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14 May 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 am sending a progress report for Moran Foundation project #0065, "Antigenic sites on an extracellular cysteine protease toxin synthesized by *Streptococcus pyogenes*: mapping with synthetic peptides." A fuller characterization of the antigenic sites is in progress with sera samples obtained from patients with several types of group A *Streptococcus* disease.

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

Progress Report: Moran Foundation Research Award 93-65

Title: "Antigenic sites on an extracellular cysteine protease toxin synthesized by *Streptococcus pyogenes*: Mapping with synthetic peptides."

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

Aims and Objectives: The objective of the proposed research is to map the antigenic sites on an extracellular cysteine protease (interleukin-1β convertase) toxin synthesized by the human pathogenic bacterium *Streptococcus pyogenes* (group A *Streptococcus*). Definition of the antigenic sites is necessary to accomplish the ultimate goal of our research, which is formulation of an efficacious vaccine against *S. pyogenes*. The so-called "Geysen" strategy for epitope analysis using synthetic overlapping peptides is being used.

Progress: Although this research has just begun, we have already made significant progress. The funds provided by the Moran Foundation were used to help defray the purchase of commerically synthesized overlapping peptides corresponding to the entire length of the cysteine protease precursor. The peptides were purchased for $9920 from Chiron Mimotopes, based in Australia. Thus far we have determined the epitope recognition site of 3 murine monoclonal antibodies raised against purified protease. The antibodies were used in solid-phase enzyme immuoassays to scan 235 cleaved biotinylated peptides, each of 10 amino acid residues, with an offset of 2, representing 13 distinct allelic variants of the full length secreted protease precursor. The analysis identified a sextapeptide epitope located in a region of the molecule that has a cluster of several amino acid substitutions (the area around residues #308-317). Moreover, the analysis revealed that the specificity of all 3 murine monoclonal antibodies to this epitope is altered in several naturally occurring cysteine protease allelic variants. The stdies suggest that allelic variation in the cysteine protease gene may be, in part, a result of host selective pressure. Importantly, the results provide a framework for the design and evaluation of synthetic peptides for potential use in group A streptococcus immunoprophylaxis research. An abstract describing these results will be presented at the 34th Intersciences Conference on Antimicrobial Agents and Chemotherapy, in Orlando, FL., in October. A copy of the abstract is appended.

Planned Studies: Beginning in May, we will begin to characterize the reactivity of patient sera (acute and convalescent phase) against the panel of overlapping synthetic peptides, in order to determine exactly which, in any, linear B-cell epitopes patients raise antibody against. Sera from patients with several types of streptococcal diseases (acute rheumatic fever, severe invasive episodes, glomerulonephritis, etc.) will be studied.

34th ICAAC, Orlando, Florida

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Start→ **Identification of Linear B-Cell Epitopes in a Conserved *Streptococus pyogenes* Extracellular Cysteine Protease (Interleukin-1β Convertase).** V. KAPUR,* L-L. LI, and J. M. MUSSER. Baylor College of Medicine, Houston, TX.

We recently demonstrated that virtually all isolates of Group A streptococci secrete a highly conserved extracellular cysteine protease that cleaves human vitronectin and fibronectin, and converts inactive IL-1β precursor to biologically active IL-1β. In addition, we have shown that serum antibodies directed against the cysteine protease or it's precursor provide the host with protection against severe *S. pyogenes* infection. To determine the location of linear B-cell epitopes in this molecule, murine monoclonal antibodies directed against the extracellular cysteine protease were prepared, and used in solid-phase enzyme immunoassays to scan 235 cleaved biotinylated peptides, each of 10 amino acid residues with an offset of 2, representing 13 distinct allelic variants of the full length secreted cysteine proteinase precursor. The analysis identified a sextapeptide epitope located in a region of the molecule that has a cluster of several amino acid substitutions, and is thought to be a potential target for the host immune response. Moreover, the analysis revealed that the specificity of murine monoclonal antibodies to this epitope is altered in several naturally occurring cysteine protease allelic variants. These studies suggest that allelic variation in the cysteine protease gene may be, in part, a result of host selective pressure, and provide a framework for the design and evaluation of synthetic peptides for potential use in Group A streptococcal immunoprophylaxis research.

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S. pyogenes | Epitope mapping | Cysteine protease

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