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19 June 1995

Philip J. Migliore, M.D.
Research Director
The Moran Foundation

Dear Phil,

Enclosed are the final reports for Moran Foundation projects 93-65 and 94-70. I apologize for the tardiness of the reports, and I hope that their lateness has not severely compromised your ability to assemble a report to the Board of Directors and to Mr. Moran.

Again, Phil, sorry for the delay.

Sincerely yours,

James M. Musser, M.D., Ph.D.

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 Moran Foundation Research Award 93-65 was to map the antigenic sites of an extracellular cysteine protease (interleukin-1β convertase) toxin synthesized by the human pathogenic bacterium *Streptococcus pyogenes* (group A *Streptococcus*). We proposed to use the so-called "Geysen" strategy for epitope analysis using commercially purchased overlapping synthetic peptides.

Progress: The proposed research is now complete. We purchased a total of 235 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 of the molecule around amino acid residues #308 to 317). All murine monoclonal antibodies we had raised to the protease had the identical reactivity. Interestingly, the analysis revealed that the specificity of the monoclonal antibodies is altered in several naturally occurring cysteine protease variants (see attached figure). The studies suggest that allelic variation in the cysteine protease gene may be, in part, a result of host selective pressure. Importantly, the results provided a framework for the design and evaluation of synthetic peptides for potential use in group A *Streptococcus* immunoprophylaxis research.

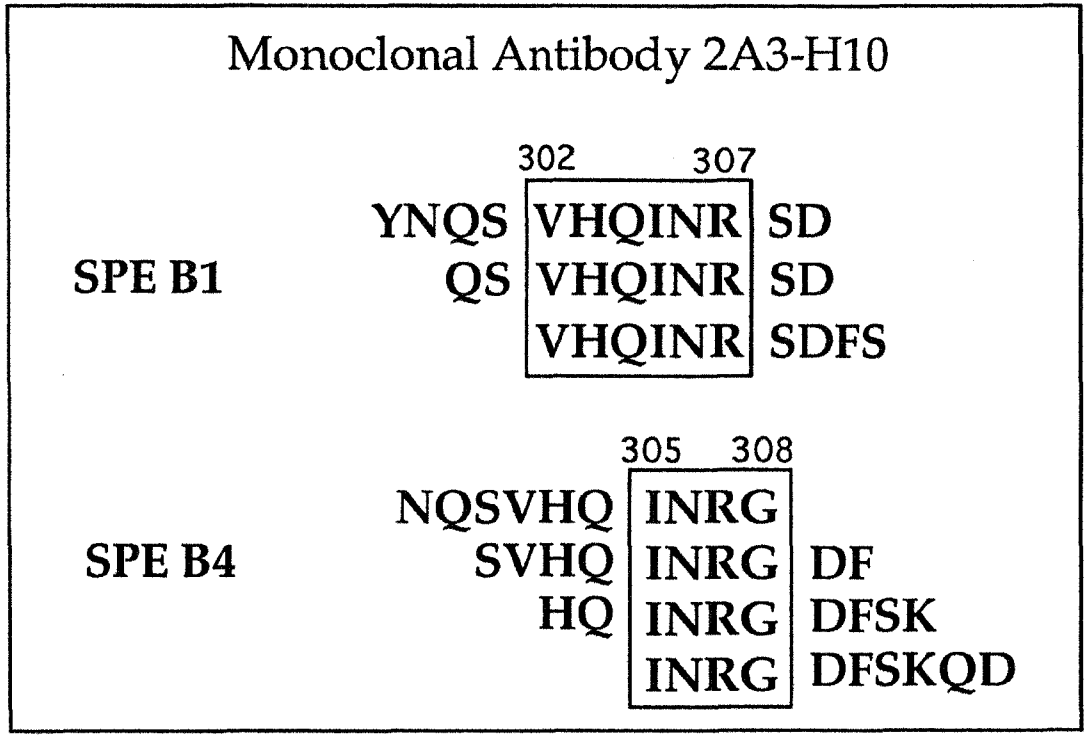
In addition to characterization of the monoclonal antibody reactivities, we proposed to study the reactivity of patient sera (acute and convalescent phase) against the panel of overlapping synthetic peptides. Unfortunately, the patient sera samples did not react strongly with the synthetic peptides, a result suggesting that in humans, conformational epitopes rather than linear epitopes are being recognized.

Presentation of data: These data were presented at the annual meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy, held in Orlando, FL (abstract #B31) (copy attached).

Acknowledgment of Moran Foundation support.

1. Support by the Moran Foundation was acknowledged in the above presentation.

2. The data are now being written in manuscript form for publication, and foundation support will be acknowledged.


Monoclonal Antibody 2A3-H10
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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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City: Houston | State: TX | Zip Code: 77030 | Country: USA

Abstract Title: Identification of Linear B-Cell Epitopes in a Conserved ...
(type in the center of the box, only the portion of the title that will fit)

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S. pyogenes | Epitope mapping | Cysteine protease

Progress Report: Moran Foundation Research Award 94-70

Title: "Synthetic peptide vaccine against Streptococcus pyogenes (group A *Streptococcus*) based on a linear B-cell epitope in the conserved extracellular cysteine protease."

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

Aims and Objectives: The objective of Moran Foundation Research Award 94-70 was to test the hypothesis that an efficacious vaccine against group A *Streptococcus* can be formulated based on a linear B-cell epitope in a highly conserved extracellular cysteine protease. The cysteine protease is made by all isolates of this pathogen and is highly conserved. We proposed to test the strategy using a mouse model of group A *Streptococcus* invasive disease.

Progress: The proposed research is complete. Three synthetic peptides were purchased that encompass amino acid residues 295 to 320 of the cysteine protease. Each peptide was chemically conjugated to cholera toxin B (CTB) subunit purchased from Sigma Chemical Co. Swiss white female mice were immunized by intranasal administration of 40 ug each of three peptide-CTB conjugates. Control mice received CTB, but no peptide. The mice were immunized i.n. once each day on days 1, 3, and 5, rested 3 weeks, and boosted i.n. with a single peptide-CTB dose. The animals were then tested 3 days later for anti-peptide antibody presence in saliva and serum by a previously described ELISA. Because only about 10% of the animals demonstrated evidence of serum antibody, and none had evidence of secretory antibody, the above immunization schedule was repeated. Unfortunately, repeat immunization failed to significantly increase the percentage of mice that seroconverted. However, on the chance that very low levels of antibody were present, the animals were challenged i.n. with a virulent streptococcal strain. There was no significant difference in the Kaplan-Meier survival curves between the immunized mice and the control animals that received CTB only. The inability to generate significant levels of seroconversion is a well-known problem with the synthetic peptide approach.

Presentation of data: Because the data did not reveal significant positive results, they have not yet been presented. When the data are presented, we will acknowledge support of Moran Foundation project 94-70