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27 April 1994

Dr. Philip J. Migliore
Chairman and Research Director
The Moran Foundation
Department of Pathology
Baylor College of Medicine
Houston, Texas 77030

Dear Phil,

I received your letter requesting an annual progress report for the Moran Foundation projects conducted in my laboratory. I am sending along a copy of the progress report for project #0062, which I submitted 3 August 1993. I have also included a final report for project #0062, and two published papers, and a submitted manuscript acknowledging Moran Foundation support. Note also that a patent application has been filed that was in small part based on some the results generated with project #0062 support. We are working with BCM Technologies to arrange licensure of the vaccine with one of the major pharmaceutical companies. Thus far, Merck & Co., American Cyanamid, BioMerieux, and Praxis Biologicals have all expressed preliminary interest.

I stress my deepest appreciation for the continuing support of the Moran Foundation. Please let me know if you need additional information.

Sincerely yours,

James M. Musser, M.D., Ph.D.
Assistant Professor of Pathology,
and Microbiology and Immunology

Final Report: Moran Foundation Research Award 92-62

Title: "Identification and characterization of natural allelic variants of a cysteine protease toxin synthesized by *Streptococcus pyogenes.*"

Principal Investigator: James M. Musser, M.D., Ph.D.

Aims and Objectives: The objective proposed was to identify the spectrum of naturally occurring allelic variants of a cysteine protease toxin expressed extracellularly by all strains of the human pathogenic bacterium *Streptococcus pyogenes* (group A *Streptococcus*), through automated DNA sequencing. Data on allelic variation was sought in order to assist the accomplishment of our ultimate goal, which is formulation of an efficacious vaccine against *S. pyogenes*. In addition, allelic variation data are important to the success of ongoing collaborative studies with investigators in New Zealand and Sweden designed to "map" the position of variant amino acids onto the three-dimensional X-ray crystallographic structure of the cysteine protease.

Proposed Question 1: What is the range of allelic variation in the protease structural gene (*speB*) among streptococcal strains? Are there certain alleles uniquely or nonrandomly associated with strains causing each type of streptococcal infection?

Progress: The two objectives were completed in entirety. Allelic variation was studied in a sample of 68 strains from global sources by automated DNA sequencing. We discovered that the *speB* gene is unusually well conserved, and allelic variation is due primarily to accumulation of point mutations, some of which result in amino acid substitutions. No compelling evidence was found for unique or nonrandom association of *speB* alleles and strains causing particular types of streptococcal infection, such as acute rheumatic fever, pharyngitis, or toxic-shock-like syndrome. These results have been communicated to the scientific community, and a manuscript describing the data is appended.

Proposed question 2: What is the 3D X-ray cystallographic structure of SPEB and where do the amino acid substitutions found in variant alleles "map" onto this structure?

Progress: These studies are being conducted in collaboration with Professors Lars Bjorck, University of Lund, Sweden, and Edward Baker, Massey University, New Zealand.

At this stage, work is progressing in Dr. Baker's laboratory on crystallization of cysteine protease. Dr. Baker's group has succeeded in obtaining crystals, but they are not yet of sufficient size and quality to commence structural analysis. However, because our group has successfully completed our component of the project, once the structural data are

generated, we will rapidly be able to map the variant amino acids onto the crystal structure.

Associated Project #1: The data generated by the study of allelic variation in *speB* permitted us to develop a rapid strategy to subtype *S. pyogenes* isolates for epidemiologic purposes. We exploited the *speB* allelic variation data to assist us in an investigation of an outbreak of invasive *S. pyogenes* disease occurring in Air Force recruits at Lackland Air Force Base, San Antonio. The outbreak and epidemiologic investigation of it using automated DNA sequencing was recently described in a paper appended to the progress report.

Associated Project #2. The data generated by study of allelic variation also proved very useful in a preliminary study examining the ability of vaccination with the cysteine protease to protect mice against lethal challenge with a heterologous strain of *S. pyogenes*. The results of the study demonstrate that active vaccination of mice with purified cysteine protease is partially protective in a mouse model of invasive streptococcal disease. A manuscript describing these critical results is now under review, and is appended to this progress report.

Manuscripts acknowledging support of Moran Foundation project 92-62.

1. Kapur, V., S. Topouzis, M. W. Majesky, L.-L. Li, M. R. Hamrick, R. J. Hamill, J. M. Patti, and J. M. Musser. 1993. A conserved *Streptococcus pyogenes* extracellular cysteine protease cleaves human fibronectin and degrades vitronectin. Microbial Pathogenesis 15:327-346.

2. Musser, J. M., V. Kapur, J. E. Peters, C. W. Hendrix, D. Drehner, G. D. Gackstetter, D. R. Skalka, P. L. Fort, J. T. Maffei, L.-L. Li, and G. P. Melcher. 1994. Real-time molecular epidemiologic analysis of an outbreak of *Streptococcus pyogenes* invasive disease in US Air Force trainees. Arch. Pathol. Lab. Med. 118:128-133.

3. Kapur, V., J. T. Maffei, R. S. Greer, L.-L. Li, and J. M. Musser. 1994. Vaccination with streptococcal extracellular cysteine protease protects mice against lethal challenge with heterologous group A streptococci. Microbial Pathogenesis, submitted.