

THE DURVASULA LAB

The Durvasula lab has played a pioneering role in the development of novel approaches to the control of infectious disease transmission. In 1997, Dr. Durvasula was part of a research team at Yale that described a strategy termed paratransgenesis, in which symbiotic bacteria of a disease-transmitting arthropod could be genetically transformed to produce molecules that killed the pathogen within the arthropod itself. This 'Trojan Horse' approach was first developed in the triatomine, or kissing bug, vector of Chagas disease, *Rhodnius prolixus*. The symbiotic bacterium of *R. prolixus*, an actinomycete named *Rhodococcus rhodnii*, was genetically transformed to export cecropin A, a peptide toxic to the Chagas parasite, *Trypanosoma cruzi*. Paratransgenic lines of *R. prolixus* which carried the genetically altered bacteria were refractory to *T. cruzi* infection (Durvasula et al, PNAS 1997). Two years later, Dr. Durvasula's group demonstrated the expression of a single chain antibody in the gut of *Rhodnius prolixus* via the engineered symbiotic bacterium. This was the first description of a functional antibody in an insect (Durvasula et al., Med Vet Entomology 1999).

For the past 10 years, the focus of the Durvasula lab has been development of paratransgenic strategies for control of a variety of infectious diseases. Dr. Durvasula is currently Associate Professor of Medicine and Infectious Diseases at University of New Mexico School of Medicine in Albuquerque, New Mexico, where he is also appointed as Chief of Medicine at the New Mexico VA Health Care System. Ongoing research activities in the Durvasula lab consist of 4 major projects:

1) Development of Paratransgenic Approaches to Control of Chagas disease

Chagas disease, or American Trypanosomiasis, remains a leading cause of heart disease south of the US border, with close to 12 million afflicted individuals, 40 million at-risk individuals and 50,000 deaths per year throughout Central and South America. It is caused by the parasite, *Trypanosoma cruzi*, and is transmitted to humans by triatomine or kissing bugs.

Projects related to Chagas disease are aimed at development of (a) molecular strategies to target *Trypanosoma cruzi* (b) techniques to deliver engineered bacteria to field populations of triatomine bugs (c) mathematical models related to optimized gene delivery (d) risk assessment tools to evaluate environmental impact of paratransgenic interventions (e) detailed analysis of triatomine bugs in terms of microbiology and effects of foreign gene expression on vector physiology

Key collaborating institutions:

University of Westminster, London, UK
Universidad Del Valle, Guatemala City, Guatemala
Centers for Disease Control and Prevention, Atlanta, GA, USA
IPM Development Corporation, Portland, OR, USA
University of Buenos Aires, Buenos Aires, Argentina
FIOCRUZ, Belo Horizonte, Brazil
Sandia National Labs, Albuquerque, NM, USA

2) Development of Paratransgenic Approaches to Control of Visceral Leishmaniasis

The spectrum of leishmaniasis has tremendous global impact with over 300 million cases per year and incidence in over 80 countries. Parasites of the genus *Leishmania* are transmitted to humans via sandfly vectors. Visceral leishmaniasis, termed kala azar, is endemic to regions of Eastern India, Nepal and Bangladesh and, in India, is caused by the parasite, *L. donovani*, and transmitted via the sandfly *Phlebotomus argentipes*.

Current projects in the Durvasula lab are aimed at (a) identification of commensal bacteria of *P. argentipes* in endemic and non-endemic regions of Eastern India (b) development of paratransgenic lines of *P. argentipes* and (c) identification of molecular targets on the surface of *L. donovani* for paratransgenic interventions

Key collaborating institutions:

Walter Reed Army Institute of Research, Bethesda, MD, USA
Rajendra Memorial Research Institute, Patna, India

3) Development of Paratransgenic Approaches to Infectious Diseases of Commercial Mariculture

World aquaculture production has increased to 51.4 million metric tons (MT) in 2002, with a value of \$60 billion. Of this, global shrimp production is greater than 5 million MT and remains the most important trade commodity. Farmed shrimp account for 1.6 million MT, representing a value of nearly \$9 billion. Disease outbreaks cause significant losses in aquaculture production throughout the world and greatly reduce export trade for affected nations. The most striking examples are shrimp viral diseases particularly White Spot Syndrome Virus (WSSV), which has devastated many parts of the world, with grave economic consequences and reduction in available food supply. The global annual economic loss due to WSSV is estimated to be \$3 billion. Antibiotic use as a prophylactic measure is tightly regulated in almost all countries due to emergence of antibiotic resistant microbes and offers no benefit against this viral disease. Effective vaccination techniques or antiviral

treatments for WSSV are currently unavailable. Given the tremendous global impact of WSSV on shrimp farming and the constraints of high-intensity cultivation, new strategies to combat WSSV are highly essential.

