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Peptide Mimotopes of *Neisseria meningitidis* Group B Capsular Polysaccharide

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The antigenic similarity between *Neisseria meningitidis* group B (NMGB) capsular polysaccharide (PS) and human polysialic acid (PSA) has hampered the development of a NMGB PS-based vaccine. But the possibility of a safe vaccine based on NMGB PS has been demonstrated by the existence of the NMGB PS-associated nonautoreactive epitope, which is distinct from those present on human PSA. To obtain peptide mimotopes of NMGB PS, we used HmenB3, a protective and nonautoreactive monoclonal antibody, to screen a phage library with 12 amino acids. We obtained 23 phage clones that bound to HmenB3 but not in the presence of *E. coli* K1 PS [which is α(2-8)-linked PSA like NMGB PS]. The clones contained 3 mimotopes and differed from previously described NMGB PS mimotopes. Immunization with a synthetic peptide of one mimotope elicited anti-NMGB antibodies in BALB/c mice. These mimotopes may be useful in the development of group B meningococcal vaccines.

Key Words: *Neisseria meningitidis*, peptide mimotope, phage display, vaccine

Neisseria meningitidis causes bacterial meningitis and sepsis in children and young adults and is responsible for significant morbidity and mortality worldwide.[1] The main virulence factor of this organism is its capsular polysaccharide (PS), which protects it against complement-mediated bactericidal activity and opsonization.[2] Since the PSs of meningococcal groups A, C, Y, and W135 can elicit serum antibodies that are protective and bactericidal, PS-based vaccines protect against diseases caused by these groups.[3] In contrast, efforts to develop a vaccine against *N. meningitidis* group B (NMGB) infections have been hampered by its poor immunogenicity and autoimmunity, which are probably due to the presence of common epitopes that cross-react with human polysialic acid (PSA).[4,5] NMGB capsular PS is composed of a homolinear polymer of α (2-8) *N*-acetyl neuraminic acid.[6] To overcome the poor immunogenicity and autoimmunity of NMGB PS, the substituted PS of *N*-propionyl group for *N*-acetyl group on NMGB PS followed by protein conjugation was tested.[7] This conjugate elicited protective anti-NMGB PS antibodies with complement-mediated bactericidal activity,[8] however, a subset of autoantibodies to human tissue was developed.[9]

A panel of monoclonal antibodies (mAbs) to NMGB PS suggested the existence of a PS-associated epitope in the bacteria that is distinct from those present in the host PSA.[9,10] We found an NMGB PS-associated epitope that binds to a mAb of HmenB3, which was obtained from mice immunized with *Escherichia coli* K1 bacteria, and which PS is structurally identical to the NMGB PS.[10] This finding generated interest in the use of molecular mimic immunogens as alternative vaccines for NMGB PS. The possibility of using peptide mimotopes as vaccine candidates was supported by the specificities of antibodies, which were induced by immunization of peptide mimotopes of *Cryptococcus neoformans* PS capsule,[11] *N. meningitidis* group C PS[12] and group B streptococcus PS.[13]

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To obtain peptide mimotopes of the NMGB PS epitope recognized by HmenB3, we chose a phage display peptide library (New England Biolabs, Beverly, Mass, USA) based on a combinatorial library of random dodecapeptides fused to a minor coat protein (pIII) of M13 phage.[14,15] After three rounds of biopanning on HmenB3-coated 60-mm-diameter culture dishes (Nunc, Roskilde, Denmark), performed as described by the manufacturer,[10] we obtained 23 phage clones bound to microwell plates (Corning Costar, Corning, NY, USA) coated with HmenB3. PhaB3L12-1, PhaB3L12-2 and PhaB3L12-3 as representatives, bound to wells coated with relevant antibody in a dose-dependent manner, and did not bind to wells coated with unrelated antibodies (Fig. 1A). This binding specificity involved PSA, since the binding of PhaB3L12-1, for example, to HmenB3 was reduced by increasing the amount of purified capsular PS of *E. coli* K1 as an inhibitor in solution but was not reduced by irrelevant PS of *Haemophilus influenzae*, polyribose-ribitol phosphate (PRP) (Fig. 1B). In addition, the binding of PhaB3L12-1 to HmenB3 was reduced by adding Naid60 (Fig. 1C); an anti-idiotype antibody obtained from mice immunized with HmenB3 F(ab')$_2$ and contains the mirror image of HmenB3.[16] These findings indicate that the chosen phage clones specifically bind to HmenB3 and mimic the structure of *E. coli* K1 PS.

