none of the mimotope peptides identified in this study bears an aromatic residue. Instead, highly charged residues, both acidic and basic, were found in both of our mimotope peptides. The presence of acidic residues probably reflects the presence of sialic acid in the original carbohydrate epitope.

The specificity and relative binding activity of the selected mimotope phage clones were further characterized with a series of ELISAs. To confirm that the sequence obtained from the phage library is specifically bound to the antibody, two different ELISAs (indirect and sandwich) were carried out to show that MAb ATVi bound to selected phage clones, but not to nonrelated clones, and that the binding was maintained regardless of whether the plate was coated first with MAb or phage. It was also revealed that binding with mimotope Vi12A (TSHHDSHGLHRV) was considerably better than with Vil2B (ENHSPVNIAHKL). It is interesting to note that mimotope Vil2A is the most abundant clone selected from the fourth round of panning. Therefore, there seems to be a correlation between the abundance of the clones represented after the fourth panning and its relative avidity. To confirm that the mimotopes interact with the same site (the paratope) with the MAb as with the original carbohydrate antigen, a competition ELISA was carried out that allows direct competition between the mimotope and ViCPS. The results from this analysis showed that the binding of MAb ATVi to phage-displayed mimotope could be quantitatively blocked by Vi-containing bacteria as well as purified CPS. The inhibition by the purified

ViCPS has an 50% inhibitory concentration (IC_{50}) value of 0.32 μg/ml.

These data demonstrate that the selected phage-displayed peptide mimotope of the Vi capsular polysaccharide is antigenic and strongly suggest that the selected phage clones bound to the antigen-binding site of the MAb mimic in part the structural epitope of Vi antigen of *S. enterica* serovar Typhi. More studies are required to determine whether the peptide mimotope possesses both experimental and therapeutic utility. It may be that the incorporation of such a mimotope peptide into vaccine preparations enhances the efficacy of vaccines in inducing antibody responses to important carbohydrate epitopes (10).

In summary, we have demonstrated in this study that it is possible to identify peptide mimotopes of disease-related immunodominant carbohydrate epitopes from a phage display peptide library by using pooled patient sera. The discovery of two major common mimotope motifs from pannings using both pooled sera and the ViCPS-specific MAb not only corroborated the findings that these mimotopes are truly relevant, but also revealed an important fact that anti-ViCPS antibodies were highly represented in pooled patient sera. The data obtained provided fundamental information on the characteristics of Vi epitopes of *S. enterica* serovar Typhi. These observations have potentially important implications and applications for the development of peptide-base diagnostic tests and peptide vaccines and may also provide a better understanding of the pathogenesis of typhoid fever.

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