Ivy Hurwitz, PhD
Research Assistant Professor
Center for Global Health
Department of Internal Medicine MSC10 5550
Univ. of New Mexico School of Medicine
915 Camino de Salud; IDTC #3130
Albuquerque, NM 87131, USA
Phone: 505-272-5969
Email: IHurwitz@salud.unm.edu

Biography: Dr. Hurwitz received her PhD from the University of Kansas Medical Center. She completed her post-doctoral training at with Dr. Steve Leeder at the Childrens' Mercy Hospital in Kansas City, MO and Dr. Nancy Cooke at the University of Pennsylvania. She joined Dr. Ravi Durvasula's lab in 1996.

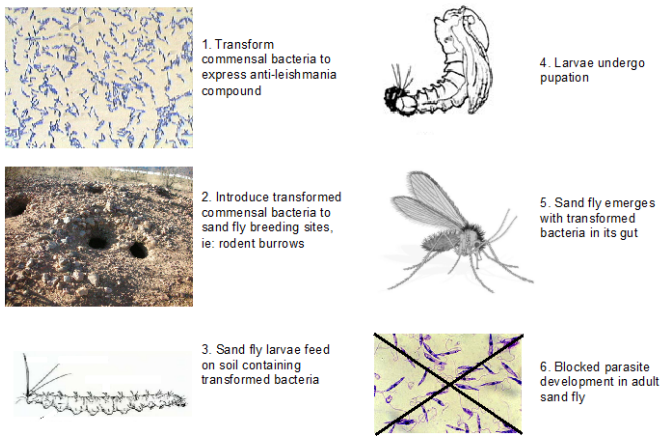
Academic and Research Interests:

Paratransgenesis

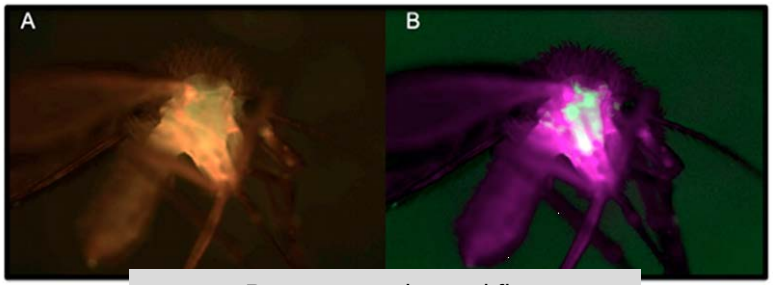
The paratransgenic approach was developed by the Durvasula lab over 15 years ago as a novel method to control vectorial transmission of infectious disease. In this approach, commensal or symbiotic bacteria of an insect or animal that are located in proximity to sites of pathogen transmission are isolated and genetically altered to produce molecules that kill the pathogen itself. Delivery of the transformed bacteria, usually at mucosal sites of pathogen transmission, disrupts the cycle of the infective organism and abrogates the disease process. Over the past several years, I have been adapting this methodology to control a number of infectious diseases.

Leishmaniasis

Leishmaniasis is caused by the protozoan *Leishmania* and is transmitted by the Phlebotomine sand fly. This disease is a leading cause of mortality in the world with close to 12 million people infected in over 80 countries, making it the second most important vector-borne parasitic diseases after malaria. There are no vaccines available and the best methods for control involve the use of chemical pesticides. However, environmental toxicity, adverse effects on human health, and the emergence of insect resistance have greatly undermined their efficacy. One focus of my research is to develop a paratransgenic approach to control this vector-borne disease. This strategy is built on the central hypothesis that aerobic bacteria isolated from the gut of the female adult sand fly can be genetically transformed to express molecules that render the fly refractory to *Leishmania* infection. In earlier work, we had identified *Bacillus subtilis* as a commensal microbe in field caught sand flies. Utilizing a modified expressing system, we engineered *B. subtilis* to express green fluorescence protein (GFP). When added

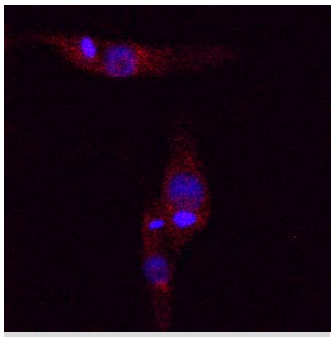


Paratransgenic strategy to control transmission of leishmaniasis



Paratransgenic sand fly

to soil-stage fourth instar sand fly larvae, we found that GFP-expressing *B. subtilis* establishes itself within the larvae and pupae stages. Further, there was evidence of transstadial passage to adult flies.

