Tufts University
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Poster Presentations

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Sponsored by the Office of the Vice Provost for Research
Title:
Menaquinones Content of Human Serum and Feces

Authors:
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Presented by:
J. Philip Karl

Departments:
Energy Metabolism Laboratory, Vitamin K Laboratory, Nutritional Immunology Laboratory, and Vascular Biology Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging

Abstract:
Bacterially-synthesized menaquinones (MKn) may contribute to vitamin K (VK) nutriture. There are limited data on interindividual variability in endogenous MK synthesis and its relation to circulating forms of VK. Serum and fecal VK concentrations were assessed in 13 healthy adults (45-65 yr) consuming a standardized diet for 14d. Phylloquinone (PK), MK4 and MK6-MK13 concentrations were measured by HPLC in fasting serum collected on day 8 and 72 hr-fecal homogenates collected days 8-10. LC/MS was used to confirm individual MKn. PK, the primary dietary form, comprised only 6 ± 3% of total fecal VK content (fecal PK [mean ± SD]; 0.45 ± 0.17 μg/g dry wt), and was not correlated with serum PK concentrations (1.85 ± 0.89 pmol/L). No MKn were detected in serum. MK4 and MK6-MK12 were detected in all, and MK13 in 7 fecal homogenates. Mean fecal MKn concentrations ranged from 0.17 ± 0.05 μg/g dry wt for MK4 to 4.95 ± 2.30 μg/g dry wt for MK10, with MK10 comprising 63 ± 19% of total fecal MKn content. Based on fecal contents, we conclude that the majority of MKn isoprenologues are present in the human colon, but with evidence of interindividual variation despite 10d of diet standardization. The clinical implications of these findings, and the role of MKn in supporting VK nutriture are yet to be determined. Funded by a grant from General Mills and USDA contract #58-1950-7-707.
Title:
Differential Cellular Uptake and Metabolism of Curcuminoids in Monocytes/Macrophages: A Possible Mechanism of Differential Effects of Curcuminoids on Lipid Uptake and Bacterial Phagocytosis

Authors:
Kiyotaka Nakagawa, Jean-Marc Zingg, Sharon H. Kim, Michael J. Thomas, Gregory G. Dolnikowski, Angelo Azzi, Teruo Miyazawa, Mohsen Meydani

Presented by:
Kiyotaka Nakagawa

Departments:
Vascular Biology Laboratory and Mass Spectrometry Laboratory, Jean Mayer USDA-Human Nutrition Research Center on Aging; Food and Biodynamic Chemistry Laboratory, Graduate School of Agricultural Science, Tohoku University

Abstract:
Objective: Curcumin (CUR) is obtained from the rhizome of turmeric (Curcuma Longa L.) and is present in dried turmeric powder. CUR has been shown to have several activities such as anti-bacterial and anti-inflammatory properties or the enhancement of phagocytosis of bacteria and parasites such as Plasmodium falciparum. Just like the phagocytotic effect, we previously showed that curcumin (CUR) can increase lipid accumulation in cultured THP-1 monocytes/macrophages (J.M. Zingg, et al., J. Cell. Biochem., 113, 833-840, 2012). CUR-induced accumulation of lipids in macrophages may be part of a mechanism aimed at the removal of lipids from the blood stream, which help to prevent diseases such as atherosclerosis. Since tetrahydrocurcumin (THC, an in vivo metabolite of CUR) had no such effect, in the present study, we have hypothesized that different cellular binding, uptake and/or metabolism of CUR and THC may be responsible for differential accumulation of lipids in macrophages.

Methods & Results: Chromatography with tandem mass spectrometry revealed that CUR was readily taken up by THP-1 monocytes/macrophages and slowly metabolized to hexahydrocurcumin sulfate. In contrast, uptake of THC was low. In parallel with CUR uptake, lipid uptake was observed in THP-1 macrophages but not with THC nor with another CUR metabolite and structurally related compounds.

Discussion: From these results, it is possible to conclude that CUR and THC are taken up and metabolized differently in THP-1 cells. The efficient cellular uptake of CUR, relative to the low uptake of the other curcuminoids (e.g., THC), may imply that CUR uptake in cells may occur via a transporter capable of distinguishing their structures. CUR-induced lipid uptake in THP-1 cells is an event that appears to be correlated to CUR transport inside the cells. These results would be useful for not only understanding
physiological function of curcuminoids for nutritional and medicinal purposes but also mechanism of phagocytosis of bacteria by macrophages.

Supported by USDA contract #58-1950-0-014 and sabbatical fellowship from Tohoku University (Japan) to Kiyotaka Nakagawa.
Title: Modulation of CD36 Scavenger Receptor Expression by Curcumin and Vitamin E Affects Cellular Uptake of Lipids and Bacteria

Authors: Jean-Marc Zingg, Kyotaka Nakagawa, Angelo Azzi, Mohsen Meydani

Presented by: Jean-Marc Zingg

Department: Vascular Biology Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging

Abstract:

Introduction: Atherosclerosis is associated with inflammation and oxidative- and lipid-mediated damage in the vascular system: the risk of these events progressively rises with age. At the molecular level, hyperlipidemia and bacterial infection have been linked as causal triggers that accelerate atherosclerosis. The CD36/FAT scavenger receptor/fatty acid translocase mediates the uptake of fatty acids in various tissues. Other molecules that are recognized and/or taken up by CD36 include oxLDL, fibrillar β-amyloid, apoptotic cells, erythrocytes infected with Plasmodium falciparum, and Gram-negative and Gram-positive bacteria. Recent research also links CD36 with several signal transduction cascades leading to inflammasome activation with sterile and non-sterile triggers. CD36 associates with Toll-like Receptors 2, 4 and 6, interacts with bacterial lipids and modulates signal transduction required for native immunity and for inflammatory processes in response to bacterial pathogens. Mice homozygous for CD36 deletion are hyperlipidemic and hypersusceptible to Staphylococcus aureus infection. In cell culture, CD36 mediates uptake of Escherichia coli and Staphylococcus aureus. The ability of nutritional components to affect the expression of CD36 and to change the inflammatory and phagocytic response to lipids and bacteria in monocytes/macrophages is largely unknown and could be an important regulatory event modulating inflammation and atherosclerosis in response to lipids and microbial pathogens.

Results: Here we show that curcumin, a natural polyphenol from turmeric spice, increases the surface and gene expression of CD36 leading to increased lipid uptake in THP-1 monocytes/macrophages. The curcumin metabolite, tetrahydrocurcumin (THC), has no effect, most likely since it is not efficiently taken up into cells. In contrast, CD36 surface expression and lipid uptake is decreased by alpha-tocopherol and more so by alpha-tocopheryl phosphate. Interestingly, phagocytosis of inactivated Staphylococcus aureus (Wood strain), as AlexaFluor 488® or 595® conjugate bioparticles is affected by these compounds in a similar manner as
observed for lipids, suggesting that binding and uptake of lipids and bacteria may share common molecular mechanisms.

**Conclusions:** It is concluded that the cellular lipid uptake and the phagocytic response to bacteria in monocytes/macrophages are modulated by curcumin and vitamin E and involve altered expression of CD36 at the cellular surface. Thus, these dietary components may not only directly modulate the cellular anti-microbial and inflammatory response but also indirectly by influencing the host-microbiome interaction. Supported by USDA contract #58-1950-014.
Title:
The Interaction between the Intestinal Microbiome and the Enteric Pathogen Cryptosporidium parvum

Authors:
Refaat Ras, Saul Tzipori, Giovanni Widmer

Presented by:
Giovanni Widmer

Department:
Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine

Abstract:
Cryptosporidiosis can be severe in immune compromised persons and is highly prevalent in infants in developing countries. The lack of effective anti-cryptosporidial drugs motivated us to explore the interaction between Cryptosporidium parvum and the host intestinal microbiome. Our aim is to understand to what extent the enteric protozoan parasite C. parvum impacts the intestinal microbiome and to assess whether parasite development can be modulated by the intestinal microbiome. Experiments were performed with laboratory-propagated C. parvum isolates of animal and human origin. Immune suppressed mice were populated with human fecal bacteria. Mice were infected with C. parvum oocysts and fecal samples were collected from infected and uninfected mice. We extracted fecal DNA and deep-sequenced 16S amplicons using an Illumina sequencer operated by the Tufts Genomics core facility. Observed changes in the composition of the bacterial microbiome revealed that the microbiome was impacted by infection with C. parvum. In some experiments changes in the fecal bacterial population became apparent 2-3 days post-infection and persisted until the infection was cleared. The taxonomic composition of the microbiome is being analyzed to identify bacterial taxa affected by the infection. To assess the impact of the intestinal microbiome on the pathogen, the microbiome was perturbed with different antibiotics. We found that oral administration of bacitracin to C. parvum infected mice mitigates the infection. Since this antibiotic does not inhibit C. parvum in culture, we hypothesize that perturbation of the bacterial microbiome in the gut is the cause of reduced parasite development. The possibility that C. parvum and the intestinal microbiome mutually influence each other opens new possibilities for understanding the importance of intestinal microorganisms in modulating the virulence of enteric infections and to find better strategies for managing cryptosporidiosis in humans and livestock.
Title:
Identification, Quantification, and Characterization of Microbiota Metabolites in Murine Gut using In Silico Analysis and Targeted Metabolomics

Authors:
Gautham V. Sridharan, Kyungoh Choi, Charmian Wu, Cory Klemashevich, Darshan Prabakaran, Long Bin Pan, Robert Alaniz, Arul Jayaraman, Kyongbum Lee

