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Dr. Philip J. Migliore
Chairman and Research Director
The Moran Foundation
Department of Pathology
Baylor College of Medicine
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Dear Phil,

It was a pleasure to see you earlier today at the shotgun wedding. Good luck with the fetal lung maturity issue.

I am sending along several items. First, the progress report for my current Moran Foundation support is enclosed. The comparative DNA sequencing of the *speB* gene encoding the streptococcal cysteine protease went extremely smoothly and moved along ahead of schedule, mainly because I was fortunate enough to have a superb Rice undergraduate student working in the laboratory since the end of May. She quickly mastered the automated sequencing technique and has been quite productive thus far this summer. Second, a manuscript describing our results is enclosed. This manuscript has been submitted to *Microbial Pathogenesis* and is under review. I have acknowledged support from the Moran Foundation, as appropriately required by the Foundation. Third, a copy of the paper describing the "Geysen technique" is enclosed. As we discussed, this technique has been widely adopted by many investigators interested in epitope mapping of microbial proteins that are candidates for use in immunoprophylaxis. Although there are some drawbacks to this technique (for example, in general only linear epitopes are readily identified), in my opinion, it is a very good approach if one has limited resources.

Although I didn't mention it in my application, my postdoctoral fellow and I have been working with Fulbright & Jaworski on a patent application for immunization with the cysteine protease, a genetically constructed nonproteolytic mutant, or synthetic peptides. The results of the Geysen technique study will be extremely important for identifying the antigenic linear epitopes for use in vaccines based on synthetic peptides.

I stress that I very much appreciate the 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

Progress 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 crystallography structure of the cysteine protease.

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

Progress: We requested funding to defray the cost of automated sequencing of the *speB* gene in 100 strains selected to represent the range of overall chromosomal diversity present in the species. Support was provided to sequence the gene in 60 strains.

Allelic variation in the cysteine protease structural gene was successfully studied in 68 strains expressing 39 M protein serotypes and 5 provisional M serologic types, and representing 50 phylogenetically distinct clones identified by multilocus enzyme electrophoresis. We have discovered that the gene is well conserved and allelic variation is due predominantly to accumulation of point mutations. Based on predicted amino acid sequences, one mature cysteine protease variant would be made by clones expressing 19 distinct M protein serotypes. Moreover, 33 of 39 speB alleles identified encode one of three mature protease variants that differ from one another at only one or two amino acids clustered in a ten-amino acid region. All 39 alleles, and virtually all strains, encode a product that reacts with polyclonal antisera specific for purified cysteine protease. No compelling evidence was found for unique or nonrandom association of certain *speB* alleles with strains causing different types of streptococcal infection.

These results have been described in a submitted manuscript by Kapur et al., "A conserved *Streptococcus pyogenes* extracellular cysteine protease cleaves human fibronectin and degrades vitronectin," that is appended to this progress report. Moran Foundation support is acknowledged.

In summary, proposed question 1 goals have been accomplished in entirety ahead of schedule.

Proposed Question 2: What is the 3D X-ray crystallographic 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 Professor Lars Bjorck, University of Lund, Sweden, and Professor Edward Baker, Massey University, New Zealand.

At this stage, work is progressing on crystallization of the cysteine protease. Dr. Baker's group has grown crystals of both the zymogen and truncated mature active form of the cysteine protease. His group has been concentrating on obtaining crystals large enough and of appropriate quality to begin X-ray diffraction studies, but at this point the structural analyses have not yet commenced. The studies are progressing in Dr. Baker's laboratory.

Importantly, based largely on our group's comparative sequencing studies, we have generated sufficient information about amino acid changes in the allelic variants that we are poised to "map" the positions of the changes onto the 3D structure when available.