American Society for Microbiology

AMERICAN SOCIETY FOR MICROBIOLOGY
Texas Branch

Fall Meeting
November 16-18, 2006
Galveston, TX

Hosted by:
UTMB
The Department of
Microbiology & Immunology
The University of Texas Medical Branch

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Texas Branch History

Our Texas Branch Constitution says, "The object of this branch shall be the advancement of the science of bacteriology, the promotion of microbiological research, and the fostering of acquaintance among workers in bacteriology and allied fields. It says also, "Any person living in the State of Texas and interested in microbiology is eligible for membership."

The Texas Branch of ASM was founded in 1941, with I.M. Lewis of the University of Texas as its first president. For 40 Years the Branch met for two weekends each year, with the microbiologists in one of our many universities serving as hosts. During the first 15 years, one meeting was at Austin and the others were in the hinterlands, but the emergence of so many active centers of microbiology dictated that Austin take its turn in hosting meetings with Galveston, Houston, Dallas, Fort Worth, Arlington, College Station, and Corpus Christi.

The typical program starts off with a seminar on Thursday evening. Several parallel sessions of research reports are held on Friday and Saturday. To encourage excellence in the Texas Branch, graduate students are encouraged to submit their abstracts for consideration for oral presentation. Those selected are rated by judges; the best general papers are awarded plaques honoring the late O.B. Williams, and the best presentations on medical subjects receive the S.E. Sulkin Award. An annual I.M. Lewis lecture has been delivered by famous microbiologists from all over the world. Since we have accumulated more heroes than we can honor in these ways, the Branch now annually honors a senior microbiologist with an outstanding record of service to our science with an elaborate plaque as a Distinguished Service Award.

At the 1956 national meeting of the Society of American Bacteriologists in Houston, a pamphlet was distributed entitled The History of Bacteriology in Texas. It was compiled by O.B. Williams of the University of Texas, Austin, and W.B. Sharp of the University of Texas Medical Branch, Galveston. At the time, graduate work in the subject was available at those two institutions, and programs had been started at Baylor College of Medicine as well as at the Southwestern Medical College in Dallas.

The amazing expansion of microbiological science in the 1960s and 1970s, especially in the areas of virology and immunology, and the leadership of ASM in uniting these fields (by expanding the journals and the meetings to serve these diverse elements) led to development of major programs in most of our university units that identified with this science. The *ASM News* of September, 1980 identified 21 educational units in the state of Texas with active programs in microbiology.

As a branch of the American Society for Microbiology, our mission is to promote microbiology throughout Texas. We cover the entire state of Texas, with the exception of the Trans Pecos region in far west Texas, which is affiliated with the Rio Grande ASM branch. Opportunities for microbiology are as big as Texas, ranging from those in medicine and infectious diseases, biotechnology, industry, and the environment to the space program. Branch membership is open to anyone interested in microbiology. While the majority of our members live in Texas, several members live in other states. In addition, we are honored that some new members have joined us from Mexico, where they are also affiliated with the Mexican Society for Microbiology.

Every person should know their beginnings – it teaches them humility and gives them pride and ambition. Every Organization should know its beginnings – for this gives us a firm base for our journey into the future.

Isaac McKinney Lewis
Founding Father

Dr. Isaac McKinney Lewis, Professor of Bacteriology in the University of Texas, died of a heart attack in Austin on March 12, 1943. He was devoted to his mother who had been a teacher throughout her lifetime, and to her he gave the credit for instilling in him the desire to secure an education. The caliber of his work is attested by the letters of commendation which he received from many leading bacteriologists, both American and foreign. The quality and loyalty of the students he sent forth in the world, his generosity in appraising the works of others in his own field of research, the courteous little notes written in his own hand, these and many acts of kindness made us all feel that a friend is gone. As a teacher Dr. Lewis excelled. His lectures, sound always in matter, were invariably well organized, well presented, and highly interesting. Among his associates it was a common experience to be told by former students that Dr. Lewis was the best teacher they had during their college career, either at the University of Texas or elsewhere. As a man he was unselfish, kind, modest almost to the point of shyness, with a lively sense of humor and sparkling wit. If differences of opinion seemed to demand it, he could set forth his own with clarity and force. Under stress, he could pack more meaning into fewer and more incisive words than most men; but he was utterly incapable of harboring ill feelings. True to the loftiest ideals, his conduct was irreproachable. Friends, colleagues, and students found him always easily approachable, sympathetic, and, in times of financial stress, generous with aid.

S.E. Sulkin

Dr. S.E. Sulkin, "Ed" to most after a first meeting, was Chair of the Department of Microbiology at the University of Texas Southwestern Medical School in Dallas, a position he held continuously for 27 years, starting 2 years after he joined the staff of what was then the newly founded Southwestern Medical College. Born in Boston he received his B.S. degree from the University of Rhode Island in 1930 and Ph.D. from Washington University, St. Louis, in 1939 under tutelage of Dr. Jacques Bronfenrenner. He was an extremely productive investigator. The field of bacteriology, immunology, and especially virology were well represented in his more than 160 publications which spanned the 37 years of his scientific life. His early interests were divided between diagnostic bacteriology (leading to the Sulkin-Willett triple sugar iron agar) and medical virology, which included his early studies on bacteriophage therapy and on St. Louis encephalitis virus. He was active until the time of this death. The S. Edward Sulkin Memorial Fund was established at Southwestern Medical School, Dallas, to provide a lasting tribute to his honor.

O.B. Williams

Dr. O.B. Williams, renowned bacteriologist who understood the game of football as well as the intricacies of the laboratory, died at age 64. Dr. Williams was Chair of the Bacteriology Department, and won awards as one of the best teachers on the UT-Austin campus. One of America's leading experts on food preservation, he was named a consultant to the US Army Quartermaster Corps to advise on this subject. In addition to his teaching career at the University, he was a bacteriologist with the National Canners Association in Washington from 1935-41. During his last year at the University's annual Honors Day, he received a Student's Association Award for Excellence in Teaching. He was listed in American Men of Science and in Who's Who in America. Dr. Williams was a charter member of the American Academy of Microbiology and former President of the Texas Branch of the American Society for Microbiology. After Dr. Williams' death on September 26, 1959, W.F. Verwey, at the 1960 Galveston meeting, made the motion setting up the O.B. Williams Award, which is presented for the best paper given by a student at the Spring and Fall meetings. This much-coveted award has done a great deal to encourage student members to report on original research. The first O.B. Williams Award was made to Mr. Harold Lewis (University of Houston student) at the spring 1961 meeting in College Station.

UTMB
The University of Texas Medical Branch

AMERICAN SOCIETY FOR MICROBIOLOGY

Texas Branch
2006 Texas Branch Meeting of the American Society for Microbiology

Thursday - November 16, 2006

6:00 - 7:00 pm **Registration**

7:00 - 9:00 pm **Session 1: Biodefense-related Microbiology**
Chair: Alfredo G. Torres (UTMB)
Speakers:
Johnny Peterson (UTMB) "Protecting Against Inhalation Anthrax"
Bernard Arulanandam (UTSA) "A Novel Contribution of Mast Cells in Innate Defenses Against *Francisella tularensis*"
Alan D. Barrett (UTMB) "A Multidisciplinary Approach to Studying Structure-function of the Dengue Virus Envelope Protein"
Stanley M. Lemon (UTMB) "Biosecurity and the National Advisory Board for Biosecurity"

Friday, November 17, 2006

8:30 - 10:00 am **Session 2: Oral Student Presentations (Basic Science)**
Chair: Rene D. Massengale (Baylor University)
O4 - Caleb Gonzalez (Baylor) "Understanding a Hypermutable Cell Subpopulation that Produces Stress-Induced Mutants of *Escherichia coli*."
O5 - Natalie Hanna (UT Arlington) "Carbon, Nitrogen or Phosphorous Stoichiometry of a Mixotrophic Protest."
O7 - Sara Lawhon (TAMU) "Role of Cobalamin Synthesis and Propanediol Utilization in Intestinal Colonization and Persistence by *Salmonella enterica* Serovar Typhimurium."
O8 - Luis Martinez (UTHSCSA) "A *Bacillus*-specific Factor Signals Nutritional Stress to the Regulators of the Stress Responsive Transcription Factor, σ^B."
O10 - Lorenzo Santorelli (Rice U) "Mechanisms of Cheating Behavior in the Social Amoeba *Dictyostelium discoideum*."
O1 - Christopher Allen (UTMB) "A Regulatory Role for RpoS in Adherent Invasive *Escherichia coli* Strain O83:H1 Under Conditions of Low-shear Stress."

Session 3: Oral Student Presentations (Medical)
Chair: Millicent Goldschmidt (UT Houston)
O2 - Jeff Barker (UTSA) "Characterization of Secreted Virulence Factors in *Francisella tularensis*."

O3 - Guillaume Duret (UH) "Modulation of *Vibrio cholerae* Porins OmpU and OmpT by Acidic pH and Deoxycholic Acid."
O6 - Sangeeta Khare (TAMU) "Gene Expression Analysis by Massively Parallel Signature Sequencing in Bovine Ligated Ileal Loop During *Salmonella* Infection."
O9 - Adam Reeves (UTHSCSA) "ClpP, a Possible Regulator of the *Bacillus subtilis* Stress Responsive, Global Transcription Factor σ^B."
O11 - Khalid Syed (UTSA) "Regulation of Hemolysin and Hemagglutinin Gene Expression by Flagellar Regulatory Factors in *Vibrio cholerae*."
O12 - Dawn Weir (Angelo SU) "Characterization of a Strain of *Staphylococcus caprae* that Harbors the Gene for Enterotoxin A."

10:30 am - 12:30 pm **Session 4: Environmental Microbiology**
Chair: Robert J.C. McLean (Texas State)
Speakers:
Mark Ott (NASA, Johnson Space Center) "Microbiology and the NASA Space Program"
Tom Wood (TAMU) "*E. coli* Biofilms: Signaling and Listening"
Frank Healy (Trinity) "Gamma-butyrolactone Autoregulatory Signaling in Plant Pathogenic *Streptomyces*"
Dittmar Hahn (Texas State) "Interactions Between Purple Sulfur and Sulfate-reducing Bacteria in the Chemocline of a Meromictic Lake"

Session 5: Cell-cell Communication and Signaling
Chair: Vanessa Sperandio (UT Southwestern Med Ctr)
Speakers:
Vanessa Sperandio (UTSMC) "Cell-to-cell Signaling Amongst Microbial Flora, Host and Pathogens: There is a Whole Lot of Talking Going On"
Marvin Whiteley (UT Austin) "Bacterial Speech Bubbles"
Heidi Kaplan (UT Houston) "Quorum Sensing During *Myxococcus* Multicellular Development"
Kendra Rumbaugh (TTUHSC) "Autoinducers as Mediator of Interkingdom Signaling"

12:30 - 2:30 pm **Poster Presentation and Lunch**

2:30 - 4:30 pm **Session 6: Foodborne Pathogens-Ecology and Control in the Intestinal Tract**
Chair: Steve Ricke (University of Arkansas)
Speakers:
Alfredo G. Torres (UTMB) "Environmental Conditions and Transcriptional Regulation in Pathogenic *Escherichia coli*: Integration between physiology and virulence"
Steve Ricke (U. Arkansas) "*Salmonella* Fermentation and Colonization Ecology in the Avian Gastrointestinal Tract"
Jackson L. McReynolds (USDA-ARS, College Station) "An Evaluation of *Clostridium perfringens*, Its Interactions in Animal and Human Populations"
Toni L. Poole (USDA-ARS, College Station) "The Ecology of *Enterococcus*

faecalis and *Enterococcus faecium* in Continuous-Flow Cultures that Model the Gastrointestinal Tract"

Session 7: Bioinformatics
Chair: Catherine Ambrose (UT Houston)
Speakers:
George Weinstock (Baylor) "High-throughput Microbial Genomics: New Technologies, Data Galore"
David A. Rasko (UT Southwestern Med Ctr.) "Advances in Comparative Microbial Genomic Analyses"
Yousif Shamoo (Rice U) "Protein-RNA Interactions: Exploring Binding Patterns with a Three-dimensional Superposition Analysis of High Resolution Structures"
Yuriv Fofanov (U Houston) "Detection of Pathogens in the Presence of Complex Backgrounds"
Stanley J. Watowich (UTMB) "Phenotype Screening to Identify Host Cell Targets that Protect Against Virus Infections"

