Thesis Title	Analysis of the Nucleocapsid Gene of Hepatitis C Virus Isolated in Thailand
Name	Rojana Kunkitti
Degree	Master of Science (Microbiology)

Thesis Supervisory Committee

Sirirurg Songsivilai, M.D., Ph.D.

Tararaj Dharakul, M.D., Ph.D.

Sunee Korbsrisate, Ph.D.

Date of Graduation 14 December B.E. 2538 (1995)

ABSTRACT

Hepatitis C virus (HCV) is the major cause of post-transfusion non-A, non-B hepatitis. The most significant consequence of HCV infection is that chronic hepatitis usually develop in more than 50% of HCV-infected individuals. The assays for detecting antibodies to HCV, using recombinant structural and nonstructural HCV proteins, have been applied for blood screening and diagnosis of viral hepatitis. The sensitivity of such assays is dependent upon the extent of sequence diversity between different isolates of HCV in different geographical regions. Recent data demonstrated that the most dominant immunoreactive epitopes were located within the amino-terminal, especially the first 40 amino acids of nucleocapsid or core protein, and the antibodies directed against these epitopes appeared early after infection. Therefore, the antigens from the nucleocapsid region were incorporated in the second- and third-generation diagnostic assays for HCV.

The objectives of this study were to isolate and sequence the core region of

hepatitis C virus genomes found in Thailand, and to express the recombinant protein containing the core protein of the Thai strain of HCV.

In the study presented in this thesis, the gene encoding the core and 5'-noncoding (5'-NC) regions of HCV isolated from 12 Thai blood donors and a chronic hepatitis patient were sequenced and the nucleotide sequences were analysed and compared with other sequences deposited in GenBank database. The alignment of nucleotide sequences of the core region of the Thai strains of HCV in comparison with sequences of HCV in GenBank database showed high degree of homology (approximately 90.5%) among the isolates. The differences at amino acid level were also observed in this study. This result also showed that, HCV genomes isolated in Thailand could be classified into 4 groups, including genotype 3a, genotype 1b, genotype 1a and the new genotype 7.

The gene encoding core protein of a Thai strain of HCV (strain BB51, genotype 1b) was expressed in *Escherichia coli* using a glutathione-S-transferase (GST) fusion protein system. The entire core protein could not be expressed using full-length core genome. However, when this region was truncated at 3'-terminal, resulting in the gene encoding only the first 123 amino acids of core protein, the expression of fusion protein was successful. GST-HCV core protein with the molecular weight of 42 kDa was insoluble but can be solubilized in sarkosyl and purified by affinity chromatography using glutathione-sepharose 4B. This purified fusion protein was verified by reacting with sera containing antibodies to HCV. The expected protein band was observed by immunoblotting. However, there were also several smaller bands in a ladder pattern. The comparison of Coomassie blue protein staining with the immunoblot assay demonstrated the same banding pattern, confirming that the smaller bands contained parts of HCV core protein. The fusion protein purified using this method gave a yield of approximately 26 µg/ml of bacterial culture.

The information obtained from this study on the analysis of nucleotide sequence

of hepatitis C viruses and the GST-HCV core fusion protein derived from the genome of Thai strain of HCV will be crucial for the development of a new generation of diagnostic assays for the diagnosis of HCV infection in Thailand.