Presented by:
Gautham V. Sridharan

Departments:
Department of Chemical and Biological Engineering, School of Engineering; Artie McFerrin Department of Chemical Engineering, Texas A&M University; Department of Microbial and Molecular Pathogenesis, Texas A&M Health Science Center

Abstract:
Increasing evidence suggest that the metabolites produced by the gastrointestinal (GI) tract microbiota are important modulators of human health and disease. However, only a handful of bioactive microbiota metabolites in the GI tract have been identified. The microbiota has the potential to carry out a diverse range of biotransformation reactions that are unavailable to the mammalian host, and to produce a broad spectrum of metabolites nonnative to the host. Isolating and characterizing individual bacteria to identify metabolites is intractable, as many species present in the GI tract cannot be cultured under standard laboratory conditions. Untargeted metabolomics approaches have been useful in profiling bodily fluids and samples directly connected to GI tract, but are not well suited to resolving the origin of a metabolite as either bacterial or host metabolism. We present here a novel metabolomics strategy that integrates in silico analysis with targeted metabolomics to facilitate identification and quantification microbiota metabolites. We model the microbiota as an integrated metabolic system comprising 46 different species reported to be abundant in the gut microbiota, and represent this system with a metabolic reaction network. Of the 1,886 distinct reactions in the microbiota network, approximately 50% are strictly bacterial, i.e. absent in the murine host, with the largest number of reactions involved in amino acid metabolism. Focusing on tryptophan (TRP) as a representative, diet-derive amino acid, we utilize a probabilistic pathway construction algorithm to predict potential metabolic derivatives present in the murine GI tract, while also discriminating between microbiota- and host-specific derivatives. We validate the model-based predictions using multiple reaction monitoring (MRM), a quantitative mass spectrometry technique, on cecum and fecal samples from mice treated with antibiotic, dextran sodium sulfate (DSS), and vehicle. We find that both antibiotic and DSS treatment significantly alters the levels of the predicted TRP metabolites. To demonstrate the potential for the predicted and confirmed TRP derivatives to play a physiological role, we characterize these metabolites as ligands for the aryl hydrocarbon receptor (AhR).
Title:
IL-17A-Mediated Protection in Acanthamoeba-Induced Keratitis

Authors:
Amol Suryawanshi, Zhiyi Cao, Noorjahan Panjwani

Presented by:
Amol Suryawanshi

Department:
Department of Ophthalmology, School of Medicine

Abstract:
Acanthamoeba keratitis (AK) is a globally emerging, intensely painful and vision impairing infection of the cornea that is difficult to treat. Although past studies have indicated a critical role of neutrophils and macrophages in AK, the relative contribution of a recently discovered proinflammatory cytokine, IL-17A that is essential for migration, activation and function of these cells into the cornea is poorly defined. Moreover, the role of adaptive immune response particularly contribution of recently discovered CD4+ T cell subsets, Th17 and Tregs, in AK is yet to be understood. In this report, using a mouse corneal intrastromal injection-induced AK model, we show that corneal Acanthamoeba infection induces a strong CD4+ T effector and regulatory T cell response in the cornea as well as local draining lymph nodes (dLN). Furthermore, we demonstrate that corneal Acanthamoeba infection induces IL-17A expression proportional to the AK lesion severity and that IL-17A expression is essential for host protection against corneal Acanthamoeba infection and associated tissue damage. Accordingly, IL-17A neutralization in Acanthamoeba infected animals resulted in a significantly increased chronic corneal AK pathology, increased migration of inflammatory cells at the site of inflammation and a significant increase in effector CD4+ T cell response in dLN. Further studies indicated that neutrophils and CD4+ T cells contribute to the source of IL-17A during early and late stages of AK, respectively. Thus, in sharp contrast to other corneal infections such as herpes and Pseudomonas aeruginosa where IL-17A contributes to corneal pathology and inflammation, findings presented in this manuscript indicate that IL-17A response after Acanthamoeba infection plays an important role in host protection against invading parasites and minimizes associated corneal tissue damage.
Title:
Galectin-1-Mediated Suppression of Pseudomonas aeruginosa-Induced Corneal Immunopathology

Authors:
Amol Suryawanshi, Zhiyi Cao, Noorjahan Panjwani

Presented by:
Amol Suryawanshi

Department:
Department of Ophthalmology, School of Medicine

Abstract:
Corneal infection with Pseudomonas aeruginosa leads to a severe immunoinflammatory lesion often causing vision impairment and blindness. Although past studies have indicated a critical role for CD4^+ T cells, particularly Th1 cells, in corneal immunopathology, the relative contribution of recently discovered Th17 and Treg cell is undefined. In this study, we demonstrate that after corneal P. aeruginosa infection, both Th1 and Th17 cells infiltrate the cornea with increased representation of Th17 cells. In addition to Th1 and Th17 cells, Treg also migrate into the cornea during early as well as late stages of corneal pathology. Moreover, using galectin-1 (Gal-1), an immunomodulatory carbohydrate-binding molecule, we investigated whether shifting the balance among various CD4^+ T cell subsets can modulate P. aeruginosa-induced corneal immunopathology. We demonstrate here that local recombinant Gal-1 (rGal-1) treatment by subconjunctival injections significantly diminishes P. aeruginosa-mediated corneal inflammation through multiple mechanisms. Specifically, in our study, rGal-1 treatment significantly diminished corneal infiltration of total CD45^+ T cells, neutrophils and CD4^+ T cells. Furthermore, rGal-1 treatment significantly reduced proinflammatory Th17 cell response in the cornea as well as local draining lymph nodes (DLN). Also, rGal-1 therapy promoted anti-inflammatory Th2 and IL-10 response in secondary lymphoid organs. Collectively, our results indicate that corneal P. aeruginosa infection induces a strong Th17-mediated corneal pathology and treatment with endogenously derived protein such as Gal-1 may be of therapeutic value for the management of bacterial keratitis, a prevalent cause of vision loss and blindness in humans worldwide.
Title:
The New England Regional Biosafety Laboratory – A Regional BSL3 Facility at TCSVM

Authors:
Donna E. Akiyoshi and Saul Tzipori

Presented by:
Donna E. Akiyoshi and Saul Tzipori

Department:
Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine

Abstract:
The New England Regional Biosafety Laboratory (New England RBL), on the campus of the Cummings School of Veterinary Medicine, serves as an important regional resource for conducting Biosafety Level 3 (BSL3) research to improve the means of detecting, preventing and treating infectious diseases that occur naturally in the environment and are transmitted among humans, and between animals and humans. The mission of the New England RBL as a regional facility, is to serve investigators within the NE region who do not have access to secure BSL3/Select agent laboratories and/or vivaria at their own institutions, with a view to: a) investigate NIAID Category A-C Biodefense and Emerging Infectious Diseases (BEID), with emphasis on BSL3/select agent pathogens, including the biology of disease, development of diagnostic tools, therapeutics and vaccines; (b) provide a facility that in addition to being secure, is also GLP-compliant; (c) train and educate graduate students and scientists from Tufts and other institutions to conduct biomedical research on BEID under BSL3 conditions and (d) provide support or collaborative opportunities with scientists with strong core expertise in infectious disease animal models and/or global public health for investigators from other academic institutions, the private sector in New England, and nationwide.

The New England RBL adds a significant scientific resource that strengthens and considerably expands the overall objectives outlined in the TII theme of Microbes and Human Condition. To accomplish its mission, the New England RBL provides state-of-the-art BSL3 laboratories and an A/BSL3 vivarium to conduct innovative biomedical research. The facility is equipped with a highly sophisticated aerobiology suite that is used for nose-only aerosol challenge of pathogens or aerosol delivery of therapeutics, and an insectary for vector-borne disease research. Currently the RBL is designed to house all rodent and invertebrate species including rabbits and ferrets, avian species and germ-free piglets. With slight modifications, small ruminants and other small animals may be housed. The Imaging and Flow Cytometry Core Facility is equipped with a Perkin Elmer IVIS Spectrum, an in vivo imaging system that monitors processes in live animals, a Zeiss LSM 700 confocal laser scanning microscope and a BD LSR Fortessa cell analyzer. This state-of-the-art equipment is complemented by
investigators within the Department of Infectious Disease and Global Health with strong scientific expertise in animal model development, evaluation of therapeutics and international health. Current research at the New England RBL includes studies on select agent bacterial toxins, Bacillus anthracis and Mycobacterium tuberculosis. CDC approval is also available for research on Yersinia pestis, Francisella tularensis and EEE; others can be added as required.
Title:
Novel Bisphosphocin Nu3 Demonstrates Rapid in vitro Killing of Bacteria-Encased in Biofilm

Authors:
Donna E. Akiyoshi, Julia Dilo, Bonnie Marshall, Stuart B. Levy, Paul DiTullio

Presented by:
Donna E. Akiyoshi

Departments:
Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine; Center for Adaptation Genetics and Drug Resistance, School of Medicine; Lakewood-Amedex Inc.

Abstract:

Background: Bacterial biofilms pose a significant treatment challenge to traditional therapies because they enable the bacteria to persist in a dormant or slow growth phase. Biofilms are increasingly becoming associated with chronic infections and implantable device failure as well as resistance of bacteria to conventional antibiotics, thus highlighting the need for more effective therapeutics. Nu3, a member of a novel class of extremely broad-spectrum antimicrobials, was evaluated for its ability to rapidly kill biofilm-encased bacteria in vitro.

