

Identification of antigenic epitopes of *Salmonella typhi* using phage display epitope library

VMB-4

Kwai Lin Thong, G Subramaniam¹, S. Devi², S. Puthuchery², M. Yu³, L.F. Wang³, T. Pang¹

Abstrak

Kami telah menyusun suatu pustaka gen target dan pustaka epitop/peptida acak yang terdapat di permukaan filamen partikel faga. Pustaka target gen dibuat dengan teknik kloning dan ekspresi fragmen-fragmen DNA *S. typhi* yang relatif pendek (100-300 pb) dengan menggabungkan dengan gen permukaan faga pIII. Dengan menggunakan biopanning assay di mana serum dari penderita demam tifoid yang telah diencerkan diimobilisasi pada suatu fase padat (misalnya pada pelat ELISA atau butir magnetik), epitop antigenik dari pustaka ini dapat diidentifikasi melalui pengikatan dan selanjutnya elusi dari faga rekombinan tersebut, serta pembacaan sekuens DNA yang relevan. Sekuens DNA yang berhasil diidentifikasi telah dihimpun dalam suatu data dasar dan beberapa epitop antigenik telah diidentifikasi. Kelebihan pendekatan ini adalah pada kemampuannya untuk menemukan seluruh spektrum epitop yang antigenik dan mampu pula menilai reaksi tanggap kebal dari penderita terhadap galur *S. typhi* yang spesifik. Penemuan tersebut di atas dapat memberi implikasi sangat penting dalam meningkatkan pemahaman akan patogenesis penyakit, diagnosis yang lebih baik dan pengembangan vaksin di masa depan.

Abstract

We have constructed a genome-targeted library of *Salmonella typhi* displayed on the surface of filamentous phage particles. The genome-targeted library was made by cloning and expressing relatively short DNA fragments (100-300bp) from *S. typhi* genomic DNA into the pIII phage coat protein genes of a phagemid. Utilizing a biopanning assay, where diluted sera from patients with typhoid fever were immobilised on a solid support (paramagnetic beads), antigenic epitopes from the genome-targeted phage library were identified following binding and subsequent elution of recombinant phages and DNA sequencing of relevant inserts. Database searching of the identified sequence was carried out and putative antigenic epitopes identified. The power of this approach lies in its ability to search for the entire spectrum of antigenic epitopes and in assessing individual patient's immune responses to particular strains of *S. typhi*. The findings may have important implications for improved understanding of disease pathogenesis, better diagnostics and future development of vaccines.

INTRODUCTION

In the past few years, there has been a surge of interest in a new technology for displaying foreign peptides on the surface of filamentous bacteriophages. This phage display technology, which was first developed by George Smith and his colleagues^{1,2}, has a wide range of applications in many disciplines of biological sciences³. One such application is the identification of antigenic epitopes from random peptides libraries displayed on the phage surface by affinity selection or biopanning using immobilised antibody molecules³. This procedure involves repetitive rounds of binding the phage particles to an immobilised antibody target, removal of non-binding and non-specifically bound phages by several washes and

recovery of bound phages by acid elution. The displayed peptide(s) responsible for binding to the antibody can be identified by directly sequencing the encoding insert in the genome of the recombinant phage.

Despite the importance of typhoid fever in the tropical developing countries, the pathogenesis of the disease and host immune response to typhoid fever remains poorly understood. Our previous studies showed that significant genetic diversity exists among recent *S. typhi* isolates from different parts of the world^{4,5} and that this diversity can be correlated with disease phenotypes⁶. Thus we would like to apply the phage display technology to ascertain whether this genetic diversity is reflected phenotypically at the level of antigenic peptides expression recognised by the host immune response during typhoid fever.

In this paper, we describe a slightly different random expression strategy for epitope mapping using phage-display technology. Rather than expressing totally

Institute of Biological Sciences,

¹ Institute of Postgraduate Studies and Research,

² Department of Medical Microbiology, University of Malaya, Kuala Lumpur, Malaysia;

³ CSIRO, Animal Health Lab., Geelong, Australia.

random synthetic peptide sequence, our approach relies on the construction of a random fragment expression library using small DNA fragment generated by partial digestion of whole genome using DNase I. Fragmented *S.typhi* DNAs are cloned into the gene III of a phagemid vector DNA. To increase the efficiency in generating recombinant phage DNA, the linker-ligation-PCR-(LL-PCR) strategy was used⁸.

METHODS AND MATERIALS

Bacterial strains, expression vectors and reagents

A strain of *S.typhi*, st111 was used. *E.coli* strain XL1blue and the phagemid vector pCP 429 were obtained from the AAHL, CSIRO, Australia. All the chemicals were of analytical grade.

Library construction

The procedures for the construction of a genome-targeted phage display library were essentially the same as those described by Wang *et al.* (1995) and Nagesh *et al.* (1996). DNA from *S.typhi* was used. Briefly, genomic DNA from *S.typhi* was partially digested with DNase I to obtain DNA-fragments of between 100-300 bp. These DNA fragments were ligated to phosphorylated *Bam*HI and *Bgl*III linkers, followed by PCR-amplification using the same linkers. The DNA insert was then cloned into the gene III of the phagemid vector, pCP429. Transfection of the recombinant vector DNA into XL1blue cells by electroporation was followed by plating onto LB/Amp plates to obtain cell count. Determination of the library size, preparation of host cells, infection and titration of the phage were all performed according to the procedures previously described⁷.

Library screening

Screening of the phage library through 'biopanning' or binding to antibodies adsorbed on streptavidin-coated magnetic beads was carried out. Serum, #034 from patient with a culture-confirmed diagnosis of typhoid fever was used for this binding studies.