5:00 - 6:00 pm **Keynote Speaker Presentation**
"The Clinical Impact of Real-Time Molecular Infectious Disease Diagnostics"
Jim Dunn
Cook Children's Medical Center
Dept. of Pathology
Lecture sponsored by the American Society for Microbiology *(formerly Waksman Foundation for Microbiology)*

6:00 – 6:30 pm **Student Award Presentations**

6:30 – 7:30 pm **Student and Faculty Reception**
[Organized by the UTMB Department of Microbiology Students Organization (MISO)]

Saturday, November 18, 2006

8:30 - 10:30 am **Session 8: Medical Molecular Mycology**
Chair: Paul J. Szaniszlo (UT Austin)
Speakers:
Garry T. Cole (UTSA) "A Multivalent Recombinant Vaccine Against San Joaquin Valley Fever"
Paul J. Szaniszlo (UT Austin) "Cell Wall Virulence Factors in the Model Black Mold *Wangiella dermatitidis*"
Michael C. Lorenz (UT Houston) "Genomic Analysis of Virulence in *Candida albicans* Reveals an Important Role for Alternative Carbon Metabolism"
Brian L. Wickes (UTHSCSA) "Genetic and Molecular Analysis of Differentiation in *Cryptococcus neoformans*"

Session 9: Education

Chair: Lillian Waldbeser (TAMU-CorpusChristi)

Speakers:

Clifford Houston (UTMB) "Producing the Next Generation of Microbiologists"(talk by the President-Elect, American Society for Microbiology)

Lillian Waldbeser (TAMUCC) "Undergraduate Research at a Teaching-Intensive University"

Joanna Mott (TAMUCC) "Environmental Microbiology: Applied Research for Undergraduates: a Real-World Training"

Lee Hughes (UNT) "The Scholarship of Teaching and Learning"

10:45 – 11:00 am **Concluding Remarks**

11:00 am **Returning Home**

2006 Fall Branch
ASM Planning Committee

Alfredo Torres, Ph.D. (Chair, local organizing committee)

Mardelle Susman, Faculty Associate

Sarah Daniel, Financial Accounting

Belinda Iles, Administrative Secretary

Jennifer Junemann, Internet Production Designer

David Niesel, Ph.D. (Chair, Department of Microbiology and Immunology)

Jennilee Robinson and Tonyia Colpitts (MISO officers)

Oral Presentations

O1 - A Regulatory Role for RpoS in Adherent Invasive *Escherichia coli* Strain O83:H1 under Conditions of Low-Shear Stress

Allen C and A Torres. Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX

Escherichia coli clinical isolates from Crohn's Disease patients have been shown to exhibit adhesive and invasive properties shared by members of the newly formed class of adherent-invasive *E. coli* (AIEC). For successful colonization and persistence in the gut, these bacteria must adapt to various physiological (e.g. acid stress) and mechanically-based (e.g. low-fluid shearing) stresses in the gut. Low-shear stress is encountered by enteric flora between the microvilli within the brush border regions of the gut. To better understand the effects of this low-shear stress (LSS) environment, AIEC isolate serotype O83:H1 (AIEC O83:H1) was cultured in high-aspect ratio vessels (HARVs) and evaluated for changes in stress resistance, adhesion, and invasion. Altered stress resistance (increased thermal stress resistance, decreased oxidative stress resistance) and enhanced adherence to Caco-2 monolayers was observed by LSS-grown cultures compared to controls. Inactivation of the *rpoS* gene, encoding the alternative sigma factor and global stress regulator, resulted with significantly higher adherence under LSS compared to the wild-type strain. Tn*phoA* mutagenesis was carried out on the *rpoS* mutant to identify RpoS-regulated membrane-associated proteins implicated in the enhanced adherence phenotype. A disruption in the *tnaB* gene, encoding a tryptophan permease, was identified in one of the mutants. Characterization of this mutant revealed a reduction in adherence activity compared to the wild-type and *rpoS* mutant strains. Adherence capabilities were restored in the *tnaB* mutant after complementation. These results indicate a regulatory role for RpoS in mediating AIEC O83:H1 stress resistance and adherence under conditions of LSS. Furthermore, members of the tryptophanase operon (*tnaB*) appear to function in the RpoS-mediated regulation of AIEC O83:H1 adherence activity.

O2 – Characterization of Secreted Virulence Factors in *Francisella tularensis*

Barker J, KE Klose. Department of Biology and South Texas Center for Emerging Infectious Diseases, University of Texas San Antonio, San Antonio TX

Francisella tularensis is a gram-negative coccobacillus that causes the disease tularemia in humans and animals. Macrophages infected with *F. tularensis* are induced to undergo Caspase-1 dependent apoptosis. Several genes present within the Francisella Pathogenicity Island (FPI) have been shown to be important for intramacrophage survival and induction of apoptosis. We have constructed a *F. novicida* strain with a mutation in the FPI gene *iglC* and have shown IglC to be important for intramacrophage survival and virulence in mice. We hypothesize that IglC may be a secreted effector protein that functions within the macrophage cytosol. Fluorescent microscopy images suggest that IglC is secreted from the bacteria into the phagosome. Mutation of two cysteine residues near the IglC C-terminus, which may represent a prenylation site, prevented *F. novicida* from growth within the macrophage, demonstrating the importance of these two residues for IglC function. Our results indicate that IglC may be secreted into the host cytosol to modulate cell functions and facilitate intramacrophage survival.

O3 – Modulation of *Vibrio cholerae* Porins OmpU and OmpT by Acidic pH and Deoxycholic Acid

Duret G, V Simonet, and AH Delcour. Department of Biology and Biochemistry, University of Houston, Houston, TX

OmpU and OmpT, the two major porins of *Vibrio cholerae*, form dynamic ion conducting channels with distinct properties such as selectivity, closing kinetics and voltage dependence. *In vivo* OmpU and OmpT are thought to be involved in the adaptation of the bacteria to the host environment as their expression is regulated by both bile and pH. Moreover, deoxycholic acid, a major bile component, inhibits growth of *V. cholera* more efficiently in cells expressing solely OmpT than solely OmpU, indicating a possible protective effect of OmpU. Using the electrophysiological techniques of patch clamp and planar lipid bilayers, we studied the effect of deoxycholic acid and acidic pH on the pore activity of each porin. We showed that deoxycholic acid inhibits OmpT but not OmpU. OmpU, on the other hand, displays modifications in kinetics and conductance at acidic pHs, resulting in porin closure. The differential sensitivity of the two porins to these environmental factors suggests that these functional modulatory properties may play important roles *in vivo*.

O4 – Understanding a Hypermutable Cell Subpopulation that Produces Stress-Induced Mutants of *Escherichia coli*

Gonzalez C, RG Ponder, M Price, PJ Hastings and SM Rosenberg. Department Molecular and Human Genetics, Baylor College of Medicine, Houston, TX

Stress-induced mutational processes are stress-induced responses to growth-limiting environments in which mutations are produced, some of which confer a growth advantage. "Hypermutation" models for stress-induced mutagenesis (SIM) state that mutation rates increase throughout the genome, promoting accumulation of both adaptive and non-adaptive mutations. Alternatively, "cryptic-growth" models state that there is no increase in mutation rate leading to adaptive mutagenesis. In the Lac assay, *E. coli* with a *lac* frameshift allele are plated onto lactose medium, on which they cannot grow, and acquisition of a compensatory frameshift mutation lets them grow and form colonies. Strong support for hypermutation models came from the observation that Lac$^+$ revertants carry high frequencies of mutations in other genes throughout their genomes, whereas Lac$^-$ cells that also starved on the lactose medium, but did not become Lac$^+$, do not. This demonstrates that there is increased mutagenesis in a subpopulation of the starving cells. Advocates of cryptic-growth models have suggested that perhaps only some, but not most, Lac$^+$ mutants arise from that subpopulation. This could make it possible that most stress-induced Lac$^+$ mutations form in cells that do not have elevated mutation rates. According to the hypermutation model, the hypermutable cell subpopulation (HMS) should produce most/all stress-induced Lac$^+$ mutants. First, we present evidence that Lac$^+$ stress-induced mutations from HMS-derived cells are not different in sequence from most stress-induced Lac$^+$ reversions, supporting the idea that most/all stress-induced Lac$^+$ revertants come from the HMS. Second, the mechanism of SIM is error-prone DNA double-strand break (DSB) repair (Ponder *et al* 2005 *Mol Cell*), and we show that DSBs that we introduce cannot promote mutation independently of the HMS. This again supports the idea that the HMS gives rise to most Lac$^+$ revertants. Global stress-induced increases in mutation rate in cell subpopulations may be an important model for microbial evolution.

O5 – Carbon, Nitrogen or Phosphorous Stoichiometry of a Mixotrophic Protest.

Hanna N, TH Chrzanowski, and JP Grover. Department of Biology, The University of Texas at Arlington, Arlington, TX

Aquatic bacteria are responsible for the movement of dissolved nutrients from the abiotic pool into the biotic pool. Protists are the main consumers of bacteria; therefore, protists are a critical link in the transfer of nutrients sequestered in bacteria to higher trophic levels. Nutrient requirements of protozoa are poorly characterized and this has limited our understanding of the nutrients protozoan consumers recycle back to the abiotic pool and transfer to higher trophic levels. This lack of understanding stems largely from the difficulties in separating undigested bacteria in food vacuoles from the protozoa themselves. In this work we explore how a bacterial prey (*Pseudomonas fluorescens*) grown in different nutrient conditions effects the nutrient ratios (carbon, nitrogen, and phosphorous) of a model protozoan predator (*Ochromonas danica*). Cultures of *P. fluorescens* were grown in batch cultures at 28°C under four different nutrient conditions: balanced (atomic C:N:P ratio 120:12:1), nitrogen-limited (200:5:1), carbon-limited (30:10:1) and phosphorus-limited (1000:50:1). Bacteria of differing nutrient composition were fed to *Ochromonas*. The element composition of *Ochromonas* was determined using a mass-balance approach. *Ochromonas* growth was variable and appeared to depend upon the medium in which bacteria were grown. However, the element composition of the protozoan appeared to match that of its prey. For example, *Ochromonas* feeding on bacteria having a C:N:P ratio of 72:12:1 was found to have a C:N:P ratio of 86:12:1. These data suggest that protozoan predators are flexible in their element composition and will adjust their element stoichiometry to match that of their prey.

O6 – Gene Expression Analysis by Massively Parallel Signature Sequencing in Bovine Ligated Ileal Loop During Salmonella Infection

Khare S, SD Lawhon, CDHaudenschild, GS Davidson, JF Figueiredo, CA Rossetti, JES Nunes, T Gull, TL Thomas, AJ Bäumler, and LG Adams. Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX

We have utilized massively parallel signature sequencing (MPSS) to compare the *in vivo* host global gene expression responses in wild type *Salmonella enterica* serovar Typhimurium (ST) infected, Type Three Secretion System (T3SS) genes SipASopABDE/E2 mutant (MT) infected, and LB control in the bovine ligated ileal loop model during the first 4 hr post-infection. MPSS captures and identifies transcript sequences and analyzes the level of expression of virtually all genes expressed in a sample by counting the number of individual mRNA molecules derived from a gene to the level of only a few transcripts. Analysis of MPSS data led to the identification of a number of genes with unrecognized roles in host response to ST and MT infections. We also found evidence of extensive antisense transcript expression as well as identified several signatures as candidate biomarkers characteristic of the early host response to ST infection.

O7 – Role of Cobalamin Synthesis and Propanediol Utilization in Intestinal Colonization and Persistence by *Salmonella enterica* Serovar Typhimurium

Lawhon SD [1], LG Adams[1], and HL Andrews-Polymenis[2]. [1]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX; [2]Department of Microbial and Molecular Pathogenesis, Texas A&M University System Health Science Center, College Station, TX

Both *Salmonella enterica* serovar Typhimurium (S. Typhimurium) and *E. coli* use cobalamin (vitamin B12) as a cofactor in the synthesis of methionine and the use of ethanolamine as a carbon source. Unlike *E. coli*, S. Typhimurium is able to synthesize cobalamin *de novo* and employs it in the use of 1,2-propanediol as a carbon source. We postulated that these two functional abilities contribute to the success of S. Typhimurium in the mammalian intestinal tract. To test this theory, we generated mutants in *cobSTU*, *cbiL*, and *pduC* and tested them in competition with the isogenic wild type in BALB/c and CBA/J mice to determine their requirement for organ colonization and persistence. We found that all five genes are required for optimal persistence in CBA/J mice and that cobS is required for colonization of the liver and cecum of BALB/c mice.