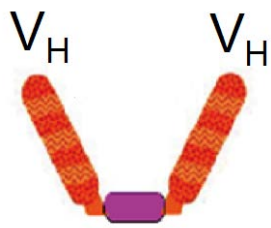


Binding of a single chain antibody to surface epitopes of *L. mexicana*

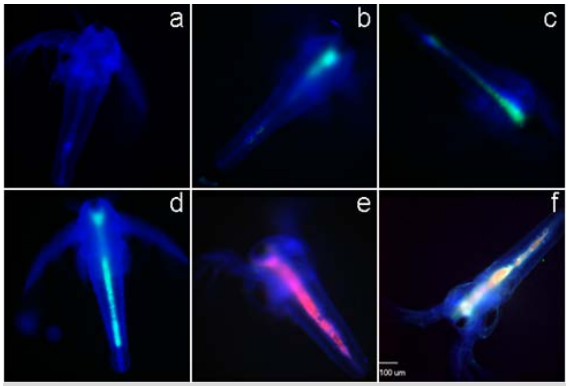
Current work on this project is focus primarily on the identification and development of anti- leishmania "effector" molecules for expression in *B. subtilis* and to validate the paratransgenic approach to control vectorial transmission of Leishmaniasis under laboratory conditions. Ongoing research includes 1) development of highly specific single chain antibodies that target surface epitopes of *Leishmania*, with the aim of blocking parasite attachment and/or development in the gut of the sand fly, 2) elucidation as to whether single chain antibodies with inherent light-induced cytotoxic properties can be utilized as effector molecules, and 3) development of an effective expression system for the anti-microbial peptides, specifically melittin, which has very potent anti-leishmania properties, in *B. subtilis.*

<u>*Clostridium difficile* infections</u>

C. difficile is an anaerobic Gram-positive spore-forming Firmicute. Nosocomial infection occurs when patients experience a reduction in their gut microbiota following antibiotic treatments. The current cost associated with *C. difficile* infections (CDI) is estimated to exceed $1 billion annually. Patients suffering from CDI are most often treated with metronidazole or vancomycin. However, strains of *C. difficile* strains that are resistant to these front-line antibiotics have emerged. This is further compounded by the appearance of hypervirulent strains of *C. difficile*, such as the NAP1/027 isolate, which not only overexpresses *C. difficile* toxin A (TcdA) and toxin B (TcdB), but also demonstrates high-level resistance to fluoroquinolone. TcdA and TcdB are the major virulence factors for *C. difficile*. There is currently no acute CDI treatment that targets TcdA and TcdB. I am currently developing a suite of TcdA and TcdB neutralizing recombinant antibodies (rAbs) for expression in the probiotic *Lactococcus lactis* as a novel non-antibiotic based therapeutic and prophylactic approach to treat CDI. Ongoing research on this project includes 1) biochemical characterization of selected TcdA rAbs for neutralizing activities, 2) validating the functionality of selected TcdA rAbs in *in vitro* and *in vivo* assays and 3) development of a CRISPR/Cas platform for stable and constitutive expression of TcdA rAbs in *L. lactis.*



Recombinant antibody consisting of two variable heavy domains linked together by a short linker



Fluorescence micrographs of *Artemia* fed with *E. coli* transformed with either EGFP or DSRed, showing expression and accumulation of the fluorescent proteins within the gut of *Artemia*.

<u>Diseases of aquaculture</u>

Worldwide production of farmed shrimp amounts to 4 million MT in 2014, and is expected to double in the next decade. However, in shrimp aquaculture, animals often succumb to diseases, especially under intensive farming practices. The most devastating bacterial pathogens are members of the genus *Vibrio*. In recent work, we have demonstrated that several species of pathogenic *Vibrios* are highly susceptible to certain antimicrobial peptides. In these studies, we will utilize a slightly modified paratransgenic approach is utilized to transfer passive immunity against V*ibrios* to shrimp larve. Ongoing work on this project includes 1) development of a reliable and effective method to convert the common probiotic *B. subtilis*, into a delivery vehicle that would target *Vibrios*, and 2) optimized feeding system with *Artemia* intermediates that transfers molecules with anti-*Vibrio* activity to the gut of shrimp larvae.

Publications:

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