Methods: The bactericidal activity of Nu3 against biofilm-encased bacterial strains of Acinetobacter baumannii, Klebsiella pneumoniae, and Staphylococcus epidermidis was evaluated using a time-kill assay to assess the post-antibiotic effect (PAE). Bacteria were grown 24 hours in tryptic soy broth with 1% dextrose (TSBD) in sets of four borosilicate glass tubes to allow formation of the biofilm on the tube wall. The medium was carefully removed and the tubes treated with 170 U/ml Nu3 for 10 min and 30 min, or sterile saline. Following the room temperature incubation, the tubes were washed with sterile saline. TSBD was added to all four tubes and the tubes were incubated at 37°C for 24 hours without shaking. After a 24-hour incubation, the cultures were visually examined for growth and appropriate dilutions were made and aliquots plated in onto TS agar plates to enumerate colonies. Minimum inhibitory concentration (MIC), minimum bacteria concentration (MBC) and time-kill studies against the planktonic forms of these bacteria were also performed to confirm the bactericidal activity of Nu3.

Results: Nu3 exhibited a rapid bactericidal effect on biofilm-encased bacteria with a 100% kill of all four bacterial strains observed at 170 U/ml and exposure time of 10 minutes. These results further support experimental data showing Nu3 is directly bactericidal through a mechanism of action involving depolarization of the cell membrane, which is in contrast to most traditional antibiotics.

Conclusion: Nu3 displays rapid bactericidal activity against both gram-negative and gram-positive biofilm-encased bacteria, highlighting its potential as a new topical antimicrobial therapy for infected wounds or prophylactic treatment prior to surgical closure or implantation of medical devices. This project was supported in part by the Tufts Collaborates! Seed Grant Program, Tufts Provost’s Office.
Title:
Translating Science into Global Ecosystem Health: Establishment of the Department of Infectious Disease and Global Health at the Cummings School of Veterinary Medicine

Authors:
Donna Akiyoshi, Hellen Amuguni, Julie Ellis, Gillian Beamer, Akram Da'darah, Stan Fenwick, Greice Krautz-Peterson, Sangun Lee, Joann Lindenmayer, Jean Muhkerjee, Maureen Murray, Felicia Nutter, Mark Pokras, Alison Robbins, Diafuka Saila-Ngita, Abhineet Sheoran, Chuck Shoemaker, Patrick Skelly, Jennifer Steele, Xingmin Sun, Sam Telford, Flo Tseng, Chris Whittier, Giovanni Widmer, Saul Tzipori

Presented by:
Patrick Skelly

Department:
Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine

Abstract:
Human, animal and ecosystem health are linked and the global well-being of all three is advanced through integrated, translational science and education. The newly created Department of Infectious Disease and Global Health (IDGH) at the Cummings School of Veterinary Medicine is committed to excellence in infectious disease and population health research and wildlife, international and conservation medicine. Through cross-disciplinary, inter-professional partnerships, IDGH faculty have formed productive teams within Tufts, with other universities and with colleagues in resource-poor regions of the world.

Positive outcomes of our efforts include contributions to global infectious disease research, vaccine development and One Health capacity building. The power of combining infectious disease research with wildlife and international medicine has been evident in the work recently accomplished through the US Agency for International Development (USAID)-funded RESPOND project focusing on stemming the emergence of global pandemic threats.

The Department of IDGH offers distinctive educational experiences in classrooms and laboratories, in clinics and in international research venues. At the Doctor of Philosophy (PhD) level, many of our students already hold Doctor of Veterinary Medicine (DVM) degrees and are drawn to the opportunity to address health challenges in a One Health context. An innovative Master of Science in Conservation Medicine program articulates expertise from IDGH with colleagues from across the school and university who seek to address ecosystem health and human and animal well-being. The department also helps support the dual DVM-Master of Public Health (MPH) degree offered as a special track within the School of Medicine’s MPH program.

Creation of this new department has enhanced our ability to address important One Health problems and to drive global health research, education and active citizenship consistent with Tufts traditions.
Title:
Yersinia Interactions with Innate Immune Cells in Different Organ Tissues

Authors:
Michelle Paczosa, Erin Green, Julia Murphy, Francisco Maldonado-Arocho, Hortensia Garcia-Rolan,
Joan Mecsas

Presented by:
Francisco Maldonado-Arocho and Hortensia Garcia-Rolan

Department:
Department of Molecular Biology and Microbiology, School of Medicine

Abstract:
Yersinia pseudotuberculosis (Yptb) is a gram-negative enteric pathogen of humans and animals. It is an extracellular pathogen that uses a type three secretion system (T3SS) to inject effector proteins known as Yops into host cells. These Yops modify host cell responses, and allow the bacteria to inactivate the immune system and cause disease. Our lab has focused on elucidating the cellular and molecular targets of these T3SS effectors in spleens, lymph tissues and lungs, and the dynamic interplay between innate immune cells and Yptb in infected tissues. Recent work has shown that in lymph tissues, spleens and lungs, neutrophils are a key target of Yop injection by Yptb. In the lungs, Yptb uses specific bacterial adhesins to target and subvert neutrophil responses whereas in the spleen, Yptb uses both adhesins and complement to target injection of Yops into neutrophils. To inhibit neutrophils, a specific effector YopH dismantles critical signal transduction networks via dephosphorylation of the adapter proteins SLP-76, PRAM-1 and SKAP-2. Work in our lab has also focused on characterizing the behavior of Yptb in host tissues using microscopic approaches. These studies have confirmed that neutrophils are a primary responder to Yptb infection, and moreover their presence influences microcolony size and the number of Yop-injected cells. Notably we have found that other immune cell types are important for controlling Yptb infection in the absence of neutrophils. Additionally we have taken a genomics approach to discover genes critical for survival under different immune environments during animal infection. Current work involves manipulating Yptb to inject therapeutics into diseased tissues and understanding how other Yops manipulate innate immune cell behavior in infected organs.
Title:
Endosymbiotic Bacteria of Shipworms (Bivalvia: Teredinidae) Secrete Compounds with Anti-Parasitic Activity

Authors:
Roberta O’Connor, Daniel Distel, Sherif Elshahawai, Margo Haygood

Presented by:
Roberta O’Connor

Departments:
Division of Geographic Medicine and Infectious Disease, Tufts Medical Center; Ocean Genome Legacy; Oregon Health and Sciences University;

Abstract:
Owing to the tremendous biodiversity in the oceans, marine bioprospecting, despite its short history, has already yielded many unique molecules with tremendous therapeutic potential. Shipworms are marine bivalves that survive by borrowing into and consuming wood, a lifestyle entirely enabled by their symbiotic bacteria. In terrestrial xylophagous animals, cellulose-digesting symbiotic bacteria reside in the digestive tract in direct contact with the substrate. In stark contrast, the shipworm digestive tract has few bacteria and the caecum, the primary site of wood digestion, lacks any bacterial community. Since digestion of lignocellulosic substrates provides a nutrient-rich environment, the lack of bacteria in this organ suggests the presence of antibiotic activity as well as celluolytic enzymes. Analysis of the genomes of shipworm symbionts reveals a significant commitment to the production of polyketide and non-ribosomal peptide secondary metabolites. The capacity to produce diverse and unique secondary metabolites combined with the demonstrated ability of shipworm symbionts to produce compounds that move through multiple cellular compartments and effect processes distant from their living quarters suggests a reservoir of unusual anti-microbial compounds worthy of investigation. In preliminary studies, we observed that culture supernatant (SN) from symbiont strain-1 inhibited intracellular growth of the apicomplexan parasites, C. parvum and T. gondii, without toxicity to the host cells. In contrast, SN from a closely related strain (symbiont-2) had no effect on parasite burden. Additionally, strain-1 SN, but not strain-2 SN, exhibited activity against two gram+ bacteria. Comparison of strain-1 and strain-2 genomes identified a non-ribosomal peptide synthase locus absent in the inactive strain. Fractionation of strain 1 SN identified two fractions containing anti-parasitic activity and indicated that the source of the activity is a small molecule. Purification of the compound and identification of its target is ongoing. These studies have tremendous potential to open up a new area of anti-parasitic drug discovery, holding the promise of new molecular targets for drug development, potentially with broad application to many pathogens.
Title:
The Role of Environmental Parameters in Characterizing Exposure to Enteric Pathogens in Urban and Rural Settings in South India

Authors:
Alexandra Kulinkina, Vinohar Balraj, M. Venkata Raghava, Elena N. Naumova

Presented by:
Alexandra Kulinkina

Departments:
Department of Civil and Environmental Engineering, School of Engineering; Community Health Department, Christian Medical College

Abstract:
Bacteriological quality of drinking water plays an important role in characterizing the risk of enteric infections. This research conducted in partnership with the Christian Medical College (CMC) in Vellore, India aims to examine the relationship between meteorological parameters, water quality and the rate of enteric infections in three rural villages and two urban slums. 2287 water samples from public taps and households were collected regularly over a 12-month period. Daily meteorological data were obtained from a local station. Harmonic regression models (HRM) adapted to time series data and implemented in R statistical software were used to assess the temporal pattern in water quality (WQ) and the effect of meteorological parameters (ambient temperature, precipitation, and relative humidity) on describing this temporal pattern. The spatial relationships between household and tap WQ were assessed using Geographical Information Systems (GiS). The likelihood of bacteriological contamination increase from tap to household was further modeled as a function of water, sanitation and hygiene (WASH) covariates and meteorological parameters using mixed effects regression models that account for clustering at the household level. Our study found that the physicochemical water quality parameters do exhibit a stable seasonal trend while bacteria are too variable to show seasonality. Overall, HRMs that incorporate meteorological data show promise in the forecasting of seasonal changes to pathogen exposure in drinking water. The mixed effects regression models showed that meteorological parameters have more bearing on the likelihood of bacteriological contamination increase at the household level than do WASH covariates. Further work is suggested to examine this pattern while accounting for storage times and including laboratory controls which may allow for isolating the effect of meteorological parameters from those of WASH covariates and natural growth and attenuation of bacteria. This research demonstrates the utility of routinely collected meteorological data in predicting exposure to enteric pathogens. Meteorological data may play a role in decision making related to drinking water storage practices, especially in low resource settings.
Title:
Bioremediation of Contaminated Aquifers: Engineering Approaches to Facilitate the Degradation of Toxic Pollutants to Benign By-Products Using Subsurface Microorganisms