Current projects in the Durvasula lab are focused on (a) development of genetically engineered algae and cyanobacteria which can be used as feed for the shrimp, *P. monodon* (b) expression of molecules with activity against viral and *Vibrio* pathogens in transformed lines of algae and cyanobacteria (c) development of paratransgenic *Artemia* which can be deployed as feed to nauplii of *P. monodon* to confer passive immunity (d) testing of engineered organisms at shrimp hatchery settings in Andhra Pradesh, India

Key collaborating institutions:

BMR Hatcheries, Visakhapatnam, India
Shree Vasudha Labs, Visakhapatnam, India
Bedford Institute of Oceanography, Dartmouth, Canada

4) Development of Paratransgenic Approaches to *Clostridium difficile* Infections

Nosocomial infection with *Clostridium difficile* is rapidly emerging as a serious threat throughout the U.S. Cases of *C. difficile* infection have doubled in the Veterans Administration Hospital System since 1994, to an estimated number of 10/1000 discharges in 2004; for persons over 65 years of age, the rate has increased to 20/1000 discharges. Additional treatment cost due to *C. difficile* infection is estimated at \$3600 per case. Incidence and complexity of *C. difficile* infection is likely to increase. An aging population, increasing levels of co-morbidity, and widespread use of broad-spectrum antibiotics will fuel the epidemic of nosocomial *C. difficile*. The most worrisome aspects of the current *C. difficile* epidemic are (1) emergence of bacterial resistance to the front-line antibiotic metronidazole and (2) the appearance of a more virulent strain of *C. difficile* with enhanced toxigenic potential and fluoroquinolone resistance. Increasingly, oral vancomycin has become the treatment of choice for nosocomial *C. difficile*, though cost and emergence of vancomycin resistance limit this approach and result in frequent relapses. The need for novel therapeutic and prophylactic approaches to *C. difficile* is urgent. We propose to develop a line of genetically modified *Bifidobacteria* –common human probiotic bacteria- that elaborate single chain antibodies capable of binding and neutralizing *C. difficile* toxins A and B. We will engineer the sFv from hybridoma lines that elaborate anti-*C. difficile* toxin monoclonal antibodies, clone genes encoding these sFv's, characterize expression and binding of sFv's, and test the efficacy of this approach in a hamster model of *C. difficile* infection. For these studies, we will use a matched system with *B. pseudolongum* and the corresponding animal (hamster) model. We hypothesize that engineered lines of

Bifidobacteria elaborating antitoxin sFv will prevent pathological changes due to administered C. difficile toxins in the hamster model. These studies are the laboratory proof-of-concept of a paratransgenic approach that may eventually be developed for human application using common, non-pathogenic, probiotic organisms.

Key collaborating institutions:

University of Westminster, London, UK

Ongoing and Recently Completed Grants

1) National Institutes of Health: 1 R01 AI066045-01

“Molecular Targeting of T. cruzi for Paratransgenic Vectors”

Ravi Durvasula, MD (PI); 4 year project period

February 15, 2007- February 14, 2011

2) United States Dept. of Agriculture, Biotechnology Risk Assessment Grant

CREES 04-2945

“ A Paratransgenic Approach to Control of Pierce’s Disease” Thomas Miller:PI

Role: Subcontractor; 2 year project period

Feb. 2005- Feb. 2007

3) University of New Mexico RAC Grant

“ Paratransgenic Approaches to Human Clostridium difficile Infections”

Role: PI

Feb. 2007- Feb. 2008

4) National Institutes of Health RO1 AI073912-01

“ A Paratransgenic Strategy for Southern Cone Vectors of Chagas disease”

Ravi Durvasula, MD (PI); 5 year project period

Pending

5) National Institutes of Health RO1, AI 48649-01

“A strategy for spreading foreign genes in Chagas disease vectors”

PI: Ravi Durvasula, MD 4 year award,

April 2001- May 2005; completed

Relevant Publications

Conover RJ, Durvasula RV, Roy S, Wang R. Probable Loss of Chlorophyll-derived Pigments During Passage Through the Gut of Zooplankton, and Some of the Consequences. **Limnology and Oceanography** 1986:31(4), 878-887