O8 – A *Bacillus*-specific Factor Signals Nutritional Stress to the Regulators of the Stress responsive Transcription Factor, σ^B

Martinez LA and WG Haldenwang. Department of Microbiology and Immunology, The University of Texas Health Science Center at San Antonio, San Antonio, TX

The transcription factor σ^B controls the general stress regulon in *Bacillus subtilis*. σ^B can be activated by either physical or nutritional stress. σ^B activation by nutritional stress occurs when a stress-responsive phosphatase (RsbP/Q) responds to activate a positive regulators of σ^B by dephosphorylation. The signal that triggers RsbP/Q is unknown; however, its activation parallels a drop in cellular ATP levels. To determine whether a drop in cellular ATP, per se, can directly activate the RsbP/Q phosphatase, we reconstituted the known components of the nutritional stress pathway in *E. coli* and subjected the resulting strain to nutritional stress. Reporter gene assays revealed that conditions that reduce ATP levels are insufficient to activate σ^B in this heterologous system. The data argue for the existence of a *Bacillus*-specific factor that transmits the presence of nutritional stress to the σ^B regulators.

O9 – ClpP, A Possible Regulator of the *Bacillus subtilis* Stress Responsive, lobal Transcription Factor σ^B

Reeves A and WG Haldenwang. Department of Microbiology and Immunology, The University of Texas Health Science Center San Antonio, San Antonio, TX

The general stress regulon of *Bacillus subtilis* is controlled by the sigma B transcription factor. Sigma B is activated by either of two phosphatases (RsbP and RsbQ) which react to nutritional or physical stress, respectively. We now report that the regulatory protease ClpP plays a role in the control of σ^B activity following exposure to physical stress. σ^B activity levels in ClpP$^-$ *B. subtilis* are similar to those observed in wildtype strains during growth and nutritional stress; however, unlike the σ^B activity profile in wildtype *B. subtilis*, σ^B activity in ClpP$^-$ *B. subtilis* fails to return to

"pre-stress" levels after elevation by physical stress. Western blot analyses reveals no effect of the loss of ClpP on any known σ^B regulator. The data implicates ClpP in the turnover of a predicted, but unidentified, activator of σ^B's physical stress pathway.

O10 – Mechanisms of Cheating Behavior in the Social Amoeba *Dictyostelium discoideum*

Santorelli L[1], C Thompson[2], E Villegas[2], J Svetz[2], C Dinh[3], A Kuspa[3,1], D Queller[1], J Strassmann[1] and G Shaulsky[2,1]. [1]Department of Ecology and Evolutionary Biology; Rice University, Houston, TX, [2]Department of Molecular and Human Genetics and [3]Department of Biochemistry and Molecular Biology: Baylor College of Medicine, Houston, TX

D. discoideum is a eukaryotic micro-organism with a unique life cycle. The amoebae live as haploid, free-living cells, feed on bacteria and divide asexually. Under starvation, amoebae aggregate to form a multicellular organism containing 10^5 cells. The cells differentiate into viable spores and dead stalk cells that will help the spores' dispersal. This cooperative system is susceptible to disruption: in a mix between two or more genetically different clones, a conflict may arise in determining which cells become spores and which become dead stalk. A clone that differentiates more spores than its fair share is called a "cheater". Our goal is to identify the genetic mechanism(s) that regulate and modulate this social behavior. To select for cheaters, 10,000 knockout mutants were subjected to several rounds of growth and development in a mixed population. The spores were transferred to the next generation, enabling cheaters to become overrepresented. Southern Blot and quantitative PCR analyses confirmed that some knockout strains increased in relative abundance during the selection. Mutants exhibiting a normal morphological phenotype were isolated after several generations and tested for cheating ability in a pairwise mixture with the parental wild type. We have isolated 140 genes that when knocked-out produce cheating behavior. Several genetic pathways are overrepresented, including ubiquitin-mediated protein modification, signal transduction and polyketide synthesis, indicating that social cheating can involve multiple pathways.

O11 – Regulation of Hemolysin and Hemagglutinin Gene Expression by Flagellar Regulatory Factors in *Vibrio cholerae*

Syed KA[1], J Liu[1], N Correa[1], S Beyhan[2], F Peng[1], F Yildiz[2], KE Klose[1].[1]Department of Biology, The University of Texas at San Antonio, and [2]Department of Environmental Toxicology, University of California Santa Cruz, CA

Vibrio cholerae, which causes cholera, is motile due to a polar flagellum. We constructed non-flagellated (non-motile) *V. cholerae* strains containing mutations in the major flagellar regulatory genes, and performed microarray experiments to analyze their transcription profiles. Our results indicated that genes with proven or putative roles in virulence (e.g. *ctx*, *tcp*, hemolysins) were generally upregulated in the flagellar mutants. Flagellar regulatory mutants show decreased hemagglutination and increased hemolysis of human type O red blood cells. Increased transcription of the Thermolabile Hemolysin gene is responsible for increased hemolysis, while decreased transcription of a specific c-di-GMP synthase is responsible for decreased hemagglutination in the flagellar mutants. The c-di-GMP synthase in turn regulates the transcription of a novel RTX-like flagellar- regulated hemagglutinin, which mediates binding to both red blood cells and chitin.

O12 – Characterization of a Strain of *Staphylococcus caprae* that Harbors the Gene for Enterotoxin A

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Classical and molecular methods were used to identify and characterize an unknown staphylococcal environmental isolate (KD unknown) that appeared to produce enterotoxin A (SEA). Test results for coagulase production, API Staph, sequence analysis of the beta subunit of the bacterial RNA polymerase (rpoB) gene, and internal transcribed polymerase chain reaction (ITS PCR) suggest that the unknown is a strain of *Staphylococcus caprae* (KD *S. caprae*). The SEA gene was amplified in KD *S. caprae*. The SEA primers used in this study also amplified the gene for enterotoxin-like toxin type P (SElP) in KD *S. caprae*. This is the first report of the polymerase chain reaction (PCR) amplification of SEA and SElP in *S. caprae*, the first report of the amplification of SElP in a staphylococcal species other than *S. aureus*, and the first report of a single staphylococcal strain harboring both SEA and SElP.

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P36# **Regulation of innate responses to infection with intracellular parasites. Vargas-Inchaustegui DA**, L Xin, T Eaves-Pyles and L Soong.

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Poster Abstracts

P1 - Host Cell Components of FMDV Replication Complexes

Agar SL and PW Mason. Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX

Foot-and-mouth disease virus (FMDV) is the etiologic agent of foot-and-mouth disease (FMD), a debilitating disease of livestock and a threat to international economic trade. Infection by this and other viruses in the family *Picornaviridae* results in the rearrangement of membranes within the host cell and the formation of a viral replication complex believed to sequester viral RNA and provide a scaffolding for viral RNA replication. In the case of poliovirus, several cellular proteins have been implicated in this process. Studies on FMDV have indicated differences from poliovirus and other picornaviruses regarding interruption of host cell protein synthesis and recruitment of cellular components. To help understand this process, we are attempting to elucidate the host cell components of the FMDV RNA replication complex. To this end, we are performing analyses of the heavy membrane fraction from BHK-21 cells electroporated with FMDV replicon RNA. Western blot analyses have indicated the presence of viral non-structural proteins in both the cytoplasmic supernatant and this heavy membrane fraction. However, to date these studies have revealed no global redistribution of cellular proteins to membrane complexes in replicon-transfected cells as compared with mock-transfected cells. To aid in these studies we have also produced an immunoaffinity column using an antibody to the polymerase protein 3D. Using this column, the viral replication complex will be isolated and host-cell components will be identified using mass spectrometry.

P2 - Mechanisms of Resuscitation of Dormant Mycobacteria

Alderson R and TP Primm. Department of Biological Sciences, Sam Houston State University, Huntsville, TX

Greater than 99% of human tuberculosis infections are latent, which can reactivate to active disease. There is a major need to understand the mechanisms that activate dormant mycobacteria. In a previous study, it was found that after 4 months of stationary phase, *M. tuberculosis* produced no colonies on solid Sauton's media yet retained viability in liquid format (Shleeva *et al* 2002). We are investigating resuscitation using a starvation dormancy model in *M. smegmatis*. In this model bacteria also failed to recover on Sauton's agar, yet grew well on 7H11. Noting that 7H11 contains casitone, we prepared Sauton's solid media containing a variety of individual amino acids or casitone. Casitone restored growth of the recovering bacteria, as did certain but not all amino acids. Thus, amino acids are critical for recovery of mycobacteria from the dormant state in a specific manner. We propose the mechanism may be analogous to activation of spores by amino acids. Mycobacteria do contain homologs of several sporulation regulatory genes. Whether amino acids are working as signaling molecules, or as nitrogen sources, or in another way is yet unclear. A clearer view of dormancy may lead to new therapeutics against latent tuberculosis.

P3 - Identification of a *Myxococcus xanthus* Gene Involved in an Envelope Stress Pathway

Ardestani S[1], M Esmaeiliyan[1] and HB Kaplan[2]. [1]Department of Natural Sciences, University of Houston-Downtown and [2]Department of Microbiology and Molecular Genetics, University of Texas Medical School at Houston, TX

Myxococcus xanthus is a Gram-negative soil bacterium that undergoes multicellular development upon starvation at high cell density. Expression of the early developmental gene *4445* that requires starvation and high cell density is regulated by the EcfA/ReaA/ReaB envelope stress sensing signal transduction system. A random genetic screen using the mini-Himar1 transposon was used to identify genes that generate input signals and function upstream in this signal transduction pathway. A gene encoding a putative D-amino acid acylase was identified. This enzyme is expected to generate D-amino acids from N-acyl D-amino acids. D-amino acids could generate envelope stress by disruption of the cell wall peptidoglycan and as a result could induce *4445* gene expression during growth and development.

P4 - Airway Epithelial Cell Response to Human Metapneumovirus Infection

Bao X, T Liu, L Spetch, D Kolli, RP Garofalo and A Casola

Human metapneumovirus (hMPV), first identified from children with respiratory symptoms in 2001, is a significant respiratory pathogen worldwide. Here, we investigated the cellular responses to hMPV in airway epithelial cells, the major target of hMPV infection and also the first defense barrier against respiratory infections. Human alveolar type II-like epithelial cells (A549) were infected with hMPV at multiplicity of infection (MOI) of 3. Cells were harvested at different time post-infection to collect cell supernatants and prepare either total RNA or cytoplasmic and nuclear proteins. Chemokines and cytokines were investigated by ELISA and Bioplex assay, as well as by ribonuclease protection assays (RPA). Our results show that hMPV infection of alveolar epithelial cells induced a significant production of chemokines and cytokines, as well as type I interferons. HMPV-induced chemokine expression required viral replication, since it could not be induced by the addition of UV-inactivated hMPV. When we compared chemokine and cytokines secretion between hMPV and respiratory syncytial virus (RSV), we found that hMPV is a weaker inducer of pro-inflammatory mediators, but a stronger inducer of type I interferons. hMPV-induced secretion of interferon-β was higher than the one induced by RSV infection. Furthermore, hMPB, but not RSV, induced IFN- α secretion in A549 cells. We also found that hMPV infection induced activation of Nuclear Factor (NF)-κB and interferon regulatory factors (IRFs) transcription factors, which have been shown to play a fundamental role in controlling the expression of these chemokines following viral infections. The signaling pathway leading to the expression of immune and antiviral genes in response to hMPV infection, as well as the role of hMPV viral proteins in cellular signaling, are currently being investigated. (Supported by NIH/NIAID T32 AI 07536)

P5 - Mode of Action of Fluoroquinolone Derivatives Against Mycobacteria

Benavidez J and TP Primm. Department of Biological Sciences, Sam Houston State University, Huntsville, TX

Tuberculosis is the leading infectious killer of adults worldwide. Caused by species of bacteria in the genus *Mycobacterium*, tuberculosis has infected nearly one-third of the world's population.

