Thesis Title APPLICATION OF RECOMBINANT DNA TECHNOLOGY FOR DETECTION OF HLA GENES POLYMORPHISM

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ABSTRACT

Presently, the analysis of HLAs (Human Leucocyte Antigens) is clinically important especially in transplantation. Serological method by lymphocytotoxicity test, currently used in the laboratory, sometimes shows a problem of cross-reaction. Therefore, new method are required for the analysis. The aims of this study are firstly to develop the Southern blotting technique using nucleic acid hybridization probes for detection of HLA-RFLPs (HLA restriction fragment length polymorphisms) and secondly to develop the non-radioactively labeled probe to replace the radioactively labeled probe.

Several molecular biology techniques were included. Followings preparation of human genomic DNA from human peripheral blood leucocytes by proteinase K digestion, phenol-chlorofrom extraction and isopropanol precipitation, good quality of all the extracted DNAs was revealed. The absorbance ratio at the 260/280 nm of each DNA sample was above 2.00 and the electrophoretic pattern showed a single high molecular weight band at $\simeq$ 23 kb. The preparation of the specific HLA DNA probes (DRA, DRB, DQA, DQB) was done by isolating recombinant DNA from the *E. coli* followed by digestion with restriction enzymes, fractination, and recovery the

specific fragment from agarose gel. Among three methods of DNA fragment isolation, the electroeluter method was more efficient than freeze-squeeze method and double slots method. The purified DNA fragments of DRA, DRB, DQA and DQB probes were labeled by random prime labeling method and two tracers, [^{32}P]dATP and b-11dUTP were compared. The dot blot hybridization of the probes indicated that the detection sensitivity of the biotinylated probe was lower than that of the ^{32}P-labeled probes followed by autoradiography for approximately 10 folds using colour staining method and 4 folds using chemiluminescent method.

To study of the HLA-RFLPs, the human genomic DNAs were digested with selected restriction enzymes and Southern blot transfer. Using the ^{32}P-labeled probes specific to various HLA genes, all the autoradiographic results showed clear sharp DNA band patterns of HLA-RFLPs without contaminated background. These findings suggested that development of the analysis was completely successful. Morover, detection of HLA-RFLPs by using biotinylated DNA probe was also performed. The results suggested that the colour staining method was not sensitive enough to detect a single copy of any HLA genes even the amount of the human genomic DNA was increased to 10 μg. The detection by chemiluminescent method showed better results unless the amount of starting genomic DNA and the biotinylated probes was increased to 20 μg and 8 folds respectively. A few bands of the HLA restricted fragments as well as moderately intensed background were observed.

In conclusion, there is still no any alternative method for non radioisotope labelling and detection method developed in the the present study to be efficient enough for detecting a single copy gene of HLA genes as sensitive as the ^{32}P-labelling followed by autoradiography.