Durvasula RV Gumbs A, Panackal A, Kruglov A, Aksoy S, Merrifield RB, Richards F and Beard CB. Prevention of Insect-borne Disease: An Approach Using Transgenic Symbiotic Bacteria. **Proc. Nat. Acad. Sci. USA** 1997: 94, 3274-3278

Beard CB, Durvasula RV and Richards FF. Bacterial Symbiosis in Arthropods and the Control of Disease Transmission. **Emerging Infectious Diseases** 1998: Vol 4:4, 581-591

Durvasula RV Gumbs A, Panackal A, Whitharn R, Taneja J, Kang A, Richards'F and Beard CB. Expression of a Functional Antibody Fragment in the Gut of *Rhodnius prolixus* via the Transgenic Bacterial Symbiont, *Rhodococcus rhodnii*. **Med. Vet. Entomology** 1999:13,1-5

Durvasula RV and Taneja J. Maintenance of triatomine bugs *Rhodnius prolixus* and *Triatoma dimidiata* under laboratory conditions. In **Maintenance of Human, Animal and Plant Pathogen Vectors 1999**. K. Maramorosch and F. Mahmood eds. 139-157. Science Publishers Inc. Enfield, NA, New Hampshire 03748 USA pp 328

Durvasula RV Panackal A, Taneja J, Gumbs A, Kruglov O, Richards F, Beard CB. A Strategy for Spreading Anti-trypanosomal Genes in Populations of the Chagas disease Vector, *Rhodnius prolixus*. **Annals of The Entomological Society of America** 1999: 92(6), 937-943

Beard CB, Durvasula RV and Richards FF. Bacterial Symbiont Transformation in Chagas Disease Vectors. In **Insect Transgenesis. Methods and Applications 2000**. AM Handler and AA James eds. 289-303. CRC Press, Boca Raton, Florida 33431 pp 397

Beard CB, Dotson EM, Pennington P, Eichler S, Cordon-Rosales C and Durvasula RV. Bacterial symbiosis and paratransgenic control of vector-borne Chagas disease. **Int. J. Parasitology** 2001:31, 621-27

Beard CB, Cordon-Rosales C and Durvasula RV. Bacterial Symbionts of the Triatominae and Their Potential Use in Control of Chagas Disease Transmission. **Annual Review of Entomology** 2002:47, 123-141

Dotson EM, Pliykatis B, Shinnick TM, Durvasula RV and Beard CB. Transformation of *Rhodococcus rhodnii*, a symbiont of the Chagas disease vector *Rhodnius prolixus*, with integrative elements of the L1 mycobacteriophage. **Infection, Genetics and Evolution** 2003: 74, 1-7

Durvasula RV, Sundaram R and Beard CB. *Rhodnius prolixus* and Its Symbiont *Rhodococcus rhodnii*: A Model for Paratransgenic Control of Disease Transmission. In **Insect Symbiosis 2003**. K Bourtzis and T Miller eds. pp 85-97. CRC Press, Boca Raton, Florida

Durvasula RV, Sundaram R, Matthews S, Sundaram P and Subba Rao DV. Prospects for Paratransgenic Application to Commercial Mariculture using

Genetically Engineered Algae. **2006. Pp 865-889 Vol 2 in Algal Cultures, Analogues of Blooms and Applications (DV Subba Rao ed)** Science Publishers, Enfield USA

Miller T, Lauzon C, Lampe D, Durvasula R and Matthews S. Paratransgenesis applied to insect-transmitted diseases: The Pierce's Disease Case. **2006 Chap 15 in Insect Symbiosis 2. Miller T and Bourtzis K, eds.** CRC Press, Boca Raton

Matthews S, Sundaram R, Beard CB and Durvasula RV. A Risk Assessment Framework to Evaluate Paratransgenic Interventions for Control of Chagas Disease. **2007 Trends in Parasitology submitted**

Contact us

Dr. Ravi Durvasula, PI

ravi.durvasula@med.va.gov
rdurvasula@salud.unm.edu

505-991-3812

Dr. Ivy Hurwitz, Senior Scientist

ihurwitz@salud.unm.edu

Scott Matthews, Research Associate

skm4150@yahoo.com

Anil Thakkapan, Research Associate

Arriving in July 2007

Bobban Subhadra, PhD Student

bsubhadra@salud.unm.edu

Division of Infectious Diseases, Dept of Internal Medicine,
University of New Mexico School of Medicine,
Room 305, CRF,
915 Camino de Salud, NE,
Albuquerque, NM, USA 87131
505- 272-4392; 505-256-2803 (fax)