A mixture of biotinylated anti-human IgG was incubated with streptavidin paramagnetic beads for at least 30 min. The suspended beads were added to an eppendorf tube containing a suspension of phage library-polyclonal antibody and was allowed to bind for at least 30 min. at room temperature. To recover the bound phage, the beads were drawn against the eppendorf tube wall with a magnet so that the free liquid in the tube could be removed. Following cap-

ture of the biotinylated antibody-phage complexes, the beads were washed extensively with TBST-0.05% Tween 20 and the bound phage eluted with 40 μ l 1N HCl (pH2.2, with glycine.), 1 mg/ml BSA, neutralised with 16 ml 1M Tris-HCl, pH9.0. The eluates were then used for infecting *E.coli* strain, XL1blue cells followed by plating on LB/Amp plates for colony counting. The remaining culture was infected with a helper phage, M13K07 and after dilution to 10 LB/Amp/Km, they were incubated at 37°C overnight with vigorous shaking. The phage particles which were harvested by PEG/NaCl precipitation can then be used for another round of biopanning to select the highest affinity clones.

PCR-colony screening

PCR was used to screen the recombinant phage clones using the following primers: primer III-5 (AA-CAGCTATGACCATG) which anneals about 150 bp upstream of the *Bgl*III site and primer III-(CCATGTACCGTAACTG) which anneals about 150 bp downstream from the *Bgl*III site. Direct PCR amplification of the phage DNA from the bacterial colonies was performed in which single colonies were transferred to a 0.5ml PCR tube containing 14 μ l water. The cell suspension was lysed by boiling for 2 min followed after immediate chilling on ice. PCR was performed for 25 cycles at 94°C for 1 min, 45°C for 2 min and 72°C for 2 min in a reaction volume of 25 μ l containing 10 pmol each of the primers, 2.5 mM MgCl₂, 0.2 mM dNTPs and 1X PCR buffer. Samples of 5 μ l were analysed on TAE-agarose gels.

Sequence and computer analyses

DNA sequencing was carried out using the Sequenase kit (USB, Cleaveland, USA) and primer 35S (5'-CCCTCATAGTTAGCGTAACG-3') which anneals 80bp downstream from the *Bgl*III site of the phagemid vector. The nucleotide sequences were subjected to the BLASTN search.

RESULTS AND DISCUSSION

We have constructed a genome-targeted phage display library by ligation of randomly fragmented DNA from *S. typhi* into phagemid vector pCP429. The resulting library contained a total of 3x10⁴ independent ampicillin resistant clones and after infection of helper phage M13K07, the library had a titer of 2.6 X 10⁹ TU/ml.

After two rounds of biopanning, the number of phage carrying peptides selected by polyclonal antibodies

from the serum of a typhoid patient had increased from 10^3 - 10^4 , giving an enrichment factor of 10.

Approximately 90 ampicillin resistant colonies were randomly selected and PCR-colony screened. Clones without the insert gave an amplicon of 300 bp in size. Fifty-five percent of the recombinant phage clones tested gave PCR products ranging in size from 350 to 600 bp.

About 40 PCR-positive clones containing insert sizes ranging from 50 to 300 bp were sequenced.

The sequences were subjected to the BLASTN nucleotide search to give us a rough idea as to the identity of the insert sequences. Some of the sequences did not come up with any sequence matches at all and we have eliminated the possibility that they were vector sequences. This could mean that either they were sequences of genes that have not been identified yet or they could be *Salmonella* specific sequences that have not been identified. The preliminary BLASTN search came up with some interesting results in that some of the sequenced clones showed fairly high homology with sequences of known bacterial genes. For instance, one of the clones, clone #5 showed homology with *rbf* gene cluster of three gram-negative bacteria and the highest homology of 87% was coincidentally with *S. enterica*. On the other hand, the nucleotide sequence of another clone, clone #35, seemed to show close homology with the *gln* ALN operon of three enterobacteria, the highest homology (94%) again was with *S. typhimurium*. However, it must be pointed out that these are only preliminary searches. We have yet to identify the correct open reading frame and to obtain the deduced amino acids sequences. In addition, we have yet to confirm the specificity of the peptides as to whether they are *Salmonella*-specific or not. Furthermore, some the sequences did not show any matches. This could mean either that they are sequences of genes that have not been identified yet or that they could be *Salmonella* specific sequences that have not been identified.

Biopanning yielded clones even when there was no specific binding of the ligate to the phage-bound ligand. Hence there is a need for independent confirmation of the specific binding. The PCR-colony screening was shown to be a useful and rapid means of selecting recombinant phage clones harbouring inserts of varying sizes. The amino sequence of the displayed peptide (determined by sequencing the inserts in the phage DNA) is the most informative data. In

addition, other screening methods such as ELISA and immunoblotting should also be carried out to confirm the antigenicity of the epitopes.

The preliminary results obtained so far showed that this methodology is a promising one. However, there is lot more work to be done. The data obtained will provide the fundamental information on the spectrum and characteristics of immunogenic epitopes of *S. typhi* which are recognized by antibodies during typhoid fever in humans. This has important applications in the development of peptide-based diagnostic tests, peptide vaccine and also to provide a better understanding of the correlation between disease phenotypes and the observed genetic diversity among *S. typhi* isolates.

CONCLUSIONS

We have shown that it is possible to construct a genome targeted phage display library of *S. typhi* by inserting fragments of the DNA into the gene III coat protein of a phagemid vector. The preliminary results obtained so far showed that the phage display technology is a promising approach in assessing the correlation between disease phenotypes and observed genetic diversity among *S. typhi* isolates.

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