The treatment for tuberculosis is intense, typically lasting 6-9 months, and the incompletion of it often leads to a rise in drug resistant strains. Twenty-four novel derivatives of the flouroquinolones, ciprofloxacin and norfloxacin, have been screened for their activity against *Mycobacterium smegmatis*, with minimal inhibitory concentrations ranging from 0.63 to 320 ug/ml. The most active compounds are being analyzed for their mechanism of action. If the derivatives act against a different target than the parent compounds, then they may be useful for treatment of resistant strains. VS9, a norfloxacin derivative, displays synergy with both tetracycline and kanamycin using a microbroth checkerboard assay, decreasing the bacteriocidal concentration of each antibiotic by 8-fold. Thus these compounds may be utilized to shorten the therapy against tuberculosis, a major research goal in the field.

P6 - Identification of Potential Virulence Determinants by *Himar1* Transposition of Infectious *Borrelia burgdorferi* B31

Botkin DJ [1,2,3], AN Abbott[1,3], PE Stewart[4], PA Rosa[4], H Kawabata[5], H Watanabe[5], SJ Norris[1,2,3]. [1] Graduate School of Biomedical Sciences, [2]Program in Microbiology and Molecular Genetics, and [3]Department of Pathology and Laboratory Medicine, University of Texas Health Science Center at Houston, Houston, TX; Laboratory of Zoonotic Pathogens, Rocky Mountain Laboratories, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT; and [5]Department of Bacteriology, National Institute of Infectious Diseases, Tokyo, Japan

The Lyme disease *Borrelia* are highly invasive spirochetes that alternate between vertebrate and arthropod hosts, and establish chronic infections and elicit inflammatory reactions in mammals. Although progress has been made in the targeted mutagenesis of individual genes in infectious *Borrelia burgdorferi*, the roles of the vast majority of gene products in pathogenesis remain unresolved. In this study, we examined the feasibility of using transposon mutagenesis to identify infectivity-related factors in *B. burgdorferi*. The transformable, infectious strain 5A18 NP1 was transformed with the spirochete-adapted *Himar1* transposon delivery vector pMarGent to create a small library of 33 insertion mutants. Single mouse inoculations followed by culture of four tissue sites and serology were used to screen the mutants for infectivity phenotypes. Mutants that appeared attenuated (culture positive at some sites) or noninfectious (negative at all sites) and contained the virulence-associated plasmids lp25 and lp28-1 were examined in more extensive animal studies. Three of these mutants (including those with insertions in the putative *fliG*-1-encoded flagellar motor switch protein and the *guaB*–encoded IMP dehydrogenase) were noninfectious, whereas four clones appeared to exhibit reduced infectivity. Serological reactivity in VlsE ELISAs correlated with the assignment of mutants to the noninfectious or attenuated infectivity groups. The results of this study indicate that random transposon mutagenesis of infectious *B. burgdorferi* is feasible and will be of value in studying the pathogenesis of Lyme disease *Borrelia*.

P7 - Kinomics and Systems Biology Reveal Differential Activation of Akt-associated Signaling Networks in Arenavirus Infection

Bowick GC, SM Fennewald, BL Elsom, DG Gorenstein and NK Herzog. Departments of Pathology and Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX

Two variants of the New World arenavirus Pichinde, P2 and P18, provide a good comparative model for arenavirus infection causing a mild self-limiting infection or a lethal hemorrhagic disease respectively. We have used this model to investigate the differential host-response to these

viruses. By identifying differentially implicated host-signaling pathways, we hope to be able to actively modulate the host response to avoid the onset of hemorrhagic pathology. We used high-throughput proteomic and kinomic methods to investigate the global host-response to infection. We used a novel systems biology approach to model the host-signaling networks and identified a number of proteins involved in Akt signaling. Using a combination of techniques, we have shown that several proteins in this network are differentially activated between P2 and P18 infection and may be targets for therapeutic intervention.

P8 - *Pseudomonas aeruginosa* Autoinducer Activates Xenobiotic Metabolism and Transport Pathways in Host Cells

Bryan A, SC Williams, and KP Rumbaugh. Departments of Cell Biology & Biochemistry and Surgery, Texas Tech University Health Sciences Center, Lubbock, TX

Quorum sensing is an intercellular communication system used by bacteria to coordinate gene expression within a population. In the opportunistic pathogen *Pseudomonas aeruginosa* there are two QS systems, Las and Rhl, which are critical for virulence. In addition to affecting bacterial transcription, $3OC_{12}$-HSL, the effector molecule of the Las system, functions as an independent virulence factor by inducing apoptosis and the expression of immunomodulatory genes in mammalian cells. We have shown that $3O\text{-}C_{12}$-HSL can enter and function in mammalian cells, and hypothesize here that it may activate xenobiotic extrusion and metabolism pathways within these cells. $3OC_{12}$-HSL enters mammalian cells and is then rapidly secreted, indicating the activation of a possible transport mechanism. $3OC_{12}$-HSL also modulates the expression of several cytochrome P450 genes, indicating that it is may be metabolized by host cells.

P9 - Bioremediation of an Industrial Effluent-contaminated Site for the Removal of PAHs

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Polycyclic aromatic hydrocarbons (PAH) belong to the class of hazardous organic compounds. They are highly toxic, recalcitrant and persistent with mutagenic or carcinogenic properties. Sites of petroleum refining, coal conversion and chemical manufacturing are most often contaminated with PAHs. Bioaccumulation and possible adverse health effects of PAH compounds require the designing of a soil treatment system that will effectively reduce their concentration. Biodegradation, especially microbial degradation is the major route through which PAHs are removed from contaminated environments although other mechanisms such as volatilization, leaching and photodegradation may also be effective. Bacterial and fungal populations are generally known to be associated with biodegradation.

The present study was undertaken at an effluent-polluted site in an industrial area where a major percentage of industries consist of synthetic organic chemicals (bulk drugs and intermediates), distilleries, oil refineries, textiles, tanneries and electroplating units. The aim of this study was to evaluate and monitor the capability of the native microflora of an industrial effluent-contaminated soil to degrade or transform PAH compounds and to characterize the metabolic by-products of the PAH biodegradation. We prepared liquid cultures of the native microflora of the soil from the site and subjected the 3-day old cultures to the PAH fraction as the sole carbon source at a concentration of 200 mg/l. The PAHs fractions were obtained from the contaminated soil and

identified by HPLC. After incubation, the flasks were evaluated by HPLC for hydrocarbon removal and also for the growth of the microbial cultures. A few strains of microorganisms were found to be more effective in biodegradation than the others. In another experiment, cultures of effective microorganisms were inoculated into two portions of soil viz. sterilized and non-sterilized and cultivated for 2, 4, 6, and 8 weeks. The PAHs were extracted from the soil and their degradation was determined by HPLC. Degradation varied with the microbial strains and also with the soil treatment. The results demonstrated that only 60% PAHs were removed during the first 4 weeks which agree with the exponential growth phase of the microbial cultures. The remaining recalcitrants included methyl-substituted compounds of fluorene, phenanthrene and anthracene. Results of this study do suggest that the development, operation, and maintenance of bioremediation projects require a significant amount of planning.

P10 - Differential Interaction of Dendritic Cells with *Rickettsia*: Impact on Host Resistance and Susceptibility to Murine Spotted Fever Rickettsiosis

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Spotted fever group rickettsioses could develop to be severe or even life-threatening diseases. To the same inoculum of *Rickettsia conorii*, C57BL/6 (B6) mice are resistant while C3H/HeN (C3H) mice are highly susceptible to fatal disease. In the present study, we examined the role of dendritic cells (DCs) in resistance or susceptibility to severe and fatal rickettsiosis. We found that bone marrow-derived dendritic cells (BMDCs) from resistant mice harbored a greater quantity of intracellular *R. conorii* than DCs from susceptible mice at early time points after infection. While rickettsiae were detected only within the cytosol of target endothelial cells, rickettsiae were localized in phagosomes as well as the cytosol of C3H and B6 DCs. Rickettsial infection drove DCs from both mouse strains to maturation status with up-regulated expression of CD80, CD86, CD40, MHC class I, and MHC class II compared to respective untreated DCs. However, DCs from resistant mice infected *in vitro* with *R. conorii* exhibited slightly higher expression of OX40L, less expression of CD40 and greater IL-12 production than C3H DCs. *In vitro* DC-T cell co-culture revealed that *R. conorii*-infected DCs from resistant mice initiated earlier activation of naïve CD4 T cells, more *Rickettsia*-specific Th1 dominant polarization, fewer antigen dependent Foxp3+ CD4+T reg cells and a greater expansion of cytotoxic T cells than those from susceptible mice. Overall, these data suggested an important role of a DC-initiated suppressive T cell response in determining host susceptibility to rickettsial disease.

P11 - Endospore Activation may be Directly Related to a Change in Spore Coat Permeability Ultimately Leading to Initiation of Metabolism in *Bacillus stearothermophilus* NGB 101

Faris R and H Foerster. Department of Biological Sciences, Sam Houston State University, Huntsville, TX

Although a great deal is understood about endospore germination very little is about "activation" (readying the spores for germination). There is no consensus definition of activation, nor has a mechanism been described. Traditionally bacterial endospores have been activated with sub-lethal heat for a short period of time prior to germination; the mechanism underlying this approach is not known. Low temperature activation of the thermophilic bacterium *(Geo)Bacillus stearothermophilus* NGB 101 can be accomplished by incubating for 4 hours at 30° C with 0.2 M

$NaNO_2$. Activation of NGB 101 can be blocked with 0.1 M KCN, a metabolic poison that irreversibly binds cytochrome c oxidase. We have detected cytochrome oxidase activity in activated spores; this finding could implicate a physiological component involved in low temperature activation. We are currently developing an assay using 5-cyano-2,3,-di-(p-tolyl)tetrazolium chloride (CTC), a redox dye that can be used as an indicator of cellular respiration, to detect physiological events during activation. Germination of NGB 101 has been observed in the absence of nutrient germinants following a shift to their optimal growth temperature (65° C) after low temperature activation. A change in spore coat permeability triggered by heat or $NaNO_2$ may allow water into the spore and trigger metabolic events in an alternate pathway to that of classical nutrient germination initiators. Evidence suggests that activation is a two pronged process involving an initial alteration in spore coat permeability allowing exogenous compounds to enter the spore and lead to the initiation of metabolism.

P12 - Protease Regulation of Pneumococcal Competence

Hammett AM and ME Sebert. Texas Woman's University, Denton, TX, University of Pennsylvania and Children's Hospital of Philadelphia, Philadelphia, PA

Streptococcus pneumoniae, also known as the pneumococcus, is a Gram-positive human pathogen that shows natural genetic competence regulated by a quorum sensing system. The pneumococcal surface protease HtrA negatively regulates signaling of this competence pathway by an unknown mechanism. One possible target of HtrA is the secreted 17-amino acid linear competence-stimulating peptide (CSP), which interacts with the ComD receptor and elicits downstream effects that stimulate competence. The goal of this study was to investigate whether HtrA negatively regulates the competence pathway by degrading CSP into peptide fragments with inhibitory properties. Substantial loss of protein and proteolytic activity occurred during in vitro concentration and refolding of the purified protease. Initial HPLC assays using a crude rHtrA preparation, however, show generation of two new peaks after incubation with CSP. Additional experiments using further purified rHtrA protease are planned to confirm the results of the HPLC assays.

P13 - Green Tea and Yerba Mate Effects on Biofilms

Huerta V, RJC McLean, and D Vattem. Texas State University-San Marcos, TX

One of the most popular plants used world wide is green tea because of its known antioxidants and other beneficial properties. Yerba Mate is a popular natural tea in South American countries such as Argentina and Paraguay; it is considered to have healing and energy supplying properties that South Americans have enjoyed for hundreds of years.

Biofilm formation has been recognized as an important virulence factor because of its actions of providing shelter for the bacteria from antibiotics and the immune response as well as facilitating attachment and colonization on a surface. This study tested the effects of these two popular teas on the growth and biofilm formation of *Vibrio cholerae,* and two strains of *Escherichia coli* (O157 H7, and B). A microtiter biofilm assay was used to detect the presence of biofilm when stained with crystal violet. A bioassay was used to screen these herbs for quorum signal inhibition. Our results can be related to quorum signaling and the expression of virulence, motility, and biofilm formation in *V. cholerae*. The tests revealed that green tea in fact inhibits the formation of biofilm in *V. cholerae* and Yerba Mate slows down this process.