Authors:
Tyler F. Marcet, Rhiana D. Meade, Kurt D. Pennell, Natalie L. Cápiro

Presented by:
Rhiana D. Meade

Department:
Department of Civil and Environmental Engineering, School of Engineering

Abstract:
Chlorinated solvents such as tetrachloroethene (PCE) and trichloroethene (TCE) are the most widespread groundwater contaminants in the United States, detectable in as many as 34% of drinking water wells across the country and present at 80% of Superfund sites. They can be highly toxic even at low concentrations (drinking water standard for PCE and TCE is 5 ppb), and their high densities and low aqueous solubility make complete remediation difficult. Bioremediation, the use of bacteria to degrade these contaminants, has come to the forefront of subsurface remediation technologies in recent years due to its high versatility, effectiveness, and relatively low cost. Despite these strengths, however, complete biodegradation of chlorinated solvents to benign ethene can be impaired or stalled at an intermediate toxic byproduct (e.g., vinyl chloride) for a number of reasons. Delivery of microorganisms when sufficient quantities are not present in the aquifer (bioaugmentation), as well as substrates critical to their growth (biostimulation) can present an enormous challenge due to subsurface heterogeneities and incomplete contaminant location data. Additionally, consumption of injected substrates by non-target microorganisms can lead to unfavorable physicochemical conditions (i.e., permeability loss due to formation of metal sulfide precipitation), inhibiting delivery of additional amendments and thus, contaminant degradation. Biogeochemical processes are currently being studied in an effort to overcome these limitations and assist site remediation managers design more effective treatment schemes. Further, techniques to address challenges of effective substrate delivery are under investigation; for example, by targeting substrate delivery at the contaminant-water interface (i.e., injection of short-chain fatty acid electron donors), microbes associated with biodegradation of chlorinated solvents may be able to gain a competition advantage over indigenous microbes not associated with the degradation process. Overall, the development of novel techniques for the stimulation and monitoring of bioremediation are critical to the success of ongoing remediation efforts.
Title:
Accuracy and Usability of Free Chlorine Residual Testing Methods

Authors:
Anna Murray and Daniele Lantagne

Presented by:
Anna Murray

Department:
Department of Civil and Environmental Engineering, School of Engineering

Abstract:
Chlorine is the most widely used disinfectant worldwide, in part because residual protection is maintained after chlorination. This residual is measured using colorimetric test kits varying in accuracy, precision, training required, and cost. Seven commercially-available colorimeters, color wheel and test tube comparator kits, pool test kits, and test strips were evaluated for use in resource-limited environments by: 1) measuring in quintuplicate 11 samples from 0.0 – 4.0mg/L free chlorine residual (FCR) in laboratory and natural light settings to determine accuracy and precision; 2) conducting volunteer testing where participants used and evaluated each test kit for usability; and 3) comparing costs. Laboratory accuracy ranged from 5.1% – 40.5% measurement error, with colorimeters the most accurate and one test strip method the least. The only variation between laboratory and natural light readings occurred with one test strip method. Volunteer participants found test strip methods easiest and color wheel methods most difficult, and were most confident in the colorimeter and least confident in test strip methods. Costs range from 3.50 – 444 USD for 100 tests. Ranking test kits by five criteria revealed that colorimeters and test tube comparator kits were most appropriate for use in resource-limited environments, although the ideal appropriate kit may well vary by context.
Title:
CD209a Expression on Dendritic Cells is Critical for the Development of Pathogenic Th17 Cell Responses in Murine Schistosomiasis

Authors:
Holly E. Ponichtera, Mara G. Shainheit, Bridget M. Larkin, Raktima Raychowdhury, Joanne M. Russo, D. Brenda Salantes, Stephen C. Bunnell, Nir Hacohen, Miguel J. Stadecker

Presented by:
Holly E. Ponichtera

Departments:
Departments of Integrative Physiology and Pathobiology and Molecular Biology and Microbiology, School of Medicine; Broad Institute of MIT and Harvard

Abstract:
In murine schistosomiasis, immunopathology and cytokine production in response to schistosome eggs is uneven and strain dependent. Infected CBA mice develop severe hepatic egg-induced granulomatous inflammation associated with prominent Th17 and Th1 cytokine responses, whereas in BL/6 mice milder lesions develop in a Th2-dominant cytokine environment. The pathogenic Th17 response in CBA mice is largely dependent on IL-1β and IL-23 produced by schistosome egg-stimulated dendritic cells (DC); by comparison, this pro-inflammatory cytokine pathway fails to materialize in low-pathology BL/6 mice. The requirements for Th17 cell differentiation induced by CBA DC have been elucidated; however, the reason for strain-dependent difference in APC reactivity to live eggs is not known. Initial gene profiling disclosed a significant difference in C-type lectin receptor (CLR) expression between CBA and BL/6 bone marrow derived DC (BMDC). CLR are pattern recognition receptors capable of binding carbohydrates, including those secreted by schistosome eggs. A dramatic increase in CD209a, a murine homologue of human DC-specific ICAM-3-grabbing non-integrin (DC-SIGN), was documented by real-time PCR and flow cytometry on tissues from infected CBA mice, including liver, spleen and granuloma cells. Functional assays determined that CBA DC, but not macrophages, B cells, or granulocytes, elicit Th17 cell differentiation in response to schistosome eggs. Gene silencing in CBA DC, and over-expression in BL/6 DC, demonstrated CD209a to be essential for IL-1β and IL-23 production and subsequent Th17 cell differentiation. These findings reveal a novel role for CD209a in mediating pathogenic pro-inflammatory Th17 responses in helminthic disease.
Title:
Generation of D-peptide Antibiotics via Mirror-Image Phage Display

Authors:
Emel Adaligil, Kalyani Patil, Krishna Kumar

Presented by:
Emel Adaligil

Department:
Department of Chemistry, School of Arts and Sciences

Abstract:
Vancomycin, a glycopeptide antibiotic is one of the last resort drugs used in the treatment of life threatening hospital infections caused by a resistant strain, such as Methicillin-resistant Staphylococcus Aureus (MRSA). Bacterial resistance to Vancomycin was first observed in 1988 with Vancomycin-resistant Enterococci (VRE); therefore, it is of concern that VRE type resistance can be spread to MRSA that is responsible for lethal infections in immune-compromised patients, such as those suffering from AIDS, and having undergone organ transplants. The aim of this project is to develop D-peptide antibiotics with a mechanism similar to that of Vancomycin via mirror image phage display using the enantiomers of cephalosporin and penicillin as phage display targets as the target to overcome bacterial resistance.
Title:
CD4-CD8- T cells, B cells and Complement are Critical for Resolution of Babesia microti Infection in the Absence of CD4

Authors:
Maude Leveque, Zachary Silver, Carrie Wilson, Jia-Shyuan Su, Sam Telford, Jeffrey Gelfand, Henry Wortis, Peter Krause, Edouard Vannier

Presented by:
Edouard Vannier

Departments:
Division of Geographic Medicine and Infectious Diseases, Tufts Medical Center; Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine; Department of Integrative Physiology and Pathobiology, Tufts University School of Medicine; Division of Infectious Diseases, Massachusetts General Hospital; Yale School of Public Health, Yale University

Abstract:
Babesiosis caused by the protozoan parasite Babesia microti is an emerging infectious disease in the United States. In patients who are immunocompromised, particularly those treated with the B cell depleting antibody rituximab, babesiosis is severe and persistent. To understand how the immunocompromised host clears B. microti, we developed a mouse model. In athymic mice, parasitemia was intense and persisted. In cd4 -/- mice, however, parasitemia was intense and resolved. In the spleens of the latter, the populations of CD8+ T cells, CD4-CD8- (DN) T cells, and B cells expanded. Parasitemia resolved in cd4 -/- cd8 -/- mice, but persisted in cd4 -/- igh6 -/- mice. In cd4 -/- mice, the rise in circulating Babesia specific IgGs was delayed but concomitant with resolution of parasitemia. All IgG subisotypes were produced. Fcg receptors were dispensable for resolution of parasitemia, but complement factor C3 was required. DN T cells that produce both IL-21 and IFN-g expanded in cd4 -/- mice, but not as fast as CD4+ T cells producing both cytokines in wild-type mice. In contrast, DN T cells producing IFN-g in the absence of IL-21 expanded as fast as their CD4+ T cell counterparts. DN T cells failed to produce IL-21 alone whereas CD4+ T cells producing IL-21 alone quickly expanded. In cd4 -/- mice, resolution of infection was not affected by neutralization of IFN-g or IL-21 but was delayed by blockade of both cytokines. Our studies indicate that DN T cells and B cells are key for resolution of B. microti infection in the absence of CD4. Despite the production of IgGs, resolution of infection requires complement activation, but not Fc receptors.
Title: Gluma Antimicrobial Effect on Five Strains of Cariogenic Bacteria

Authors: Minh Bui, Brian Klein, Ronald Perry

Presented by: Minh Bui

Departments: School of Dental Medicine; Sackler School of Graduate Biomedical Sciences, School of Medicine

Abstract:

AIM: Gluma desensitizer is one of many topical agents used to prevent post-operative hypersensitivity. This project aims to see if Gluma could have an additional antimicrobial effect on five strains of common bacteria associated with caries by assessing the zone of inhibition.

