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Moran Foundation
2003 Progress Report
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Project Title:

"Whole Genomic Approaches to *Helicobacter pylori*:Epithelial Cell Interactions"

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Award Period: November 1st, 2002 - June 30th, 2003

Hypothesis and Specific Aims

This proposal aims to identify novel bacterial genes that modulate the immune system and regulate cytokine gene expression in innate immune effector cells, specifically monocyte-macrophages.

Aim: **Identify *Helicobacter pylori* genes upregulated during co-culture of *H. pylori* and human gastric epithelial cells (GECs).** Genomic microarray-based expression profiling will be used to identify *H. pylori* genes induced in *H. pylori.*

Research Accomplishments During Award Period

We have partially addressed the specific aim of this proposal. The Moran Foundation award enabled us to initiate our microarray studies in the laboratory. Our initial studies focused on validation of our approaches to study gene upregulation with human Affymetrix arrays. We have purchased and installed all of the relevant software for microarray-based gene expression profiling in our laboratory. A combination of dChip and GeneSpring (v. 5.1) programs have been installed and are currently being used to study human macrophage responses to bacterial lipopolysaccharide (LPS). One Ph.D. candidate in the lab, Michael Dillon, was recently trained by Silicon Genetics to perform informatics and advanced statistical analyses with GeneSpring. These capabilities enable us to perform studies of host responses using Affymetrix arrays and bacterial gene expression profiling using custom and commercial arrays.

Experiments regarding bacterial LPS-induced gene expression will be described briefly. LPS stimulation of macrophages initiates the macrophage activation program in a dose and time-dependent manner. The aim of this study is to utilize Affymetrix microarray technology to screen the global gene expression profile initiated by low-level LPS stimulation of RAW 264.7 macrophage-like cells. RAW 264.7 cells were grown to confluence and activated with a very low concentration [2 ng / 5 X 10^4 cells] of *E. coli* LPS, serotype O127:B8 (Sigma). The cells were incubated for five hours, washed and harvested. RNA was extracted from the cultures by way of phenol-chloroform extraction and EtOH precipitation. RNA quality and concentration were determined by way of Agilent Bioanalyzer microfluidics-based LabChip analysis and absorbance spectrophotometry, respectively. RNA from 5 experimental days was divided into two

pools, A and B, (pool A = 3 experimental days and pool B = 2 experimental days) for both the control (unstimulated) and experimental (LPS stimulated) samples. These two sets of pooled RNA were then submitted, as biological replicates, for microarray analysis to the BCM Microarray Core Facility.

Data analysis was performed via two computer-based programs DNA Chip Analyzer (dChip; Wong et al., Harvard University), which is being used for data normalization, outlier detection and model based analysis, and GeneSpring (Silicon Genetics), which is being used to perform statistical analysis (Welch's T-test) and visualization of results. Results obtained from these experiments indicate statistically significant ($p < 0.05$) expression changes of greater than 1.2 fold for 463 genes, with 188 of these genes being significantly up-regulated and the remaining 275 genes, which passed the criteria, being significantly down-regulated. As would be expected, an increase in mRNA levels of pro-inflammatory cytokines and chemokines has been detected. For example, initial analysis has shown that chemokines CXCL-2 and CXCL-10, increased 24- and 8-fold respectively ($p \leq 0.05$), CCL-5, 7, 4, and 3 showed increases between 5- and 18-fold ($p \leq 0.05$) with CCL-5 having the highest (18 fold) increase. IL-1α and IL-1β mRNA levels increased approximately 7-fold and TNF-α mRNA levels increased 5-fold. Continued interpretation of these results is in progress, and will be followed by real time RT-PCR analysis of interesting findings for verification of mRNA expression levels.

Experiments with *H. pylori* microarrays are pending as of this progress report. *H. pylori* arrays have been purchased with Moran support from MWG Biotech. Experiments have been designed and RNA isolated from pathogenic strains of *H. pylori*. Hybridization experiments and informatics studies are pending.

This project is still active.

Relevant Abstracts and Publications During Award Period

Abstracts

Alyamani AJ, Brandt P, Fox JG, Suerbaum S, **Versalovic J.** Characterization of *Helicobacter hepaticus* catalase. American Society for Microbiology 103rd General Meeting, Washington, DC. 5/03.

Tuazon OM, Barraquio WL, DeUngria CA, **Versalovic J.** Denaturing high performance liquid chromatography exhibits the presence of *Helicobacter pylori* 23S rDNA mutations in gastric biopsies. The Philippine Society for Microbiology 32nd Annual Convention, Tadaytay City, Philippines. 5/03.

Dillon MG, Pena JA, and **Versalovic J.** Microarray based gene expression profiling of LPS-stimulated murine macrophages. Baylor College of Medicine Graduate School Symposium, 2003. Submitted.

Future publications will acknowledge the support of the Moran Foundation.