P14 - Quorum Sensing-Based Signaling and Immunomodulation of Mammalian Cells

Jahoor A, A Bryan, SC Williams and KP Rumbaugh. Departments of Cell Biology & Biochemistry and Surgery, Texas Tech University Health Sciences Center, Lubbock, TX

Quorum sensing is an intercellular signaling system used by bacteria to regulate gene expression in response to changes in cell population density. The Gram negative opportunistic pathogen *Pseudomonas aeruginosa* utilizes QS to produce virulence factors. Individuals with cystic fibrosis are commonly colonized with *P. aeruginosa*, leading to significant morbidity and mortality. The acyl homoserine lactone $3OC_{12}$-HSL functions is a LasR ligand in *P. aeruginosa*, but also enters eukaryotic cells and alters host immune responses. $3OC_{12}$-HSL increases mRNA levels for the inflammatory modulators (IL-1a , IL-6, IL-8 (KC), and Cox-2) in fibroblasts, endothelial cells, and lung epithelial cells, including the cystic fibrosis (CFTR null) cell line JME/CF15. Our data indicate that $3OC_{12}$-HSL may modulate host reponses in these cells by acting as ligands for the mammalian nuclear hormone receptors PPARγ and PPARβ.

P15 - A New Hemolysin from a Clinical Isolate of *Aeromonas hydrophila*

Khajanchi BK, TE Erova, J Sha, and AK Chopra. Department of Microbiology and Immunology, UTMB, Galveston, TX

A clinical strain SSU of *Aeromonas hydrophila* produces a potent cytotoxic enterotoxin (Act) which possesses cytotoxic, enterotoxic, and hemolytic activities. Recently, in this strain, we detected a new gene, which encoded a hemolytic protein of 440-amino acid residues with a molecular mass of 49 kDa. We first detected this hemolysin based on our observation that the cytotoxic enterotoxin gene (*act*) minus mutant of *A. hydrophila* SSU still had residual hemolytic activity. The new hemolysin gene (designated as *hlyah*) was cloned, sequenced, and over-expressed in *Escherichia coli*. The *hlyah* gene exhibited 96% identity with its homolog found in a recently annotated genome sequence of an environmental isolate ATCC 7966 of *A. hydrophila* from our laboratory. The *hlyah* gene did not exhibit any homology with other known hemolysins (*hly)* and aerolysin (*aerA)* genes detected in *Aeromonas* isolates. However; this new hemolysin exhibited significant homology with hemolysins (TlyC) of *Treponema hyodysenteriae* and *Rickettsia typhi* as well as with the cystathionine beta synthase (CBS) domain of *Shewanella*. The Hlyah protein was activated only after treatment with trypsin and the resulting hemolytic activity was not neutralizable with antibodies to Act. We also investigated the presence of this new *hlyah* gene in various *Aeromonas* species isolated from clinical and water sources to demonstrate its possible role on *Aeromonas* virulence.

P16 - DNA Methyltransferases of *Y. pestis*

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Yersinia pestis, the causative agent of plague, exists in nature at ambient temperatures within the flea vector or in the mammalian host at 37°C. Five strains of *Y. pestis* have been sequenced so far. *Y. pestis* possesses three chromosomal putative Type II DNA methyltransferases (MTases), namely M.Ype391, M.Ype2088, M.YpeDam and one M.Ype64 that is plasmid pMT1-encoding. Bacterial DNA MTases do not only participate in restriction-modification events, but also play

regulatory roles in the cell. We successfully constructed both knock-out *ype391M*- and overproducing *ype391MOP* mutants of *Y. pestis*, and found that methylation provided by this unique to *Y. pestis* methyltransferase was not essential for the viability of the microbial cell and can provide a temperature- and Ca2+ ions - induced methylation of the plague genome. DNA microarrays encompassing the entire genome of *Y. pestis* (obtained from the Pathogen Functional Genomics Resource Center of The Institute for Genomic Research) were used to characterize global regulatory changes in response to the methylation state of DNA provided by M.Ype391. The functional distribution of genes affected by the methylation state was significantly different from that described by us earlier during the study of the global expression changes occurring after the temperature shift (Motin et.al. J. *Bacteriol.* 2004. 186:6298-6305). The overexpression of M.Ype391 had little effect on the transcription of the genes located on any of the plasmids pMT1, pCD1 and pPCP1. Also, in contrast to the previous study, in which most of the genes affected by the temperature shift belonged to small molecule metabolism, we observed now that the most prominent changes took place in the genes of macromolecule metabolism and cell processes. Indeed, both of these categories accounted for 47% of all of the genes which changed their transcription in response to methylation conditions. Thus, the methylation provided by M.Ype391 had a significant impact on DNA replication, RNA synthesis and modifications, transport, and particularly on the cell envelope. In addition, a significant number of broad regulators changed their transcriptional profile, which indicated that the total number of genes affected by methylation status could be larger than currently detected if other time points or conditions were analyzed. Interestingly, the vast majority of the members in the "other" category were hypothetical genes, which signified that methylation can have an influence on functions currently not known for the plague microbe.

P17 - The Effects of Junin Virus Infection on Human Endothelial Cell Adherens Junctions

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Junin Virus (JV) is the etiologic agent of Argentine hemorrhagic fever, a classic arenaviral hemorrhagic fever exhibiting increased vascular permeability, the mechanisms of which are still unclear. It is widely accepted that mediators produced by infected inflammatory cells contribute significantly to increased vascular permeability during arenaviral hemorrhagic fevers, but endothelial cells are also targeted, to varying degrees and direct infection may play a crucial role in pathogenesis. In endothelial cells adherens junctions are established to modulate permeability and we hypothesize that organization of these junctions is disrupted during JV infection, in the absence of cytopathology. Therefore, we determined the direct effects of JV infection on the adherens junctions of human umbilical vein endothelial cells (HUVEC).

Using coimmunoprecipitations, western blotting, electric cell-substrate impedance sensing (ECIS) and confocal microscopy, we investigated changes in adherens junction protein complexes, protein expression levels, protein phosphorylation states, and electrical resistance during infection with Candid #1, the vaccine strain of JV.

We show that early during Candid #1 infection: HUVECs exhibit no cytopathic effects; VE-cadherin/β-catenin complexes are decreased; β-catenin expression levels do not change; VE-cadherin staining is reduced early and recovers; tyrosine phosphorylation of several adherens junction proteins is altered and electrical resistance of the monolayer is decreased.

Our data are the first evidence that endothelial cell responses to direct JV infection contribute to disorganization of endothelial cell adherens junctions which could play a major role in the pathogenesis of JV and perhaps other hemorrhagic fever viruses.

P18 - Indole and Acyl-Homoserine Lactones are Inter-species *Escherichia coli* Biofilm Signals Mediated by SdiA

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Through a series of global transcriptome analyses, confocal microscopy, isogenic mutants, and dual-species biofilms, we show indole is a non-toxic signal that controls *Escherichia coli* biofilms by inducing the sensor of the quorum sensing signal autoinducer-1 (SdiA) which in turn represses motility and influences acid resistance (e.g., *hdeABD, gadABCEX*). Isogenic mutants showed the associated proteins are directly related to biofilm formation, and SdiA-mediated transcription was shown to be influenced by indole. The reduction in motility due to indole addition results in the biofilm architecture changing from scattered towers to flat colonies. Additionally, there are 12-fold more *E. coli* cells in dual-species biofilms grown in the presence of *Pseudomonas* cells engineered to express toluene *o*-monooxygenase (TOM, which converts indole to an insoluble indigoid) than in biofilms with pseudomonads that do not express TOM due to a 22-fold reduction in extracellular indole. Also, indole stimulates biofilm formation in pseudomonads; hence, indole is an interspecies signal that may be manipulated by oxygenases of another bacterium to control biofilm formation, and pseudomonads respond to signals they do not synthesize. Further evidence that the indole effects are mediated by SdiA and AI-1 quorum sensing is that the addition of *N*-butyryl-, *N*-hexanoyl-, and *N*-octanoyl-L-homoserine lactones repress *E. coli* biofilm formation in the wild-type strain but not with the *sdiA* mutant; therefore, *E. coli* changes its biofilm in response to signals it cannot synthesize. The indole microarrays were also used to discover the uncharacterized protein YmgB mediates acid-resistance (renamed as AriR for regulator of acid resistance influenced by indole), and to discern that indole reduces acid resistance.

P19 - Essential Role of RIG-I and MAVS in Human Metapneumovirus-induced Cellular Signaling

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Human metapneumovirus (hMPV) is a recently identified pathogen responsible for a significant portion of respiratory tract infections in young children, elderly, and immunocompromised patients, causing bronchiolitis, croup, asthma exacerbation, and even pneumonia. While there is an emerging literature on the clinical and epidemiological reports of hMPV infections, very little is known regarding the cellular signaling elicited by this pathogen in airway epithelial cells, the major target of hMPV infection. In the present study, we show that RIG-I (retinoic acid inducible gene I), an RNA helicase binding double stranded viral RNA, is the main pattern recognition receptor (PRR) leading to interferon regulatory factors (IRF) activation and subsequent expression of important inflammatory and antiviral genes. hMPV infection readily induced the expression of RIG-I, both at the mRNA and protein level in A549 cells, a type II-like alveolar epithelial cell line. Overexpression of dominant negative RIG-I significantly reduced hMPV-induced Interferon(IFN)-β and RANTES gene transcription, as well as activation of an IRF-driven reporter gene plasmid. SiRNA downregulation of RIG-I expression confirmed the important role of this molecule in

hMPV-induced cellular responses. MAVS (mitochondrial antiviral signaling protein), a CARD-domain containing protein, was recently identified as the adaptor protein linking RIG-I to activation of downstream molecules such as IKKε and TBK-1, which are involved in IRF phosporylation. hMPV infection induces the mitochondrial expression of MAVS and overexpression of a protein lacking the N-terminal CARD domain significantly reduced IFN-β, RANTES and ISRE-driven promoter activation in response to hMPV infection. Together these results demonstrate that the RIG-I-MAVS signaling pathway plays a fundamental role in the initiation of epithelial cell responses leading to the production of important immune and antiviral molecules involved in the innate immune response to hMPV infection.

P20 - Genetic Stability of RVF MP-12 Virus in Cell Culture Systems

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Rift-valley fever is a viral disease endemic to Sub-Saharan Africa and causes several epidemics among human and lives stock. RVFV is a member of the genus Phlebovirus, family of Bunyaviridae. It is an envelope RNA virus and has a tripartite negative stand genome. It is a mosquito born virus.

In spite of its danger there is no known human vaccine at present. Only live attenuated vaccine so far produce is a RVFV MP-12 attenuated vaccine. To verify the genetic stability in terms of safety of MP- 12 for lack of reversion to the virulent virus in cell culture, we did this study.

P21 - Linking Phylogeny and Phenotype in Environmental Bacterial Antagonistic Interactions

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Microbial mats are composed of a diverse array of microbes that form self-sustaining communities that significantly contribute to the biogeochemistry of terrestrial and shallow water habitats. While previous studies have focused upon physio-chemical properties that control the distribution of species, we investigated antagonistic interactions by bacteria as mechanism in regulating their distribution. A predominance of Gram-positive bacterial isolates were obtained from 5 distinct vertical layers of a core taken from a microbial mat found in a hypersaline pond. Screening for antagonistic interactions suggested distinct patterns of inhibition observed between layers. Comparison of phylogenetic and phenotypic (i.e. antagonism or sensitivity) patterns revealed linkage of the former to sensitivity patterns.