METHODS: Three experiments were conducted with prepared plates with strains of Streptococcus mutans (ATCC 25175), Porphyromonas gingivalis (BAA-308), Porphyromonas gingivalis (ATCC 33277), Lactobacillus rhamnosus GG (ATC 53103), Lactococcus lactic (CMB8): Gluma pipetted directly onto plates, impregnating Whatman and Fisher filter paper discs with Gluma then placed on plates, and filling 1μl and 0.5μl Gluma into wells punched out using tips of micropipette tip and a Pasteur pipette tip. Plates were packaged in pouches and incubated anaerobically for at least forty-eight hours then retrieved and zones of inhibitions were assessed and measured. Statistics was not done because the study's aim was to verify the presence or absence of the zones of inhibition.

RESULTS:

<table>
<thead>
<tr>
<th>Strains</th>
<th>Zones of Inhibition (mm) for Gluma pipetted onto plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Mutans</td>
<td>10 11 12 13</td>
</tr>
<tr>
<td>CMB8</td>
<td>9 11 12 12</td>
</tr>
<tr>
<td>Lactobacillus rhamnosus GG (LGG)</td>
<td>8 10 10 11</td>
</tr>
<tr>
<td>Porphyromonas gingivalis (PG) W83</td>
<td>12 13 14 16</td>
</tr>
<tr>
<td>Porphyromonas gingivalis (PG) 33277</td>
<td>5 5 6 10</td>
</tr>
</tbody>
</table>

All five strains demonstrated susceptibility by the zones of inhibition with clear borders of antimicrobial capability up to a certain point where the Gluma could not diffuse out. Results were listed from smallest to largest values.
<table>
<thead>
<tr>
<th>Strains</th>
<th>Zones of inhibition (mm) for Fischer filter paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Mutans</td>
<td>9     10  10  10  11</td>
</tr>
<tr>
<td>CMB8</td>
<td>8     9   9   10  10</td>
</tr>
<tr>
<td>LGG</td>
<td>7     9   9   10  11</td>
</tr>
<tr>
<td>PG W83</td>
<td>9     9   10  10  11</td>
</tr>
<tr>
<td>PG 33277</td>
<td>9     9   10  10  12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strains</th>
<th>Zones of inhibition (mm) for Whatman filter paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Mutans</td>
<td>8     9   10  10  10</td>
</tr>
<tr>
<td>CMB8</td>
<td>5*    10  10  11  13</td>
</tr>
<tr>
<td>LGG</td>
<td>7     9   9   10  10</td>
</tr>
<tr>
<td>PG W83</td>
<td>5*    9   9   10  10</td>
</tr>
<tr>
<td>PG 33277</td>
<td>9     10  12  12  14</td>
</tr>
</tbody>
</table>

All discs but two, notified with the asterisk, demonstrated clear inhibition zones. Fischer filter paper discs created zones of similar diameters, whereas Whatman discs had a larger range. Prepared wells filled with Gluma.

<table>
<thead>
<tr>
<th>Species</th>
<th>Micropipette tip well + 1μl</th>
<th>Micropipette tip well + 0.5μl</th>
<th>Pasteur tip well + 1μl</th>
<th>Pasteur tip well + 0.5μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMB8</td>
<td>19</td>
<td>20</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>LGG</td>
<td>15</td>
<td>15</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>S. Mutans</td>
<td>15</td>
<td>17</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

Plates yielded results with distinct borders only for three species. Zones were bigger for the wells created by the micropipette than the Pasteur pipette.

**CONCLUSIONS:**

From the results of all three experiences, Gluma demonstrated an antimicrobial effect on the bacteria tested regardless of how the desensitizer was used. Based on the promising results, clinician could consider Gluma for the preparations of those patients susceptible to caries in addition to using it only as a desensitizer.
Title:
An Adenovirus Vector Expressing a Single VHH-Based Neutralizing Agent (VNA) Protects Piglets from Fatal Systemic Complications Induced by Infection with Shiga Toxin Producing E. coli

Authors:
Abhineet S. Sheoran, Ocean Cohen, Jean Mukherjee, Jacque M. Tremblay, Michelle Debatis, Gillian Beamer, Igor Dmitriev, David T. Curiel, Charles B. Shoemaker, Saul Tzipori

Presented by:
Abhineet S. Sheoran

Departments:
Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine; Department of Radiation Oncology, Washington University

Abstract:
Infection with Shiga toxin (Stx)-producing Escherichia coli (STEC) is the most significant cause of hemolytic uremic syndrome (HUS), the leading cause of acute renal failure in children. Of the two antigenically distinct toxins, Stx1 and Stx2, Stx2-producing E. coli strains are more frequently associated with HUS than strains that produce both Stx1 and Stx2; while Stx1 alone has rarely been associated with HUS. There is no effective treatment or prophylaxis for HUS available clinically. Human monoclonal (HuMAb) against Stx2, produced by our team were shown to be highly effective when administered after bacterial challenge. The systemic administration of VHH-based neutralizing agents (VNAs) that can be economically produced and target both Shiga toxins may be a superior approach for therapies that prevent or treat STEC sequelae such as HUS. Camelids produce heavy chain-only antibodies and their antigen-binding VH domains (VHH) bind antigens without light chain domain pairing. We recently produced a panel of VHH domains that neutralize Stx1 and/or Stx2 in cell-based assays. Linking two VHHs targeting the same toxin into a single protein results in substantial improvements in neutralization potency compared to the monomer VHH components. We linked three neutralizing VHHs (one specific for each Stx and one cross-specific for both Stxs) to produce one VNA that neutralizes both Stx1 and Stx2 (VNA-Stx1/Stx2) with extreme potency. This VNA is highly protective in mice exposed to lethal doses of either Stx1 or Stx2. VNAs are highly amenable to gene therapy strategies that employ genetic delivery vehicles to promote de novo serum expression of toxin neutralizing activities. We produced a non-replicating adenovirus vector that promotes secretion of functional VNA-Stx1/Stx2 (Ad-VNA-Stx1/Stx2) from infected cells. This virus was parenterally administered to mice 4h prior to Stx2 challenge and protected all mice from fatal intoxication. Furthermore, parenteral injection of the Ad-VNA-Stx1/Stx2 about a day prior to oral STEC challenge protected 5/5 gnotobiotic (GB) piglets from Stx2-mediated neurological complications and death, whereas 0/5 untreated controls survived
the systemic complications. We are currently determining the protective efficacy of Ad-VNA-Stx1/Stx2 in relation to the time of treatment after the onset of diarrhea of STEC infected GB piglets. In conclusion, genetic delivery of VNA-Stx1/Stx2 shows excellent potential to prevent Stx-mediated fatal systemic complications.
Title:
A Broadly Applicable Therapeutic Platform that Employs a Simple VHH-Based Neutralizing Agent (VNA) and a Universal Effector Antibody (efAb) to Replicate the Pathogen Neutralization and Serum Clearance Functions of Polyclonal Antibodies

Authors:

Presented by:
Chuck Shoemaker

Departments:
Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine;
Department of Microbial Pathogenesis, University of Maryland; Laboratory of Bacterial Diseases,
National Institute of Allergy and Infectious Diseases; Wadsworth Center, New York State Department of Health; Department of Radiation Oncology, Washington University

Abstract:
We have developed and successfully tested a novel therapeutic platform that performs two major effector functions of conventional polyclonal antibodies, pathogen neutralization and pathogen serum clearance. Our platform performs both of these functions by administering two simple recombinant proteins. One component is a “VHH-based neutralizing agent”, or VNA, that binds pathogens at two or more unique sites and potently neutralizes their activity. Each VNA is a heteromultimer of camelid heavy-chain-only antibody (Ab) V_H (VHH) binding domains. Linking VHHs into VNAs commonly results in major improvements in pathogen neutralization potency compared to the monomer VHHs. The second component is the “effector Ab” (efAb), a monoclonal Ab that binds to multiple copies of an epitopic tag present on each VNA. Co-administration of the VNA and the efAb results in decoration of the pathogen with multiple mAbs which, in addition to neutralization, promotes rapid pathogen clearance from serum. The same efAb can be used in all therapeutics developed using this platform. Our platform has been successfully used in animal models to protect animals from many different pathogen targets, including: C. difficile infection (CDI); exposure to C. diff toxins TcdA and TcdB; Shiga toxin-producing E. coli (STEC) infection; exposure to Shiga toxins Stx1 and Stx2; exposure to two serotypes of Botulinum neurotoxin (BoNT); exposure to ricin, and; exposure to anthrax. In some animal models, employing the VNA alone has proven fully protective in the absence of efAb. VNAs containing up to four linked VHHs have
successfully been used to treat exposures to two different pathogens with a single, easily-produced protein. Genetic delivery using a single treatment with an adenovirus that promotes in vivo VNA expression protects mice from lethal BoNT/A challenge for more than two months and protects gnotobiotic pigs from the lethal sequela of STEC infection. Use of this platform should permit rapid development of economic therapeutics for virtually all pathogens responsive to antibody treatments, including many microbial and viral pathogens. The resulting products should have much improved safety and shelf-life properties compared to conventional serum-based products. Genetic delivery options should permit protection from multiple pathogen risks for long periods with a single treatment and make possible oral delivery for treating enteric diseases.
Title:
Identification, Characterization, and Cloning of Polypeptide N-acetylgalactosaminyl Transferase 4 of Cryptosporidium parvum

