June 7,1995

Philip J.Migliore,M.D.
Chairman and Research Director
The Moran Foundation
Dept.of Pathology
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Dear Dr. Migliore

Hereby I am submitting the status report of the research project for the identification of T lymphocyte receptor gene sequences and the development of a molecular diagnostic test for Hypersensitivity Pneumonitis. I would like to take this opportunity to thank the Moran Foundation for the financial support of this research.

Sincerely

Sri Rajagopalan,Ph.D.
Asst.Professor of Pathology

Title of Research

Identification of T lymphocyte receptor gene sequnces and the development of a molecular diagnostic test for Hypersensitivity Pneumonitis.

Status report : June 7,1995

The set out goal of the research is to analyse the T cell receptor gene sequences in the T lymphocytes of individuals suffering from Pigeon breeders' disease, a form of hypersensitivity pneumonitis. If certain similarities in the T cell receptor gene sequences are observed, it is hoped that the nucleic acid methodology would provide a way for the diagnosis of various forms of hypersensitivity pneumonitis that lead to inflammation in the lung.

Towards this goal, the following experiments were conducted in this study.

I) Evaluation of the T cell receptor Typing Amplimer kits :

A commercially available T cell typing kit(Clontech Laboratories Inc., Palo Alto,Ca.) was evaluated. Three different T cell lymphoma lines were analysed for T cell receptor alpha and beta sequences. Total RNA isolated from these cell lines maintained in culture were subjected to reverse transcription (RT) using random hexamers. The product of the reaction was used in PCR reactions containing 22 primers representing 22 v alpha families and 25 primers representing 25 v beta families. The PCR products were analysed on 4% agarose gel electrophoretically. Discrete fragmets were observed in 3 of the 22 alpha families and 2 of the 25 beta families for one of the cell lines(H9) indicating usage of restricted v cell family of genes in receptor rearrangement. The other two cell lines each yielded one discrete fragment for the alpah and beta family respectively.

II) Analysis of peripheral blood of patients suffering from Pigeon Breeder's Disease for T Cell receptor gene:

Buffy coat preparations from 17 individuals suffering from Pigeon Breeder's disease were extracted with RNAZol B to obtain total RNA. The RNA was reverse transcribed and the product was subjected to PCR using 22 v alpha and 25 v beta primers in separate reactions. The PCR products were analysed by gel electrophoresis. In none of the 17 specimens analysed discrete fragments were seen on gel indicating the absence of monoclonality. Simultaneous analysis by RT-PCR for terminal deoxynucleotidyl transferase, a gene active in T lymphocytes yielded positive result. This indicates that the T cells in the peripheral blood of patients analysed so far does not contain monoclonal populations.

III) Analysis to be done : We are in the process of analysing lymphocyte specimens from bronchial lavage of patients. The rationale behind this approach is that bronchial lavage might contain a higher proportion of sensitised T cells compared to peripheral blood.

This analysis is expected to be completed by late August of this year. At that time a comprehensive analysis as to the use of T cell receptor sequences as a window for hypersensitivity pneumonitis can be made.