P22 - Inactivation of pH-regulated Genes in *Borrelia burgdorferi*

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Borrelia burgdorferi, the causative agent of Lyme disease, alters its gene expression in response to highly disparate environmental signals encountered in its tick vector or mammalian host. In particular, *bba64*, *bba65* and *bba66* are three linear plasmid 54 (lp54)-encoded genes that exhibit

maximal differential expression in response to host-specific pH. Therefore, we hypothesized that *bba64*, *bba65* and *bba66* are required for infectivity of the mammalian host. An *in vitro* mutagenesis strategy to inactivate the three pH-regulated genes was used. A plasmid designated pMM2 carrying a 4.6 kb region of lp54 extending from *bba64* to *bba68* was used as the target plasmid in conjunction with a customized transposon carrying Str^R under the control of a borrelial promoter P_{flgB}. Putative plasmids with insertional inactivation at the 5' end of *bba64*, *bba65* and *bba66* respectively, were selected based on restriction enzyme profile and used to transform a non-infectious lp25⁻ strain of *B. burgdorferi* B31. Borrelial transformants were selected in the presence of 50µg/ml streptomycin on BSKII agarose overlays and analyzed by PCR using primers specific to each of the ORF. Mutant strains of *B. burgdorferi* had a 1.6kb increase in their respective amplicon size in comparison to the amplicons from their isogenic parental controls due to the presence of the antibiotic resistance marker. Southern blot hybridization analysis, using P_{flgB}-Str^R as a probe, indicated the presence of the antibiotic selection marker only in the mutants and not in the isogenic parental strain. We are in the process of complementing each one of the above borrelial mutants with an intact copy of the gene that has been inactivated along with the minimal region of lp25 that will restore infectivity. The above strategy will help to determine if inactivation of pH-regulated lipoproteins will attenuate the infectivity *B. burgdorferi* in the murine model of Lyme disease.

P23 - Environmental Factors Affecting Abundance and Distribution of a Marine Human Pathogen, *Vibrio vulnificus*

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Vibrio vulnificus is a marine human pathogen that thrives in warm brackish waters. While the contributions of salinity and temperature to *V. vulnificus* dynamics have been well established, influences of other environmental factors are unknown. Numerous outbreaks have occurred along the Texas Gulf Coast over the last several summers, where waters experience warm temperatures, low salinities and low oxygen concentrations. Matagorda Bay, the test site of our current study, was selected because it provides a gradient of environmental conditions and was the site of numerous *V. vulnificus* infections. We are also examining Powderhorn Lake, a small body of water adjacently connected to Matagorda Bay, as a possible point-source for *V. vulnificus*. Seasonally collected samples are being analyzed using real-time Q-PCR and epifluorescent microscopy.

P24 - Flavitrack: A Database Approach to Identifying Flavivirus Evolution and Functional Determinants

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Flaviviruses are vector borne human pathogens that cause severe encephalitic and hemorrhagic diseases such as tick-borne encephalitis, and Dengue, West Nile and Yellow fever. There are currently no effective antiviral drugs and few vaccines against flaviviruses. We use combined sequence and structural analysis to track viral evolution, determine functional areas of proteins and conserved antigenic sites. To aid in this effort, we established a database of flavivirus genomes to enable structure based sequence comparison and rapid phylogenetic evaluation of these diverse

pathogens. Flavitrack (http://carnot.utmb.edu/flavitrack) contains over 475 complete genomic sequences from 39 different flaviviruses, plus related information on known mutations, structure and literature references. Each sequence has been assigned a unique identifier, a "license plate" that summarizes its date and place of isolation, phenotype, and lethality. This enables us to run very large sequence alignments and interpret the data with regard to vector and symptom specificity within viral subclasses and strain evolution. Primary applications for Flavitrack include sequence retrieval, BLAST comparison of a sequence to others in the database, obtaining sequence alignments, and building cluster or phylogenetic trees. In addition, Flavitrack provides access to our in-house program PCPMer to quantitatively evaluate and visualize areas of the viral proteins affected by mutations. Flavitrack will also eventually contain direct visualization methods for structures or 3-D models for all flavivirus proteins, allowing combined sequence/structure analysis to characterize common B- and T-cell epitopes.

P25 - Inference and Comparison of Metabolic Networks of Four *Vibrio* Species

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Vibrio is a gram-negative genus of bacterium that contains pathogenic and non-pathogenic strains. *Vibrio parahaemolyticus* RIMD 2210633, *V. vulnificus* YJ016 and *V. vulnificus* CMCP6 are three pathogenic *Vibrios* responsible for diarrhea and other symptoms of food poisoning. Nonpathogenic *Vibrio* species, including *V. fischeri* ES114, are usually bioluminescent symbiotes living within the light emitting organs of marine animals such as squid. The recent release of a complete genomic sequence for *V. fischeri* ES114 provides an opportunity for comparison studies among pathogenic and non-pathogenic *Vibrio* strains. The genomic sequence data forms a data set that could reveal much about the mechanisms and evolution of pathogenesis in *Vibrio*. One approach to understanding these variations is through examination of metabolic pathways. Losses and gains of genes must have direct effects on the enzymes present in the cell, and therefore on the metabolic pathways available to the cell. In order to integrate the gene gain/loss data with information about the metabolic and regulatory networks in these four strains, we built pathway genome databases (PGDBs) for them using the Pathway Tools software. The resulting PGDBs were scored based on the pathways proposed and their functional categories. There were 350 to 400 pathways represented in the four PGDBs, with numerous pathways shared between species. In order to determine whether the pathways of interest were complete and to account for the amount of pathway overlap, an *XYZ* scoring system was employed. The divergence found in the metabolic networks across these *Vibrio* species could shed light on adaptive mechanisms in response to environmental challenges and life style changes.

P26 - Recombinant Plasminogen Activator of *Yersinia pestis* and its Substrates/ Inhibitors for Anti-plague Therapy

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The growing resistance of bacterial populations to antibiotics that kill the microorganism has promoted an interest in developing antibacterial compounds that target the virulence mechanism of

pathogens. Such chemical attenuation will enable a host to clear the infection, and inhibitors of virulence could be also useful as prophylactic and therapeutic agents, in combination with traditional drugs and vaccines. *Y. pestis*, the etiological agent of plague, contains a surface-located protease called plague plasminogen activator (Pla). This protease is involved in *Y. pestis* systemic infection, and therefore, inhibitors of this enzyme can potentially serve as anti-plague chemical agents.

P27 - The Characterization of Septin Genes and the Synergistic Roles of WdCdc10p and the Chitin Synthases During Yeast Budding in the Pathogenic Black Yeast, *Wangiella* (*Exophiala*) *dermatitidis*

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Septins are a highly conserved family of proteins that show significant homology within and between species, excluding plants. Also, septins are critical for proper yeast morphogenesis and cytokinesis, especially during septal region development and septation. Apparently, in budding yeast, a septin-based hierarchy of proteins is required to localize chitin in the bud neck prior to septum formation. However, this process has not been clarified in other fungi. *Wangiella dermatitidis*, a well-known causative agent of human phaeohyphomycosis, is a melanized asexual fungus that predominantly grows as a budding yeast in rich nutrient conditions. Interestingly, some chitin synthase mutants (w*dchs*Δ) show defects in septum formation. Therefore, we hypothesized that the septins of *W. dermatitidis* may functionally interact with some of its chitin synthases (WdChsp). To test this hypothesis, we first cloned the four major vegetative septin homologs of *S. cerevisiae* from *W. dermatitidis*, designated *WdCDC3*, *WdCDC10*, *WdCDC11*, and *WdCDC12*. We then characterized the functions of each encoded *W. dermatitidis* septin by the heterologous complementation expression and mutagenesis. Consistent with other fungal septin mutants, all the *wdcdc*Δ mutants displayed defects in cell separation and morphology. Yet, each septin carried out a distinct function. In addition, the introduction of w*dcdc10*Δ*-1* mutation into each of five different *wdchs*Δ mutants revealed that interactions occurred among WdCdc10p and WdChs3p, WdCdhs4p, and WdChs5p. These results confirmed that in *W. dermatitidis* septins are important for proper morphogenesis, cytokinesis, and especially septum formation through the interactions with some chitin synthases.

P28 - Trehalose Metabolism as a Novel Drug Target in *Mycobacterium tuberculosis*

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Trehalose is a disaccharide generated under stress conditions, protecting proteins from denaturation. *M. tuberculosis* uniquely contains constitutively high trehalose levels and has three biosynthetic pathways. Deletion of those pathways genetically is lethal, thus trehalose is essential in *M. tuberculosis*. Given there is no trehalose synthesis or utilization in humans, trehalose metabolism may be an excellent novel drug target. We have obtained 8 strains of *M. smegmatis* which are mutants in various trehalose metabolic genes. Interestingly, strains with gene deletions in only one pathway are more growth impaired at low trehalose levels than the mutant strain lacking all 3 pathways. In concert, while the triple mutant has mild (i.e. 2-fold) increases in sensitivity to certain antibiotics, individual mutants have more profound effects (8-fold). We propose that the mutations result in major changes in cell wall biosynthesis, which in turn increase

antibiotic sensitivities. Development of compounds which can inhibit trehalose metabolism should increase sensitivity to current drugs, thus shortening therapy.

P29 - Improving Nucleic Acid-Based Pathogen Detection Assays Through the Estimation of the Total Genomic Diversity of Complex Backgrounds

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With the support of HSARPA BIAD-2, we are currently developing new DNA technology for the estimation of the effective genomic sizes of complex environmental and clinical samples. Based upon statistical properties of the presence, frequencies, and location of particular short subsequences(8-20 nucleotides) within genomic sequences, we suggest that PCR primers and/or oligonucleotide probes on microarrays (or any other high-throughput sequence-detection platform) can be used to estimate the effective genome size in any given environmental or clinical sample. We believe that this technology developed will lead to significant improvement of the quality of probes and primers for existing and future detection systems, especially by reducing the frequency of false positives and supporting robust detection of engineered threats.

P30 - *pdpD* is Not Essential for *F. tularensis* Virulence

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The Francisella Pathogenicity Island (FPI) has been shown to contain genes necessary for intramacrophage survival and virulence of *F. tularensis*. The FPI shares a high degree of homology among the various subspecies, and is duplicated in subsp. *tularensis* and *holarctica*. One major difference in the FPI between the subspecies is found in the *pdpD* gene. This gene is essentially missing from the FPI of subsp. *holarctica*, and is larger by 150 bp in subsp. *novicida* than in subsp. *tularensis*. An earlier study identified *pdpD* as being critical for subsp. *novicida* intramacrophage growth and virulence in mice. We constructed a subsp. *novicida* strain (KKF52) with an insertion/deletion of *pdpD* (Δ*pdpD*::*ermC*), and were surprised to find that this strain was only slightly attenuated for growth in macrophages and maintained relatively high virulence in mice. The *pdpD* mutation in strain KKF52 removes amino acids 207-587 from PdpD, whereas the *pdpD* mutation previously described in strain JL12 removes all 1245 amino acids from PdpD; both mutations have an *ermC* cassette inserted into the deletion. Western immunoblot with antisera against IglC showed that strain KKF52 expresses IglC, whereas strain JL12 does not, suggesting that the *pdpD* mutation in JL12 has polar effects on the downstream genes, which are known to be important for intramacrophage survival and virulence. One reason for the polar effects of the JL12 *pdpD* mutation might be the presence of an *iglABCD* promoter in the portion of *pdpD* deleted in this strain; however, RT-PCR analysis suggested that the promoter for the *iglA* gene is at the beginning of *pdpD*, indicating that *pdpDiglABCD* are transcribed as an operon. Further analysis should clarify the polar vs. non-polar nature of these two *pdpD* mutations, but we conclude that *pdpD* is not essential for, but contributes to, intramacrophage survival and virulence.

P31 - Non-nucleotide Inhibitors of Anthrax Edema Factor Identified by an *ab initio* Screening Method

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Many pathogenic bacteria produce adenylyl cyclase toxins, such as the edema factor (EF) of *Bacillus anthracis*. These disturb cellular metabolism by catalyzing production of excessive amounts of the regulatory molecule cAMP. To identify novel lead compounds that would take advantage of the diversity of compound libraries, a structure based method was developed, using an *ab initio* fragment docking method and scoring with with HINT (a hydropathic free energy scoring tool based on $LogP_{o/w}$). Fragments that bound with high affinity to the active site of EF were used to design a 3D-model pharmacophore with flexible distance constraints between the active groups. This was used to select similar molecules from the >250,000 compounds in the NCI library. Compounds were docked with FlexX and AutoDock to select compounds with the highest binding scores to EF *in silico*. The selected molecules were then used to select over 10,000 compounds from the 2.7 million commercially available ones in the ZINC database. These were docked to the EF active site and ranked according to their binding/docking scores followed by selection based on physicochemical properties. A final list of 19 compounds was identified for purchase and tested in the biological assay for inhibition of cAMP production induced by EF in mammalian cells. The three lowest energy compounds from this screen were effective inhibitors of EF and other bacterial toxins. Thus, the *ab intio* screening process was able to reduce millions of compounds to a small list of candidates for assay, greatly enhancing the selection process of advanced lead compounds for drug design.