Authors:
Maria DeCicco and Honorine Ward

Presented by:
Maria DeCicco

Department:
Department of Immunology, School of Medicine; Division of Geographic Medicine and Infectious Diseases, Tufts Medical Center

Abstract:
Cryptosporidium is an apicomplexan parasite of global importance that causes diarrheal disease in humans and animals. Two main species account for 90% of all human cases of cryptosporidiosis, C. hominis (Ch) affecting humans and the zoonotic C. parvum (Cp) affecting both humans and other mammals. Immunocompromised individuals such as AIDS patients and malnourished children experience the most severe disease, but to date, there are no effective therapies or vaccines that exist to treat or prevent cryptosporidiosis in these patients. Nitazoxanide, a drug with wide antiparasitic properties is the only drug approved for treatment in immunocompetent individuals. Several proteins involved in attachment and invasion are mucin-like O-glycoproteins, which are surface proteins or are secreted from the apical complex of the sporozoite during gliding motility. Gp40, a glycoprotein that our lab has studied, has been shown to elicit an immune response in animal models and infected humans. A monoclonal antibody 4E9 as well as IgG from infected patients recognize αGalNAc glycopeptides derived from gp40, but not their non-glycosylated equivalents. In addition, αGalNAc has been shown to be indispensable for the function of the glycoproteins that contain this glycan as αGalNAc specific lectins and antibodies block attachment and disrupt sporozoite infectivity.

My project is focused on the family of enzymes responsible for catalyzing the first step of mucin-like O-glycosylation, the polypeptide α-N-acetylgalactosaminyl transferases (ppGalNAc-Ts). ppGalNAc-Ts are found in many organisms from Drosophila and C. elegans to mice and humans and have largely conserved domains and motifs. There are twenty ppGalNAc-Ts that have been identified in humans, and to date, the only ppGalNAc-Ts that have been identified in protozoa are in Apicomplexans including Toxoplasma, Cryptosporidium, Eimeria, and Neospora. Previously we have cloned, expressed and characterized Cp-ppGalNAc-T1.
Ongoing work is focused on characterizing Cp-ppGalNAc-T4 and expressing the recombinant protein in a mammalian expression system. The recombinant protein will be used to study enzymatic activity, identify endogenous substrates, and further understand the role of Cp-ppGalNAc-T4 in infection.

Our long-term goal is to determine if effective interventions can be developed, by targeting the enzymes that are responsible for glycosylating the crucial attachment and invasion glycoproteins. The overall objective of my project is to characterize Cp-ppGalNAc-T4 and identify specific inhibitors of Cp-ppGalNAc-T1 and T4 that can inhibit both enzymatic activity and O-glycosylation and subsequently block Cryptosporidium infection in vitro. Thus far, I have identified the complete sequence of Cp-ppGalNAc T4 and have characterized the protein in silico.
Title:
Identification of New Therapeutic Targets for Schistosomiasis using RNAi

Authors:
Akram Da’darah, Greice Krautz-Peterson, Qiang Wang, Patrick Skelly

Presented by:
Akram Da’darah

Department:
Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine

Abstract:
Schistosomes are parasitic platyhelminths (blood flukes) that can cause a chronic, often debilitating, disease called schistosomiasis that affects several hundred million people around the world. Infection is water-borne; free-swimming larval forms penetrate the skin and invade the vasculature of their hosts. In the laboratory, we focus on the molecular and cellular biology of the schistosome outer covering (the tegument). This surface constitutes a major site of host-parasite interaction and molecules making up this surface are likely accessible therapeutic targets.

Among the surface molecules identified in the parasite surface membranes are a collection of enzymes which, we hypothesize, act to impair host immune signaling and hemostasis. Using RNA interference (RNAi) to suppress the expression of genes encoding these surface enzymes, we have shown that one, ecto-ATP diphosphohydrolase 1 (SmATPDase1), can cleave the pro-inflammatory DAMP ATP and a second, alkaline phosphatase (SmAP), can generate the potent anti-inflammatory mediator, adenosine. Another host-interactive schistosome tegumental enzyme, the phosphodiesterase SmNPP-5, is rapidly upregulated following invasion of the definitive host. Suppressing the expression of the gene encoding SmNPP-5 greatly impairs the ability of schistosomula to establish infection. Thus SmNPP-5 can be considered a virulence factor for schistosomes.

Other host-interactive enzymes include a carbonic anhydrase (SmCA) which likely functions to regulate pH homeostasis and the transport of CO₂, and an acetylcholinesterase (SmAChE) which may impact host vascular physiology. Suppressing the expression of these genes too impairs parasite infectivity. This work has identified that these are essential molecules for normal parasite development and are therefore important therapeutic targets. We have begun drug screens to identify chemicals that can impede the function of these surface proteins (to mimic the RNAi effect) and debilitate the worms.

In addition our work is designed to generate a comprehensive understanding of the role of these surface proteins in promoting parasite survival by controlling the biochemistry of their immediate external environment.
Title:
Neutrophils in Pulmonary Tuberculosis

Authors:
Samuel Major and Gillian Beamer

Presented by:
Gillian Beamer

Departments:
Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine

Abstract:
Tuberculosis (TB), due to Mycobacterium tuberculosis (M.tb) remains a substantial global health problem. TB kills 1-2 million people each year and is newly diagnosed in 8-9 million more patients. Severe immune deficiencies (genetic or acquired) are known risk factors for TB. However, most TB (80%) occurs in patients that are immunologically responsive to M.tb antigens and disease manifests specifically in the lungs. This discrepancy in patient profiles indicates that we do not know how disease develops in most TB patients. It is important to understand because these patients are sick and are contagious to others. Growing evidence shows that lung-damaging inflammation is associated with neutrophils and may contribute to pulmonary TB. However, discovering roles for neutrophils in TB has been hampered by two limitations. First, there have been technical challenges in generating sufficient numbers of unactivated neutrophils for in vitro and in vivo testing. Second, there may be a disconnect between the clinical situation and the experimental models: Human studies typically focus on sick patients with active TB while experimental studies typically focus on neutrophils prior to or during early M.tb infection when mice are not sick, or use immune deficient hosts, or derive data from gene association studies. Here, we show that the mouse experimental TB disease model can recapitulate features of pulmonary TB in humans, and that neutrophils are specifically associated with disease in mice. We can now produce ex vivo normal neutrophils that will be used in future studies to understand the mechanisms by which neutrophils alter immunity, or contribute to lung damage.
Title:
The Intestinal Microbiota, Microbial Translocation and Inflammation in Chronic HIV Infection

Authors:
Duy Dinh, Gretchen Volpe, Chad Duffalo, Anne Kane, Christine Wanke, Honorine Ward

Presented by:
Duy Dinh

Department:
Division of Geographic Medicine and Infectious Diseases, Tufts Medical Center

Abstract:
In the current era of effective antiretroviral therapy (ART), HIV infection has become a chronic disease. Non-infectious complications such as cardiovascular disease, diabetes, metabolic syndrome, obesity and accelerated aging, all associated with chronic inflammation, are being seen with increased frequency in these patients. While ART is successful at suppressing viral replication to below the limits of detection in blood, there is concern that ongoing unexplained immune activation and inflammation are leading to these adverse outcomes. However, the specific mechanisms underlying persistent immune activation and chronic inflammation in these patients are not known.

The gut microbiota is critical for maintaining intestinal homeostasis and is known to play vital roles in mucosal barrier function and modulation of immune and inflammatory responses. Dysbiosis (an imbalance in the composition of the microbiota) has been implicated in the pathogenesis of chronic inflammatory conditions such as inflammatory bowel disease, diabetes and obesity (Brenchley and Douek, 2012). However, the role of the gut microbiota in persistent immune activation and inflammation in individuals with chronic HIV infection on suppressive ART has not been explored. Our hypothesis is that inflammation in these individuals is a consequence of microbial translocation across the gut due to intestinal dysbiosis. The goal of this pilot study was to determine if there is an association between intestinal dysbiosis and microbial translocation and systemic inflammation in individuals with chronic HIV-infection on suppressive ART.
Title: The Expression of Protective Capsular Polysaccharides by the Opportunistic Pathogen A. baumannii Responds Phenotypically to Antibiotic Stress

Authors: Eddie Geisinger and Ralph R. Isberg

Presented by: Eddie Geisinger

Department: Department of Molecular Biology and Microbiology, School of Medicine

Abstract: Nosocomial infections with multidrug-resistant bacteria raise morbidity and mortality in hospitalized patients and pose an increasing challenge to clinicians. The most common agents of nosocomial infections are successful because they can withstand the antimicrobial effects of both the host innate immune system and antibiotic treatment. How bacterial defense phenotypes are modulated by host and nosocomial factors such as antibiotics is poorly understood, although such information may lead to novel approaches to treat nosocomial infections. Here we demonstrate that the opportunistic pathogen Acinetobacter baumannii reversibly augments expression of its key virulence determinant, capsular polysaccharide, upon antibiotic-induced stress. We have identified multiple novel regulatory loci in the bacterium that control basal- and stress-induced capsular polysaccharide expression. In addition, we have developed mouse and tissue-culture models to interrogate the roles of stress-responsive capsular polysaccharides in lung infection and phagocyte interactions.
Title:
Phenotypic Specialization of Y. pseudotuberculosis within Microcolonies

