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• 計畫中文名稱	抗 B 型肝炎病毒抗體的選殖及其基因的研究		
• 計畫英文名稱	Characterization and Genetic Study of Anti-hepatitis B Virus Antibodies		
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• 英文關鍵字	Hepatitis B virus (HBV)；Combinatorial antibody library；Fab fragment		

• 中文摘要

The objectives of this study are (1) to amplify the entire human heavy and light chain variable region gene repertoire using polymerase chain reaction from anti-HBV antibody positive individuals (2) to clone and express a repertoire of Fab fragments on the surface of VSCM13 phage (3) to enrich a panel of recombinant phages with HBV-specific binding activity. In the subsequent study, we will concentrate on (1) the characterization of the obtained Fab fragments using a competitive inhibition assay; and (2) the determination of the nucleotide sequences of various (V) regions utilized by the heavy and light chain genes of the isolated monospecific Fab molecules. Based on the proposed study, we present here the results generated from our laboratory during the previous year. Several antibody libraries containing light chain genes have been constructed in the newly modified pComb3H vector. However, due to unknown factors, we were unable to ligate the PCR-amplified heavy chain products into the isolated recombinant DNAs to complete the construction of antibody libraries. Accordingly, the light chain gene repertoire in the pComb3H was removed and ligated into the original pComb3 phagemid. Thereafter, the heavy chain genes were ligated into the light chain containing recombinant DNAs. Biopanning against several antigens including HBsAg, HBcAg and HCV NS3 protein shows specific enrichment based on the eluted phage virion. Ten to several hundred folds of enrichment was observed. Western blotting analysis showed that the Fab antibodies with correct molecular weight were expressed in 10 randomly selected clones. Sequence determination of 2 clones containing heavy and light genes demonstrated both clones use a V gene of VL1 family. However, 2 different heavy V genes were employed in their usage. Further experiments such as immunofluorescence staining and competitive enzyme-linked immunoabsorbent assay are required for confirming their antigen binding specificity.

- 英文摘要

The objectives of this study are (1) to amplify the entire human heavy and light chain variable region gene repertoire using polymerase chain reaction from anti-HBV antibody positive individuals (2) to clone and express a repertoire of Fab fragments on the surface of VSCM13 phage (3) to enrich a panel of recombinant phages with HBV-specific binding activity. In the subsequent study, we will concentrate on (1) the characterization of the obtained Fab fragments using a competitive inhibition assay; and (2) the determination of the nucleotide sequences of various (V) regions utilized by the heavy and light chain genes of the isolated monospecific Fab molecules. Based on the proposed study, we present here the results generated from our laboratory during the previous year. Several antibody libraries containing light chain genes have been constructed in the newly modified pComb3H vector. However, due to unknown factors, we were unable to ligate the PCR-amplified heavy chain products into the isolated recombinant DNAs to complete the construction of antibody libraries. Accordingly, the light chain gene repertoire in the pComb3H was removed and ligated into the original pComb3 phagemid. Thereafter, the heavy chain genes were ligated into the light chain containing recombinant DNAs. Biopanning against several antigens including HBsAg, HBcAg and HCV NS3 protein shows specific enrichment based on the eluted phage virion. Ten to several hundred folds of enrichment was observed. Western blotting analysis showed that the Fab antibodies with correct molecular weight were expressed in 10 randomly selected clones. Sequence determination of 2 clones containing heavy and light genes demonstrated both clones use a V gene of VL1 family. However, 2 different heavy V genes were employed in their usage. Further experiments such as immunofluorescence staining and competitive enzyme-linked immunoabsorbent assay are required for confirming their antigen binding specificity.