dentification of antigenic epitopes of *'almonella typhi* using phage display epitope library

VMB-4

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ɔstrak

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

stract

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

ΓRODUCTION

he past few years, there has been a surge of inter- in a new technology for displaying foreign pep- s on the surface of filamentous bacteriophages. ; phage display technology, which was first devel- d by George Smith and his colleagues[1,2], has a e range of applications in many disciplines of bio- cal sciences[3]. One such application is the identi- tion of antigenic epitopes from random peptides ıries displayed on the phage surface by affinity ction or biopanning using immobilised antibody ecules[3]. This procedure involves repetitive ıds of binding the phage particles to an immobi- i antibody target, removal of non-binding and -specifically bound phages by several washes and recovery of bound phages by acid elution. The dis- played peptide(s) responsible for binding to the anti- body can be identified by directly sequencing the en- coding insert in the genome of the recombinant phage.

Despite the importance of typhoid fever in the tropi- cal developing countries, the pathogenesis of the dis- ease and host immune response to typhoid fever re- mains 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 phenotypes[6]. Thus we would like to ap- ply 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

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