Authors:
Kimberly M. Davis and Ralph R. Isberg

Presented by:
Ralph R. Isberg

Department:
Department of Molecular Biology and Microbiology, School of Medicine

Abstract:
Many enteric bacterial pathogens can spread from the intestines to deep tissue sites, where infections become difficult to treat with antibiotics. Yersinia pseudotuberculosis is an example of an enteric pathogen that spreads to deep tissue sites, such as the spleen, where it replicates to high numbers despite the recruitment of host phagocytes. Within the spleen, bacteria replicate extracellularly to form clusters, called microcolonies. Bacteria around the periphery of microcolonies come into direct contact with neutrophils, and must prevent phagocytosis and extracellular killing, however interior bacteria only contact other bacteria. We hypothesize this drives phenotypic specialization of individual bacteria, based on their spatial location relative to host cells. These studies focused on the bacterial nitrogen stress response, and the production of host nitric oxide. We utilized bacterial fluorescent reporter constructs to visualize expression of the nitric oxide detoxifying gene, hmp, at the single cell level. Fluorescence microscopy of infected tissues was used to visualize reporter expression. Bacteria located around the periphery of microcolonies responded to nitrogen stress by expressing high levels of hmp. In contrast, hmp expression was not detected from interior bacteria, indicating peripheral bacteria may effectively eliminate the nitric oxide gradient in tissue. hmp expression by peripheral bacteria promoted bacterial survival within tissue sites and impacted virulence, based on experiments with a Δhmp strain. Immunofluorescence microscopy was used to visualize the location of nitric oxide-producing host cells relative to microcolonies. Neutrophils in contact with the microcolony do not express nitric oxide, instead this is produced from host cells distant from the microcolony. Contact between host cells and peripheral bacteria also led to heightened virulence factor expression (YopE), relative to interior bacteria. Yop expression is required for virulence in the spleen, and high expression of Yop genes may also define peripheral bacteria. These studies identify the existence of multiple subpopulations of bacteria present within a single microcolony, and also suggest cooperative behavior must be established to support bacterial replication within tissue sites.
Title:
Host Cell Contribution to Type III Secretion in Yersinia pseudotuberculosis

Authors:
Kerri-Lynn Sheahan and Ralph R. Isberg

Presented by:
Kerr-Lynn Sheahan

Department:
Department of Molecular Biology and Microbiology, School of Medicine

Abstract:
Type III secretion (T3S) is a highly conserved effector delivery mechanism in many different Gram-negative bacteria including Yersinia pseudotuberculosis. Previous studies of Yersinia spp. type III secretion have focused on characterization of 6 Yop (Yersinia outer proteins) effectors and the components of the translocation apparatus. However, the role of the host cell in Yop effector translocation, cellular trafficking and subsequent localization with target is unknown. To investigate the contribution of the host cell to T3S, we have designed a pooled RNAi screen to discover host genes required for the cytotoxic effects associated with the Yersinia translocated substrate YopE, a GTPase activating protein that inactivates the small Rho GTPases. Fluorescence Resonance Energy Transfer (FRET) of a Rho GTPase Biosensor can measure the efficiency of RhoA inactivation by YopE in RNAi depleted cells challenged with Y. pseudotuberculosis. RNAi knockdown of host cell genes critical for YopE intoxication should block YopE inactivation of the Rho GTPases. Therefore, cells maintaining an active Rho GTPase after incubation with YopE can be positively selected by fluorescence activated cell sorting (FACS) in order to identify the candidate genes disrupting YopE cytotoxicity. Flow cytometry experiments confirm that the Rho Biosensor accurately measures the activation state of RhoA as shown by analyzing genetic point mutations or following incubation with a Y. pseudotuberculosis strain secreting just YopE. As a proof of principal, we demonstrate that FRET positive cells can be enriched from a mixed population of cells. Candidate genes are identified as shRNAs enriched in the FRET positive cells versus input cells as determined by Illumina sequencing. Overall, we have performed a unique screen to elucidate the role of host cell factors in T3S of Y. pseudotuberculosis. The results obtained from this screen should provide a breadth of new information pertaining to T3S in Yersinia spp., which can also be extrapolated to T3S systems in many other bacteria.
Title:
Subversion of the Host Cell Cycle by Legionella pneumophila

Authors:
Dennise A. De Jesús Díaz and Ralph R. Isberg

Presented by:
Dennise A. De Jesús Díaz

Department:
Department of Molecular Biology and Microbiology, School of Medicine

Abstract:
The ubiquitous Gram-negative pathogen Legionella pneumophila modulates endocytic and immune host processes to ensure formation of the vacuole compartment where it replicates intracellularly. However, we hypothesize that, as an intracellular pathogen, L. pneumophila would exploit additional host networks to replicate. Analyses of host cell cycle dynamics upon Legionella infection revealed that L. pneumophila infected cells couldn’t progress through the cell cycle independently of the cell cycle stage the host cell is at the time of infection. In order to prevent host cells from cycling, Legionella requires the presence of a functional Type 4 Secretion System, as cells infected with a L. pneumophila mutant defective for translocation have the ability to progress through the cell cycle. Interesting, Legionella intracellular growth is enhanced in host cells present at either G1 or G2 of the host cell cycle, but reduced in cells present in S-phase. Our results demonstrate that L.pneumophila preferentially infects growth-arrested hosts because S-phase of the host cell cycle is an unstable environment for bacteria replication; the Legionella containing vacuole is permeable at this stage allowing the detection of bacteria from the cytosol. Thus, by stopping cell cycle progression L. pneumophila prevents detrimental consequences and preserves its ability to replicate.
Title:
Malaria Heat Shock Protein 101 (HSP101) is a Substrate of Plasmodium falciparum Signal Peptide Peptidase; Identification of a Novel Therapeutic Target

Authors:
Michael Baldwin and Athar Chishti

Presented by:
Michael Baldwin

Department:
Department of Integrative Physiology and Pathobiology, School of Medicine

Abstract:
Previously we described the identification of a Plasmodium falciparum signal peptide peptidase (PfSPP), thought to localize to the micronemes and apical regions, where it plays a functional role at the blood stage of malaria infection. This single gene encoded enzyme, with no other paralogs, is conserved in other apicomplexan parasites, and expressed during the merozoite, gametocyte, and sporozoite stages of the malaria parasite life cycle. Our previous studies demonstrated the pharmacological inhibitors of mammalian SPP prevent malaria parasite growth at the late-ring/early trophozoite stage of intraerythrocytic development. Eukaryotic SPPs are generally expressed in the endoplasmic reticulum (ER), and play an important role in development. Consistent with its role in development, PfSPP functions at the ER in Plasmodium falciparum where it cleaves membrane-bound signal peptides, generated following the activity of signal peptidase. We co-localized PfSPP to the ER with a known ER-marker using immunofluorescence microscopy, and confirmed its ER localization by immunogold electron microscopy. An antibody raised against the C-terminus of PfSPP enabled its isolation from parasite lysate, and demonstrated its existence as both a monomer and dimer. We performed a bioinformatics screen and identified several candidate PfSPP substrates in the parasite genome. Using an established transfection-based luminescence assay, we demonstrate that malaria heat shock protein 101 (HSP101) functions as a substrate of PfSPP. This finding reveals the first known substrate of PfSPP, and may provide a novel pharmacological target for use in a multi-targeted antimalarial therapy.
Title:
Single Molecule Assays for Early Breast Cancer Detection

Authors:
Shazia Baig, Stephanie M. Schubert, David R. Walt

Presented by:
Shazia Baig and Stephanie M. Schubert

Department:
Department of Chemistry, School of Arts and Sciences

Abstract:
The goal of this project is to use single molecule detection of proteins to develop a fingerprint for early detection of breast cancer. Protein biomarkers that have the potential to indicate earlier diseased states likely exist in serum at concentrations currently below the detectable limits of standard methods, such as ELISA. The sensitivity of the previously established single molecule array (SiMoA) detection platform employs a digital counting methodology, enabling the detection of very low concentrations of proteins in bodily fluids, such as blood. This approach facilitates the development of a simple, minimally invasive sampling method that could provide biological information about a newly formed tumor. As such, SiMoA could be used to not only detect, but also help with diagnostic and prognostic analysis of breast cancer. This work outlines efforts to develop single molecule assays for biomarkers of interest in breast cancer as well as preliminary results from mouse models.
Title:
A Novel Regenerative Therapeutic Strategy to Reverse Advanced Heart Failure in Chronic Chagas Disease and Other Cardiomyopathies

Authors:
Ryan Salvador, Daniel Aridgides, Mercio Perrin

Presented by:
Ryan Salvador

Department:
Department of Developmental, Molecular and Chemical Biology, School of Medicine

Abstract:
Development of regenerative therapeutic strategies to reverse the progression of advanced heart failure is a most urgent clinical need in this century. Trypanosoma cruzi is a protozoan parasite that causes chronic cardiomyopathy in millions of people worldwide, estimated to cost about $7.19 billion per year. The only cure for the cardiomyopathy, characterized by intense inflammation and fibrosis, is hearts transplant, but this therapy is impractical due to the extreme difficulty in securing heart for transplant. However, cardiomyopathy occurs in only 30% infected individuals as the other 70% remain asymptomatic and show no cardiac pathology, implying the existence of strong regenerative mechanisms in Chagas disease progression. We have identified in T cruzi itself a surface molecule that potently promotes renewal of cardiac progenitor/stem cells (CPCs) ex vivo, concomitantly inducing secretion of anti-inflammatories, particularly TSG-6. Intravenous administration of a recombinant form of this unique CPC renewal and anti-inflammatory stimulating factor in mice bearing chronic Chagas cardiomyopathy dramatically reverses cardiac inflammation, inflammatory cytokines and fibrosis. These results suggest 1) that the T cruzi-modeled CPC renewal factor is a mechanism underlying pathology-free progression in Chagas disease progression, and 2) a novel regenerative therapeutic opportunity to reverse heart failure not only in Chagas but also in other cardiac diseases such as postmyocardial infarction.
Title: SiMoAs for the Ultra-Sensitive Detection of the Host Immune Response to Microbial Infections