P32 - Functional and Biological Characterization of a New Type III Secretion System Effector from an Emerging Human Pathogen *Aeromonas hydrophila*

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Our laboratory is the first one to report human infections with *Aeromonas hydrophila* by direct consumption of contaminated water by DNA fingerprinting of the isolates. In addition to gastroenteritis, *A. hydrophila* can lead to cellulitis, formation of bullae lesions, and ecthyma gangrenosum. We first fully characterized the type III secretion system (T3SS) from a diarrheal isolate SSU of *A. hydrophila* and identified a new effector, designated as AexT-like, from this pathogen (see our other poster). This effector exhibited limited homology to ExoT/S (31%) of *Pseudomonas aeruginosa* and to AexT (40%), isolated recently from a fish pathogen *A. salmonicida*. Since T3SS effectors are directly translocated into host cells, we used the HeLa Tet-off system to investigate the effect of *aexT-like* gene in the eukaryotic cells. We cloned the full-length and the DNA encoding the first 231 amino acid (aa) and 232-512 aa residues, representing NH_2- and COOH- terminal domains of AexT-like effector into pBI-EGFP vector, which allows expression of the target gene and the enhanced green fluorescence protein from a bidirectional promoter. All three constructs were transfected into HeLa Tet-off system cells by

electroporation, and the presence of various domains of AexT-like proteins was confirmed by Western-Blot analysis and flow cytometry using specific antibodies to AexT-like effector. HeLa cells transfected with plasmids harboring the full length and NH_2-terminal domain of AexT-like protein exhibited rounding of the cells within 8 hr after transfection. The host cells transfected with the plasmid construct possessing the COOH-terminal domain also exhibited cell rounding but was significantly less compared to that caused by the full-length and NH_2-terminal domain of AexT-like protein. Disruption of actin filaments in HeLa cells due to expression of the AexT-like full length and the AexT-like NH_2-terminal domain was evident by phalloidine staining. In addition, HeLa cells transfected with AexT-like full length and NH_2-terminal domain showed increased apoptosis compared to HeLa cells expressing the COOH-terminal domain of AexT-like protein. Since both ExoT/S and AexT possess ADP-ribosyltransferase activity, we believe that cell cytotoxicity could be attributed to this enzymatic activity. We purified different portions of the AexT-like protein from *E. coli* as His-tagged fusion proteins. Our data indicated that the full-length and the NH_2-terminal domain of AexT-like protein had more ADP-ribosyltransferase activity compared to the COOH-terminal domain of this effector. Different motifs contribute to this enzymatic activity of AexT-like protein compared to that of ExoT/S and AexT. We have recently performed microarray analysis on cDNA isolated from HeLa Tet-off system cells expressing the *aexT-like* gene to understand global transcriptional profiling induced by AexT-like effector to better define its mechanism of action.

P33 - Simulating the Endogenous Environment of *Mycobacterium tuberculosis*

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According to the lipolytic theory, mycobacteria that are infecting animals metabolize phospholipids from the surrounding lysed cells that are encasing the infection. This is in contrast to typical clinical media, which rely on glucose as the carbon source. *M. smegmatis* grown on lipids as sole carbon source display differences in drug sensitivity to multiple commonly utilized drugs. Colonies growing on lipid-containing plates appeared smooth, domed, and three times smaller compared to the typical rough, filamentous colonies that form on standard media. When compared in light microscopy, the lipid-grown bacteria are elongated. We suggest that when the lipids are being metabolized, major rearrangements are made in the acid fast cell wall. Permeability and cell wall composition are currently being analyzed. A group of experimental compounds was discovered to have enhanced activity against the lipid-grown cells. These compounds may have utility as antimycobacterial agents. The redesign of *in vitro* drug sensitivity methods to include lipid-based media may better predict *in vivo* activities of drugs under investigation.

P34 - A New Type III Secretion System Effector Protein in an Emerging Human Pathogen *Aeromonas hydrophila*

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Aeromonas hydrophila is a gram-negative bacterium and is associated with gastroenteritis and wound infections. Our laboratory first reported the complete sequence of the type III secretion system (T3SS) from a diarrheal isolate SSU of *A. hydrophila*. We identified an effector protein of the T3SS which exhibited limited homology to a T3SS-associated effector ExoT/S of

Pseudomonas aeruginosa. A homolog of ExoT/S, designated as AexT, was also recently reported from a fish isolate of *A. salmonicida*. Importantly, the *aexT* gene from the diarrheal isolate SSU of *A. hydrophila* is unique as its carboxy terminal region (represented by 280 amino acid [aa] residues) has no homology with any known proteins in the NCBI data base. The amino terminal region of this unique effector (represented by 231 aa residues) exhibited 67% homology with AexT. Because of the unique characteristics of this effector, we designated it as AexT-like. The DNA fragment encoding the *aexT-like* gene was cloned and characterized. We demonstrated that AexT-like effector is indeed secreted through the T3SS. The *aexT-like* gene (full length and portions encoding NH_2- (231 aa residues) and COOH- (representing 232-512 aa residues) terminal regions were hyper-expressed from the T7RNA polymerase/promoter-based pET-30a system as His-tagged fusion proteins in *E. coli*. The full length effector as well as its NH_2-terminal portion exhibited ADP-ribosyltransferase activity, the COOH-terminal portion also had this activity, albeit much weaker. This activity eventually leads to host cell toxicity. The motifs within the aa sequence that could be involved in ADP-ribosyltransferase activity were identified. Although ADP-ribosyltransferase activity has been reported for ExoT/S and AexT, this activity was confined to only the COOH-terminal region. The full-length AexT-like protein was affinity purified using the nickel charged resin and sequenced to confirm the identity of the effector. Swiss Webster mice were immunized with the purified protein to generate antibodies and also to evaluate its *in vivo* effects. Hyper-immunized mice were protected when challenged with the wild-type (WT) bacterium at 2-3 LD_{50} dose; all the unimmunized mice died within 48 hr. In addition, the *aexT*-minus mutant of *A. hydrophila* was much less virulent in mice compared to the WT bacterium. Finally, animals that survived challenge with the WT bacterium had circulating antibodies to AexT, thus delineating an important role of this toxin in the disease process. In summary, we identified a new functional effector protein-encoding gene from a clinical isolate of *A. hydrophila*.

P35 - Roles of YafO and YafN Putative Toxin/Antitoxin Proteins in Stress-Induced Mutagenesis in *Escherichia coli*

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Stress-induced mutational processes (SIM) are mutation mechanisms that operate specifically under growth limitation, sometimes forming rare adaptive mutants that can grow in the growth-limiting environment. SIM may accelerate evolution during stress. In the Lac assay, *E. coli* carrying a *lac* frameshift allele are starved on lactose medium, and a cell subpopulation with increased mutation rate (Torkelson *et al.* 1997 *EMBO J*) sometimes produces Lac^+ revertants. The SIM mechanism is specific to stationary phase/starvation, requires RpoS and SOS stress responses, and DinB error-prone DNA polymerase, and proceeds *via* error-prone DNA double-strand break repair specifically during stationary phase (Ponder *et al.* 2005 *Mol. Cell*). *dinB* is the first gene in an SOS-inducible operon containing *dinB*, *yafN*, *yafO*, and *yafP*. Here, we report that *yafN* is required for 90% of the Lac^+ SIM, whereas *yafO* and *yafP* are not required. Remarkably, a *yafN yafO* double mutant shows a complete restoration of SIM to wild-type levels. Therefore, *E. coli* requires *yafN* for SIM only if *yafO* is present. YafO and YafN might form a toxin-antitoxin gene pair similar to *E. coli relE* and its cognate antitoxin, *relB*. *yafN* shows homology to *relB*, and overexpression of *yafO* stops cell proliferation, suggesting a possible toxin. Like RelBE, YafNO might function as a regulator of its own operon and we suggest might promote a temporary stasis/ senescence when induced. Because there is no decrease in cfu in the whole population, any

putative YafO-induced stasis or killing would have to be specific to a mutating cell subpopulation. We suggest that this state is not incompatible with SIM, but that in the absence of the putative YafN antitoxin, the YafO toxin could be expressed in hypermutable subpopulation cells and prevent their subsequent proliferation if adaptive mutations are formed in them.

P36 - Regulation of Innate Responses to Infection with Intracellular Parasites

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Although *Leishmania braziliensis* (*Lb*) and *L. amazonensis* (*La*) are endemic in the same geographic regions in South America, their infections can lead to very different clinical manifestations. *Lb* infection can lead to mucocutaneous leishmaniasis, a disfiguring disease characterized by excessive T- and B-cell responses. On the other hand, *La* infection can cause diffuse cutaneous leishmaniasis, an anergic form characterized by profound suppression in cellular immunity and uncontrolled replication of intracellular parasites. Most inbred strains of mice are susceptible to *La*, but genetically resistant to *Lb* infection; the underlying mechanisms remain largely unknown. Given the important role of dendritic cells (DCs) in leishmanial infections and activation of parasite-specific CD4 T cells, we hypothesize that *Lb* infection efficiently triggers innate responses in DCs and macrophages and that this innate immunity, in conjunction with the subsequent Th1 responses, leads to rapid killing of parasites in the local tissue. To test this hypothesis, we infected murine DCs, as well as murine and human macrophages, with *Lb* and *La* and measured infection rate, cell activation status and cytokine secretion. Our key findings are: (1) although both species of parasites were comparable in infecting mouse DCs and human and mouse macrophages, *La* parasites tended to have prolonged survival within the cell; (2) *Lb*-infected DCs displayed a $CD11c^{high}CD45RB^{-}CD83^{+}CD40^{+}CD80^{+}$ (activated DC) phenotype, while *La*-infected cells showed a "semi-activation" status, with much lower numbers of activated DCs; (3) *Lb*-infected DCs produced 3-fold higher levels of IL-12p40 and were more potent in priming naïve $CD4^{+}$ T cells when compared to *La*-infected counterparts; (4) although IL-10 production was detectable in *Lb*-infected DCs, its level was considerably lower than that of IL-12p40, suggesting the potential of *Lb*-infected DCs in activating Th1-type responses. These observations support our hypothesis and call for detailed analysis of intracellular events triggered by two parasites species.

P37 - Optimization of the Conditions for Secretion by the *Burkholderia Mallei* Type Three Secretion System and Use of the Effector Proteins in an Optimized Balb/c Infection Model

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Burkholderia mallei, the etiologic agent of glanders disease is a gram-negative, capsulated, non-spore forming, non-motile bacillus. *B. mallei* was used as a biological weapon during the American Civil War, World Wars I and II, and reported to be weaponized by the Soviet Union in Afghanistan. Recently, *B. mallei* has come under renewed investigation as a possible biological threat and is classified as a category B agent. Currently, there is no effective vaccine for glanders in animals or humans. Scientific reports describing pathogenesis associated with glanders is limited to identification of a type III secretion system (TTSS) and capsule production. Characterization of

effector proteins has been widely investigated in other organisms but limited in the case of *Burkholderia* species. Through a plasmid based *yplA* reporter system, we established an approach for TTSS secretion characterization with the goal to utilize these effectors as putative protective epitopes in a BALB/c mouse model. The reporter system is based on the ability to secrete YplA, a *Yersinia* TTSS effector phospholipase protein, production of the secreted YplA reporter protein was confirmed by immunoblots of culture supernatants and by lipase activity on indicator agar plates. BALB/c mice vaccinated with a linear expression element homologous to the *B. mallei* TTSS effector protein BopA showed increased survival when challenged with wild type *B. mallei* intraperitoneally. Additionally, survival of BALB/c mice vaccinated with non viable *B. mallei* was increased compared to non vaccinated mice, demonstrating the feasibility of our mouse model. Further, our results indicate that a TTSS reporter system can be used to assay secretion in *B. mallei*.

P38 - Developmental Effects of Bacteria on *Drosophila melanogaster*

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It has been observed that the absence of bacteria may play a significant role in modulating lifespan in the *D. melanogaster* mutant, DJ817. The purpose of this study is to begin the exploration into which bacteria are involved in producing this phenotype. Methods first involved collecting eggs from DJ817 and w1118 then removing as much microbial life as possible. Larvae were then allowed to develop: (1) without any added bacteria, (2) after adding *E. coli*, and (3) after adding specific bacteria previously isolated from the wild-type adult fly gut. Autoclaved fly food was used to ensure experimental control. Test results were determined by observing the size and number of emerged larvae as compared to those from eggs that were washed and then allowed to develop on normal food. Any observed difference in development would likely account for the modulated lifespan phenotype in DJ817.

