

Area of Research: **Infectious diseases/Immunology**
Mentor: **Patrick Skelley & Chuck Shoemaker**

Project Description

Research on parasitic helminths involves investigation of the molecular and cellular biology of host parasite interactions with an emphasis on the identification and characterization of surface-exposed vaccine candidates. In addition, gene manipulation technologies for these parasites, using RNA interference (RNAi), are being optimized.

Area of Research: **Infectious diseases**
Mentor: **Jean Mukherjee**

Project Description

Role of Antibody in Protection Against *Listeria monocytogenes*: *Listeria monocytogenes* is a gram-positive facultative intracellular bacillus which is responsible for ~2500 cases of food-borne illness, including ~500 deaths, each year within the U.S. Based on the finding that infection induces only low titers of *Listeria*-specific antibody and passive immunization with such antiserum does not confer protection in murine models of listeriosis, the role of antibody in protection against listeriosis had traditionally been discounted. Recent studies however, suggest that antibodies against three major virulence factors, namely, Internalin A (InIA), Internalin B (InIB), and Listeriolysin O (LLO) can mediate protective activities. Thus, the objective of this project is to generate and identify neutralizing, *Listeria*-antigen specific monoclonal antibodies (mAbs), including those against InIA, InIB, and LLO, to facilitate studies directed at further evaluating the role of antibody in protection against listeriosis. Projects that are available for summer student participation, include, isolation of recombinant InIA, InIB, and LLO, production of polyclonal and/or monoclonal antibodies to recombinant InIA, InIB, and LLO, and *in vitro* and *in vivo* characterization and functional evaluation of polyclonal and/or monoclonal antibodies against InIA, InIB, and LLO.

Area of Research: **Infectious diseases**
Mentor: **Jean Mukherjee**

Project Description

Conjugate Vaccine Development: The conjugate vaccine technology is applicable to any pathogen that has capsular polysaccharide or lipopolysaccharide on its surface. Although poorly immunogenic when administered alone, when conjugated to an immunogenic carrier protein, capsular polysaccharides and lipopolysaccharides elicit high titers of protective

antibodies. Because conjugate vaccines are comprised of highly defined purified antigens, they exhibit superior safety and efficacy relative to vaccines developed via traditional approaches. The focus of this project is to develop conjugate vaccines for use in companion and/or food animals. Projects that are available for summer student participation, include, characterization of potential vaccine strains, conjugate vaccine preparation, and *in vivo* and *in vitro* testing of candidate conjugate vaccines. This project will involve laboratory based research, which may include bacterial cultivation, polysaccharide/lipopolysaccharide isolation and purification, a variety of serological assays (enzyme-linked immunoassays, i.e. ELISAs, immunodiffusion, Western blot analysis), conjugation reactions, *in vitro* opsonophagocytosis assays, and *in vivo* challenge studies.

Area of Research: **Infectious diseases**

Mentor: **Sam Telford**

Project Description

Companion animals as sentinels for flaviviral transmission: companion animals such as dogs or cats are exposed to bites of ticks, mosquitoes, and other ectoparasites more frequently than are their human owners. Accordingly, they may serve as sensitive sentinels for active transmission of certain vector-borne pathogens. We seek to determine whether serologic evidence of exposure to West Nile virus may be detected in serum samples from companion animals, and whether the prevalence and site of such exposure coincides with what is known from other reported measures (such as crow deaths, mosquito surveillance, etc).

Method: discarded serum samples (ca. 1500) from Idexx Laboratories are available for the year 2000. These derive from routine diagnostic procedures, and are accompanied by data such as the location of the submitting practice and date of submission, as well as purpose of testing. Other such samples will be solicited from Idexx. Sera shall be screened for IgG antibody by the indirect fluorescent antibody test using antigen slides prepared by the Telford laboratory (antigen is inactivated by beta-propiolactone and the procedure is therefore not hazardous) and a standard protocol.

Endpoint: should serological evidence of exposure be documented, the findings may provide the rationale for further studies of the potential for WNV to cause illness in dogs and cats, for example, by chart review. In addition, such a conclusion would imply that veterinary clinics may serve a public health purpose as a secondary means of surveillance for local WNV activity.

Area of Research: **Infectious diseases**

Mentor: **Sam Telford**

Project Description

Prevalence and diversity of helminths in insular white-footed mouse

populations: Nematode and cestode species occur in characteristic associations (guilds) with particular hosts. White-footed mice are universally infected by *Capillaria hepatica*, an ecologically successful generalist nematode, but other helminths may be apparent. It may be that the prevalence of *Capillaria* may modulate the guild representation of other helminths that may be expected, such as hymenolepidid tapeworms.