Authors: Shonda Gaylord, Danlu Wu, Trinh Dinh, Milena Milutinovic, Sama Abdul-Aziz, David R. Walt

Presented by: Shonda Gaylord and Danlu Wu

Departments: Department of Chemistry Department, School of Arts and Sciences; Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine

Abstract: Advances in diagnostic sensitivity, enabling reliable diagnosis in the acute stage of infection, can aid in clinical precision, patient care, disease control and prevention, and outbreak surveillance. The potential for early diagnosis of infections relies on the ability to detect ultra-low concentrations of circulating pathogens or related biomarkers in clinical samples. Both cytokines and immunoglobulins are secreted by the host immune system in response to a foreign agent and can be used as diagnostic markers of infection. Sub-femtomolar detection of prostate specific antigen (PSA), botulinum toxin, HIV p24 protein, and bacterial genomic DNA has recently been demonstrated by capturing single protein molecules or fragmented DNA onto magnetic beads using specific capture agents. These assays, coined single-molecule arrays (SiMoAs), are identical to conventional sandwich and indirect ELISAs in that they use beta-galactosidase as a reporter enzyme. Each magnetic bead isolated into individual 46-fl reaction wells on a 50,000 microwell array in the presence of substrate, which generates a high local concentration of fluorescent product if the bead carries a labeled immunocomplex. This technique provides ultra-sensitive detection technology with sub-milliliter clinical sample volumes.

Here we present the development and application of SiMoAs for nine cytokines and IgG/IgM for dengue infection. We are able to detect the target cytokines at sub-femtomolar concentrations, i.e., 200- to 1000-fold more sensitive than conventional ELISA technology. Currently, six previously undetectable cytokines including GM-CSF, TNF-alpha, IFN-gamma, IL-2, IL-4 and IL-10 are detectable in healthy human serum samples using the SiMoAs. We are also able to detect dengue immunoglobulin in samples, positive for DEN-1 infection by qRT-PCR, but negative using conventional ELISAs. Our findings demonstrate how the ultra-sensitivity provided by SiMoAs can be used for the early diagnosis of infectious disease, more specifically, for the presence of biomarkers that were previously undetectable. This research may allow for the identification of different infectious diseases by observing unique cytokine fingerprints. It may reduce the “transient window period” in diagnosing acute infections using serological assays.
Title:
Combinatorial-Designed Lipid Nanoparticles for Intracellular Delivery of Biomacromolecular Therapeutics

Authors:
Ming Wang and Qiaobing Xu

Presented by:
Ming Wang

Department:
Department of Biomedical Engineering, School of Engineering

Abstract:
The clinical success of intracellularly targeted biomacromolecular therapeutics has been limited by poor stability, as well as the impermeability of cell membrane to drugs. An efficient and safe tool to deliver these biotherapeutics into the cytosol of targeted cells are highly desirable. We developed libraries of cationic lipid-based nanoparticles (termed “lipidoid”) for the intracellular delivery of various gene drugs (e.g. DNA, mRNA, and siRNA), as well as cytotoxic proteins. An efficient delivery of therapeutic siRNA or cytotoxic proteins into cancer cells inhibits cell proliferation in vitro and suppresses tumor growth in a murine breast cancer model. Moreover, the combinatorial approach in developing these nanoparticles allows the structure-function relationships study of lipid nanoparticles based intracellular delivery.
Title:
Versatile Substrates and Probes for IgA1 Protease Activity

Authors:
Santosh Choudary and Joshua Kritzer

Presented by:
Santosh Choudary

Department:
Department of Chemistry, School of Arts and Sciences

Abstract:
Bacterial meningitis is an often-fatal disease caused by diverse Gram-negative and Gram-positive bacteria, including Haemophilus influenzae, Neisseria meningitidis and Streptococcus pneumoniae. Though several virulence factors associated with the bacterial pathogenesis have been identified, the specific roles of many of these proteins are not yet well understood. One such protein, IgA1 protease (IgAP) is over-produced by meningitis-causing bacteria. IgAP exclusively cleaves the hinge region of the major immunoglobulin on mucosal surfaces, IgA1. It has been hypothesized that the proteolysis of IgA1 helps in immune suppression and immune evasion by the bacteria. Recent literature shows that IgAP also cleaves host proteins involved in extrinsic apoptosis and lysosomal trafficking, providing additional evidence to support IgAP as a virulence factor. The lack of IgAP inhibitors has hindered research on IgAPs and their roles in bacterial virulence. In our current work, we identified peptide sequences that are self-cleaved by membrane-bound IgAPs during secretion from N. meningitidis and H. influenzae IgAPs. These peptides were synthesized, and tested as substrates for IgAPs from diverse human pathogens. A single peptide, NG2, was found to be a surprisingly common substrate for all IgAPs tested. A Förster resonance energy transfer (FRET) probe, F2.2, was synthesized based on this peptide. High-throughput assays using probe F2.2 were also developed in anticipation of the first screening effort for the discovery of small-molecule inhibitors of IgAPs. By developing novel probes and inhibitors, we will be able to explore the roles of IgAPs in adherence, colonization and infection of epithelial cells by these pathogens, and determine the value of inhibiting IgAP as part of a combination antibacterial strategy. This probe was also used to demonstrate sensitive IgAP detection in buffer and in human cerebrospinal fluid, which may be useful in developing a diagnostic assay for rapid diagnosis of bacterial meningitis.
Title:
A Novel Chimeric Vaccine against Clostridium difficile Infection

Authors:
Xingmin Sun, Yuankai Wang, Weijia Nie, Hyeun Bum Kim, Diane Schmidt, Saul Tzipori

Presented by:
Xingmin Sun

Department:
Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine

Abstract:
Not Available
Title:  
A VHH-based Neutralizing Agent (VNA) Targeting Both TcdA and TcdB Toxins Protects Mice and Gnotobiotic Piglets from the Pathology of Clostridium Difficile Infection

Authors:  
Diane J. Schmidt; Jacqueline M. Tremblay; Xingmin Sun; Hyeun Kim; Weilong Liu; Yuankai Wang; Lianfa Shi; Abhineet Sheoran; Hanping Feng; Charles B. Shoemaker; Saul Tzipori

Presented by:  
Diane J. Schmidt

Department:  
Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine

Abstract:  
Clostridium difficile infection (CDI) is one of the leading causes of nosocomial infection worldwide and is becoming an increasing problem as a community acquired infection as well. Symptoms of CDI range from mild diarrhea, pseudomembranous colitis, toxic megacolon, to organ failure and death. The emergence of antibiotic resistant strains and the increase in relapse or recurrence have complicated treatment of the disease and increased hospital stays, morbidity, and mortality among patients. Therefore it is critical to develop new therapeutic treatments for this disease.

We immunized alpacas with atoxic mutant toxin A or B antigens (the two major virulence factors of CDI). B cells were isolated from the immune alpaca and phage display libraries were generated displaying heavy-chain-only Ab VH (VHH) domains. The libraries were panned against native TcdA or TcdB toxins yielding about 20 unique, toxin-binding VHHs. VHHs were identified that recognize each of the major toxin domains on TcdA and TcdB, including the glucosyltransferase, transmembrane and receptor binding domains. These VHHs were tested in a cytotoxicity neutralization assay and approximately half neutralized toxin activity in vitro. Several VHH heterodimers were produced in which two neutralizing VHHs were expressed as a single protein separated by a flexible spacer peptide. These heteromultimeric VHH-based neutralizing agents (called VNAs) were generally found to have substantially enhanced toxin neutralization potency in cell assays compared to a pool of the component monomer VHHs. To create a single VNA capable of neutralizing both TcdA and TcdB, a VHH heterotetramer (ABBA) was produced containing the two most potent neutralizing VHHs to each toxin. ABBA was expressed and purified and shown to neutralize both TcdA and TcdB with high potency. ABBA has now been tested as a therapeutic agent in animals and demonstrated excellent efficacy in preventing disease symptoms in both mouse and gnotobiotic piglet models of CDI.
Title:
Tufts Comparative Pathology Services and Animal Histology Core

Author:
Lauren Richey

Presented by:
Lauren Richey

Department:
Division of Laboratory Animal Medicine (DLAM), Tufts University

Abstract:
The Tufts Comparative Pathology Service (CPS) and Animal Histology Core (AHC) are shared resources for animal researchers across the Tufts and Tufts Medical Center research community. Our team of professionals includes a board certified veterinary pathologist specializing in comparative pathology, two certified histologists, and a certified laboratory animal technologist trained in veterinary pathology. The CPS and AHC provide training in anatomy, tissue collection, preparation of tissues for histology, and evaluation of tissue for pathology. We provide histology and pathology expertise during project design and budget development, experimental disease studies, phenotyping of genetically engineered mutant animals, toxicology studies, and unexpected morbidity or mortality events. Histology services for animal tissues include research immunohistochemistry, cryosectioning, special stains, and rodent embryonic and fetal sectioning. Tufts CPS and AHC consults and services can be utilized on any project involving normal or abnormal animal tissues to save investigators time and money, while benefitting from the tips, experience, and knowledge of our staff. This specialized resource combines technical expertise, broad-based comparative anatomy and pathology knowledge, project design, specialized instruments and equipment, and quality assurance to fill just a few project requests or to assist more intensively as needed.