P39 - Development of a Clinically Relevant *In Vitro* Model for *Staphylococcus aureus* Biofilms on Orthopaedic Biomaterials

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A clinically relevant *in vitro* model of a *S. aureus* osteomyelitis biofilm infection was developed for use in studies to evaluate the effectiveness of various antibiotics against these biofilms. This approach was novel in that i) the *S. aureus* strain used was isolated from an osteomyelitis infection, ii) the cells were incubated in a synthetic synovial fluid (SSF), iii) the substrate was wafers of polymethylmethacrylate (PMMA), a common orthopaedic biomaterial, and iv) there was static incubation in a microtiter dish at 37°C and 5% CO_2. The SSF was changed every 24 hrs. Confocal laser scanning microscopy was used to generate a three dimensional image that was analyzed using the COMSTAT computer program. Using this model it was determined that inhibition of biofilm growth by vancomycin required more than 32 times the MIC.

P40 - NSm and 78-kDa Proteins of Rift Valley Fever Virus are Dispensable for Viral Replication in Cell Cultures

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Rift Valley fever virus (RVFV), which belongs to the genus *Phlebovirus*, family *Bunyaviridae*, causes severe epidemics among ruminants and is also recognized as a human pathogen that causes a syndrome of fever and myalgia, a hemorrhagic syndrome, ocular disease, and encephalitis. RVFV major structural envelope proteins Gn and Gc, a minor structural protein 78-kDa protein, and a nonstructural protein NSm are all encoded in the M gene. Biological functions of the NSm and 78-kDa proteins are totally unknown. The mRNA of the RVFV M segment has five in-frame translational initiation codons upstream of the region encoding Gn and Gc proteins (the preglycoprotein region). Past expression studies by others demonstrated that the NSm and 78-kDa proteins were translated from the first AUG and second AUG, respectively. To determine whether the NSm and/or 78-kDa proteins are essential for RVFV replication, the first AUG, second AUG, and both first and second AUGs in the preglycoprotein region of a plasmid expressing the RVFV MP-12 strain M RNA segment were mutated. Using an RVFV reverse genetics system, we employed these mutated M RNA-expression plasmids to recover three different types of mutant viruses, one lacking NSm expression, another lacking 78 kDa protein expression and the last one lacking expression of both. All three mutant viruses were successfully recovered, and they retained the introduced mutated sequences after ten virus passages in Vero cells at a low multiplicity of infection. All three mutant viruses and its parental virus rMP-12 showed similar plaque sizes and morphology in Vero E6 cells and had similar growth kinetics in Vero, C6/36, and MRC5 cells. A competitive replication assay showed that rMP-12 did not have any advantage of virus replication over the mutant viruses lacking expression of both NSm and 78-kDa proteins. These results convincingly demonstrated that NSm and 78-kDa proteins are viral accessory proteins that are not essential for RVFV replication in cultured cells.

P41 - The Effects of Cytokine Stimulation on Rickettsiae-infected Endothelial Cells; Behavior and Integrity

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Rickettsia rickettsii primarily targets the microvascular endothelium leading to meningoencephalitis and interstitial pneumonitis as the primary complications associated with Rocky Mountain spotted fever. This study aimed to better define the role of cytokine stimulation of the endothelium in leading to increased microvascular permeability in a cell culture model of the Blood-Brain Barrier. Specifically we were interested in defining the role of anti-rickettsial nitric oxide and reactive nitrogen species in modulating the structural integrity of cell-cell junctions. The results described herein suggest that nitric oxide produced to help control rickettsial proliferation has a mild effect on the host cell when compared to the cumulative effects of the cytokine stimulation needed to incite NO production. This work helps us better understand the mechanisms of increased microvascular permeability as they relate to rickettsial pathogenesis.

P42 - IL-1Beta Promotes the Activation of CD11c^{high}CD45RB^{-} DC Subset and Consequential CD4^{+} T Cell Priming but Accelerates *Leishmania amazonensis* Infection in Mice

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Diffuse cutaneous leishmaniasis is associated with infection with *Leishmania amazonensis* (*La*) and is characterized by profound suppression in cellular immunity. Most inbred strains of mice are also susceptible to *La*, developing progressive skin lesions; however, the underlying mechanisms remain unclear. One possibility is that La parasite modulates antigen-presenting cells, which favors the generation of pathogenic Th cells that are capable of triggering leukocytes infiltration but are insufficient to fully activate their microbicidal activities. Our previous studies have revealed a remarkable impairment in cytokines or chemokines expression during the course of *La* infection. To further explore the molecular basis underlying impaired Th1 cell activation, we used stationary promastigotes of *La* or *L. major* to infect bone marrow-derived DCs of C57BL/6 mice, assessed the activation of DC subsets, and evaluated the capacity of these DC subsets in priming CD4^{+} T cells *in vitro*. In comparison to *L. major*-infected DCs, *La*-infected DCs secreted less IL-1a and IL-1b and generated lower frequencies of IL-12-producing CD11c^{high}CD45RB^{-}CD83^{+}CD40^{+} DC subset. DC-T cell co-culture indicated that *La*-specific Th cells had the IL-12^{low}IFN-g^{low}IL-10^{high}IL-17^{high} phenotype. Although the addition of exogenous IL-1b at the time of *La* infection markedly enhanced DC activation and consequential CD4^{+} T cell priming, it did not change the phenotype of pathogenic Th cells. Local injection of IL-1b following *La* infection accelerated lesion progression and parasite burden with more activated CD4^{+} T cells. Our results suggest that intrinsic defect in DC activation is responsible for immunodeficiency in *La*-infected hosts, and that this parasite may have evolved unique mechanisms to interfere with effectors of the innate and adaptive immune system.

P43 - Characterizing the Response of Human Macrophage HL-60 Cells to *Bacillus anthracis* Infection

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Bacillus anthracis is a Gram-positive, spore-forming bacterium that is classified as a Category A select agent due to its hardy nature and high mortality rate when inhaled. The 'Trojan Horse' method of utilizing host macrophages (MØ's) as a location for spores to germinate in and for transport to regional lymph nodes is well characterized for murine MØ's, but this phenomenon in human MØ's has not been well studied. Additionally, the effect of the *B. anthracis* adenylyl cyclase, edema toxin (EdTx), on human MØ's has not been well described. Immunofluorescence studies revealed that human HL-60 cells differentiated into macrophages were capable of rapidly phagocytosing spores. Once internalized, a significant portion of spores germinated as quickly as 15 minutes. In early stages of the infection, *B. anthracis* survival was observed, but by 4 hours there was significant killing. Interestingly, a small percent of intracellular *B. anthracis* remained viable, even at 24 hours, possibly explaining how severe infection is established in humans. The effects of spore infection on MØ viability, migration, and nitric oxide (NO) production were also assessed. EdTx treatment resulted in increased production of cAMP by HL-60 cells. Further, EdTx was a potent inhibitor of MØ phagocytosis and migration, resulting in a level of suppression

similar to the actin cytoskeleton disrupting agent, Cytochalasin B. These results reveal additional ways *B. anthracis* may modulate the human immune response to prolong its survival within the host.

P44 - Role of Pulmonary Macrophage and Dendritic Cells in SARS-CoV-Induced Host Inflammatory Responses: An *in vitro* Model

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Severe acute respiratory syndrome (SARS), caused by a novel human coronavirus (CoV), designated SARS-CoV, is a highly contagious respiratory disease with the lungs as a major target. The exact molecular and cellular mechanism(s) of SARS pathogenesis remains largely unknown. However, like other acute respiratory viral diseases, SARS is likely an immune-mediated lung disease. Pulmonary macrophage (MΦ), airway epithelium, and dendritic cells (DC) are three key inflammatory mediators of the host innate defense mechanism against respiratory infections. Pulmonary MΦ are situated at the inner epithelial surface and alveoli of the respiratory tracts, whereas DC reside abundantly underneath the epithelium and in the loose connective tissues around the vessels of the airway system. Such strategic anatomic locations within the airway system make it highly relevant and important to investigate their inter-relationship with highly polarized pulmonary epithelial cells in the course of SARS-CoV infection, which is the objective of this study. We utilized the Transwell system to establish polarized bronchial Calu-3 cell cultures and cytokines that were secreted to either apical or basolateral chambers. We found that cytokines released by polarized, SARS-CoV-infected Calu-3 cells are potent in modulating various MΦ's and DC's intrinsic functions. Specifically, they induced cytokine production by co-cultured MΦ and DC as well as CD40 and CD86 expression on the surface of DC. However, they also compromised DC's T cell priming and MΦ's phagocytic abilities. These results suggest that the interplays among permissive lung epithelial cells, pulmonary MF and DC are critically involved in the regulation of acute inflammatory responses in the course of acute SARS-CoV infection.

P45 - In Situ Function and Transcriptional Regulation of the Phospholipase A_2 Activating Protein Gene

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Inflammatory mediators, including cytokines and prostaglandins, play central roles in the pathogenesis of bacterial sepsis. Phospholipase A_2 activating protein (PLAA) is a novel activator of phospholipases that regulates the production of prostaglandin E_2 (PGE_2), tumor necrosis factor alpha (TNF- á) and interleukin 1 beta (IL-1 â). In order to identify signaling cascades initiated by PLAA, we performed GeneChip analysis to examine global transcriptional profiles of HeLa cells overexpressing *plaa*. The expression of 55 genes was significantly and consistently altered by *plaa* overexpression. Many of these genes are involved in immune response (such as interleukin 32, chemokine receptor 4 and clusterin), signal transduction (such as phospholipase C beta 4 and phosphodiesterase 1A) and transcription regulation (such as kruppel-like factor 5 and nuclear receptor 2F1). The expression changes of 13 genes were verified by real time-reverse transcriptase-PCR. The induction of interleukin 32 (IL-32) was also confirmed by enzyme-linked immunosorbent assay. The *plaa* gene expression can be rapidly induced by bacterial components

(e.g., LPS and cholera toxin) and cytokines (e.g., TNF-á and IL-1â), indicating that PLAA activity is tightly controlled by transcriptional regulation. To understand how the *plaa* gene expression is regulated, we used luciferase reporter system to search for transcriptional regulatory sequences from its 5' genomic region. We identified two transcriptional regulatory elements in Hela cells; one stimulatory element that was located between 252 to 293 bp upstream of the ATG start codon and the other inhibitory element that was located between 483 to 786 bp upstream of the potential transcriptional start site. This is the first study of *in situ* function and transcriptional regulation of the *plaa* gene.

P46 - Studying the Association of VEGF and Anti-VEGF Aptamers

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Vascular endothelial growth factor (VEGF) plays an important role in cancer growth by stimulating new blood vessel formation. The development of anti-VEGF drugs is becoming an important issue in cancer therapy. Aptamers are synthetic nucleic acid ligands selected *in vitro* from large combinatorial libraries and are becoming one of the promising candidates for anti-VEGF drugs. In this study, we have begun to establish a reliable model to investigate the association of VEGF and anti-VEGF DNA aptamers, as well as to address the molecular mechanism of VEGF action. To optimize expression and purification of $VEGF_{165}$, we constructed an intein mediated purification with an affinity chitin-binding tag (IMPACT-CN) system, a 6x His tag system, as well as a T7 RNA polymerase-based vector expression system for native $VEGF_{165}$. Purified $VEGF_{165}$ was confirmed by Phast gradient gel and the activity was further confirmed by chicken chorioallantoic membrane (CAM) assay. To understand the details of the aptamer-target recognition using DNA aptamers that target VEGF, fluorescence anisotropy was used to monitor biophysical interactions of $VEGF_{165}$ and anti-VEGF DNA aptamers. More importantly, the CAM assay showed that the anti-VEGF DNA aptamers inhibited angiogenesis induced by $VEGF_{165}$. All findings suggested that anti-VEGF DNA aptamers can be used as drug candidates to treat VEGF-promoted tumor growth. Our further experiments are ongoing to explore more efficient anti-VEGF DNA or RNA aptamers.

Notes