The nucleotide sequences of the inserted DNA in all cloned phages were determined, and were translated into amino acid sequences (Table 1). Of the 23 phage clones obtained with HmenB3, 22 clones were found to express the sequences NKVIWDRDWMYP (4.3%, 1/23) and NKVIWEADWAFS (91.4%, 21/23), represented by PhaB3L12-1 and PhaB3L12-2, respectively, whereas PhaB3L12-3 expressed HHSHSILQSDWF (4.3%, 1/23). NKVIWxxDWxxx was the most recognizable consensus sequence (95.7%, 22/23) and the DW sequence was identified in all clones. These peptide sequences have

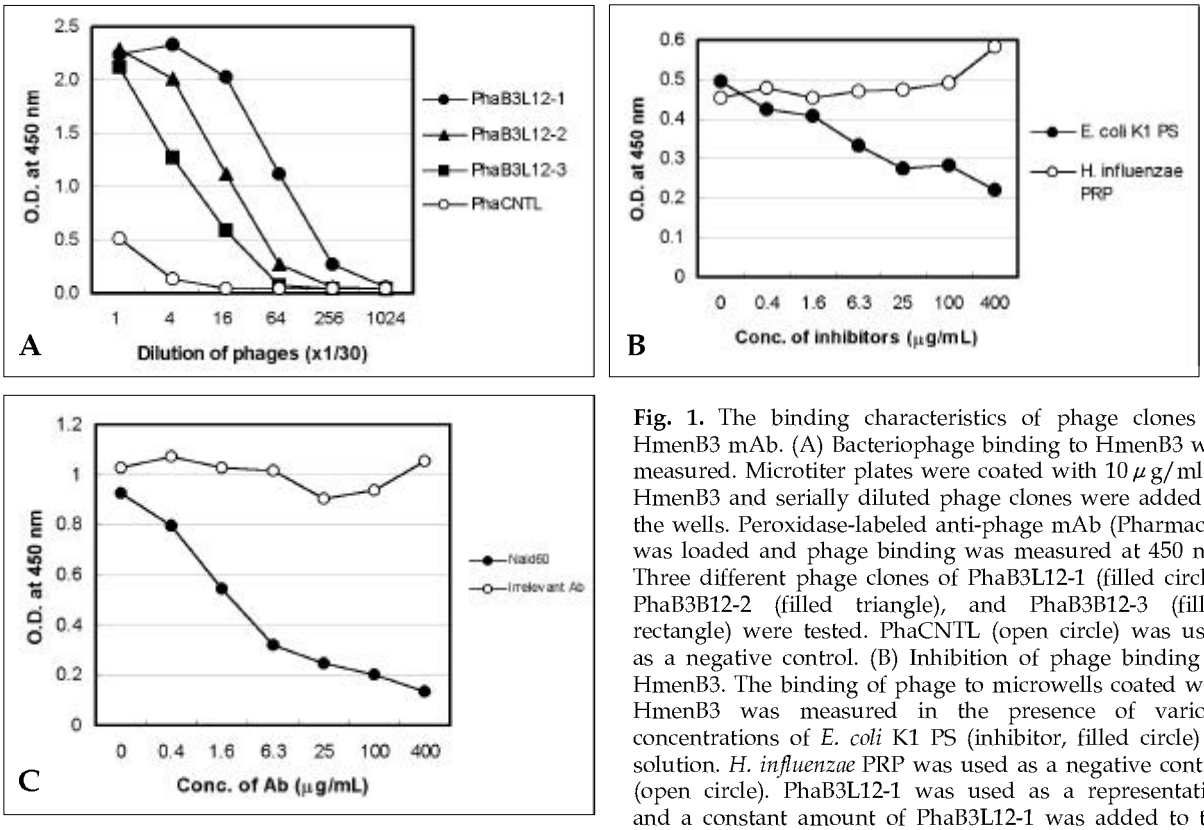

Fig. 1. The binding characteristics of phage clones to HmenB3 mAb. (A) Bacteriophage binding to HmenB3 was measured. Microtiter plates were coated with 10 μg/ml of HmenB3 and serially diluted phage clones were added to the wells. Peroxidase-labeled anti-phage mAb (Pharmacia) was loaded and phage binding was measured at 450 nm. Three different phage clones of PhaB3L12-1 (filled circle), PhaB3B12-2 (filled triangle), and PhaB3B12-3 (filled rectangle) were tested. PhaCNTL (open circle) was used as a negative control. (B) Inhibition of phage binding to HmenB3. The binding of phage to microwells coated with HmenB3 was measured in the presence of various concentrations of *E. coli* K1 PS (inhibitor, filled circle) in solution. *H. influenzae* PRP was used as a negative control (open circle). PhaB3L12-1 was used as a representative and a constant amount of PhaB3L12-1 was added to the microwells. (C) The amount of PhaB3L12-1 binding to microwells coated with HmenB3 in the presence of various concentrations of Naid60 (inhibitor, filled circle), which is an anti-idiotype antibody to HmenB3. The binding of PhaB3L12-1 to HmenB3 was reduced in the presence of Naid60. Irrelevant mAb was used as a negative control (open circle).

Table 1. Peptide Mimotope Sequences of Phage Clones Bound to HmenB3

Phage	Sequence[a]	No. of clones[b]
PhaB3L12-1	NKVIWDRDWMYP	1/23
PhaB3L12-2	NKVIWEADWAFS	21/23
PhaB3L12-3	HHSHSILQSDWF	1/23

[a]Peptide sequences of clones from dodecapeptide insert after a third biopanning by using HmenB3.
[b]Number of identical peptide sequences/total number of sequences.

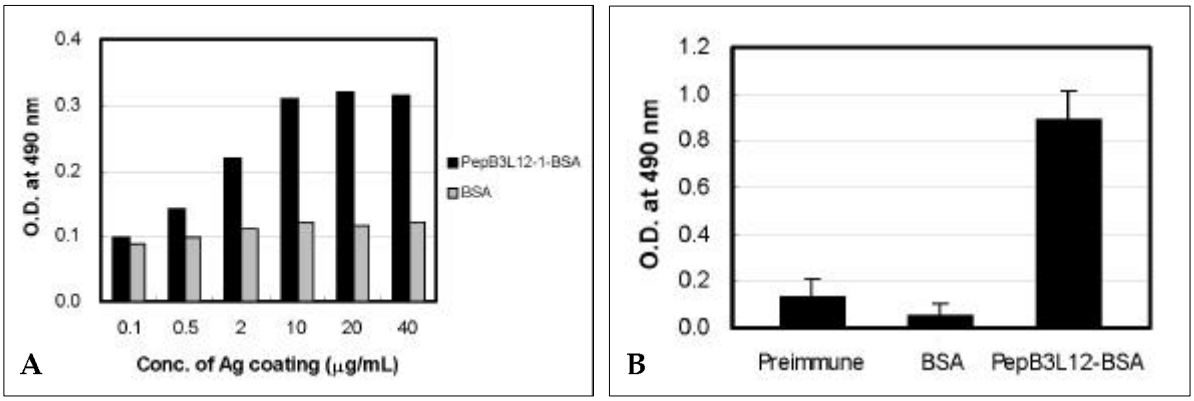

Fig. 2. A. The binding of HmenB3 to PepB3L12-1. Microwells were coated with various concentrations of PepB3L12-1-BSA (black bar) and a constant amount of HmenB3 was added to observe the Antigen binding capacity of HmenB3 by ELISA. Unconjugated BSA was used as a negative control (gray bar). B. Anti-NMGB antibody levels in PepB3L12-1-BSA-immunized mice. Groups of five BALB/c mice were injected with 50 μg of PepB3L12-1-BSA emulsified with complete Freund's adjuvant and boosted three times with incomplete Freund's adjuvant at 2-wk intervals. The production of anti-NMGB antibody was tested 7 days after the final injection using mouse sera at a dilution of 1 : 100. Heat-killed NMGB was used as a coating antigen.[10] Carrier BSA was injected into control mice group. Mean O.Ds. ± S.E are shown. $p<0.01$ for the comparison of PepB3L12-1-BSA- with BSA-immunized or preimmune group.

not been previously reported and are completely different from the several kinds of known peptide mimicry of NMGB PS, which determined for surrogate antigen of anti-idiotype Antibody[17] and from phages displaying 8-mer and 7-mer peptide libraries.[1,10]

To confirm the binding of the peptide insert of the selected phage clones to HmenB3, we used the synthetic peptide CGANKVIWDRDWMYPG named PepB3L12-1 (Korean Basic Science Institute, Seoul, Korea). The corresponding peptide mimotope sequence of the PhaB3L12-1 clone is underlined and the amino acids CGA at the N-terminal were added for conjugation with carrier protein. We conjugated PepB3L12-1 to maleimide-activated bovine serum albumin (BSA) (Pierce, Rockford, IL, USA) to enhance its solubility and absorption to ELISA plate wells. BSA was used as a negative control. After absorption to microwells containing various concentrations of PepB3L12-BSA, an immunoassay was performed using a 1: 100 dilution of mouse ascites, which contained HmenB3. The binding of HmenB3 to PepB3L12-1 was increased as the amount of PepB3L12-1 increased (Fig. 2A). In addition, the binding of HmenB3 to PepB3L12-1-BSA-coated microwells was inhibited by purified *E. coli* K1 PS and Naid60, but not by irrelevant PS (PRP) (data not shown), confirming that PepB3L12-1 is a peptide mimotope of *E. coli* K1 PS.

To investigate the immunogenicity of the synthetic peptide, we intraperitoneally immunized 2 groups of five female BALB/c mice of 6-8 wks-old with 50 μg of PepB3L12-1-BSA emulsified in complete Freund's adjuvant, and boosted three times with incomplete Freund's adjuvant at 2-wk intervals. Serum samples were collected 7 days after the final injection to determine the produc-

tion of anti-NMGB Ab by ELISA assay on NMGB-coated 96-well plates.[10] PepB3L12-1-BSA elicited significant anti-NMGB Ab production in mice (Fig. 2B). A phage clone of PhaB3L12-1 inhibited the binding of anti-NMGB Ab in pooled sera to NMGB-coated microwells (data not shown), confirming that PepB3L12-1 is one of the peptide mimotopes responsible for anti-NMGB PS antibody production. The feasibility of using a peptide mimotope as a surrogate antigen for a nominal antigen is important in vaccine development. Several approaches have been investigated to increase the weak immunogenicity of the peptide, for example genetic immunization combined with immunomodulating cytokine.[18,19] We are currently investigating bactericidal activity using PepB3L12-1-immune mouse sera and its cross-reactive binding with host tissue.

In conclusion, we report the development of a novel 12-mer peptide mimotope of NMGB PS that can elicit antibody binding to NMGB bacteria.

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