Method: formalin fixed fecal samples have been obtained from white-footed mice trapped for Lyme disease ecology studies in coastal New England from 1986-2002. Diversity and prevalence of helminths will be determined by microscopic examination of wet mount slides, with or without concentration techniques, mainly by the identification of ova.

Endpoint: prevalence of *Capillaria* and other helminth species can be compared for mouse populations on Nantucket, Martha's Vineyard, and Great Island (W. Yarmouth) within and between years. Statistical analysis may demonstrate threshold transmission indices that would allow the perpetuation of the less common helminths.

Area of Research: **Infectious diseases**

Mentor: **Sam Telford**

Project Description

Prevalence of spirochetes within dog ticks: Dog ticks (*Dermacentor variabilis*) have been ignored with respect to their possible role in maintaining pathogenic spirochetes such as *Borrelia burgdorferi* sensu lato (Lyme disease spirochetes). Emerging evidence suggests that each ecologically successful tick species may have its "own" borrelia. It may be that such spirochetes may be detected if statistically robust samples are screened for evidence of infection.

Method: DNA from dog ticks (ca. 3000 samples) collected on Martha's Vineyard during a tularemia investigation has been stored for future analysis. Evidence of infection will be sought using PCR with genus and group specific primers and protocols that have already been tested for their utility.

Endpoint: Should a borrelia or other spirochete be detected, further studies would be required (such as by DNA sequencing, life cycle elucidation) to determine their identity and public health significance.

Area of Research: **Infectious diseases**

Mentor: **Giovanni Widmer**

Project Description

Molecular biology of *Cryptosporidium parvum*: My laboratory is working on several projects related to the molecular biology and genetics of *Cryptosporidium parvum*, a pathogenic protozoan which is frequently found in neonatal ruminants and also infects immunocompromised individuals. Currently, areas of research include the genetics of *Cryptosporidium parvum*, the improvement of cell culture methods for *C. parvum*, and the development of a genetic transformation system for this parasite. Students interested in working in the lab, will participate in one of these projects and acquire research experience in one or several of the following areas: recombinant DNA, cell culture, animal models for *C. parvum*, flow cytometry, real-time PCR. Depending on the status of each project, the interested student may be able to choose a project. Some projects use laboratory animals, particularly mice. Students willing to work with mice are preferred.

Area of Research: **Infectious diseases**

Mentor: **Donohue-Rolfe**

Project Description

Enterohemorrhagic *E. coli* (EHEC) is an emerging pathogen that first appeared in the United States in 1982 but since that time has spread throughout the world. EHEC strains clinically cause hemorrhagic colitis in humans and can be responsible for a serious clinical sequelae, hemolytic uremic syndrome (HUS) or simply kidney failure. Cattle asymptotically carry the organism in their intestines. Hamburger meat which has been contaminated with EHEC in the slaughter house is the major source of human EHEC infections. The most notorious EHEC serotype is *E. coli* O157:H7 which is responsible for the majority of multi-person outbreaks of enterohemorrhagic colitis worldwide.

EHEC strains all produce Shiga toxin, a toxin initially discovered in *Shigella* strains. Shiga toxin is probably directly involved in both causing bloody diarrhea and HUS development in humans. The toxin consists of two subunits an A chain, responsible for protein synthesis inhibition of eukaryotic cells, and a B chain responsible for binding the toxin to the cell surface of a eukaryotic cell.

A project that I would like to have a student develop is to generate a mutation in the A subunit of Shiga toxin so that the toxin molecule is produced normally but is lacking the protein synthesis inhibitory activity. This mutated toxin would be created within a wild-type *E. coli* O157 strain and activity of the toxin would be tested initially in cell culture. The mutant would also be tested in a suitable infection model for its ability to cause systemic disease and for its ability to block the entry of normal toxin. Development of such toxins that possess full cell binding capabilities but lack the toxic activity may yield future therapeutic

treatment regimens that may protect people from the life threatening complications of the disease.

Area of Research: **Infectious Diseases**
Mentor: **Alison Robbins**

Project Description

Rabies Epidemiology and Control/Emerging Infectious Disease: TUSVM in conjunction with the Massachusetts Department of Public Health (MDPH), USDA/Wildlife Services, and the Centers for Disease control are conducting a rabies control program for raccoons. We vaccinate free-ranging raccoons with an orally effective vaccine contained in a bait that is distributed in the environment. Our purpose is to prevent the spread of rabies to Cape Cod. We trap and draw blood from raccoon and other wildlife in the vaccination zone to evaluate the effectiveness of the program. We track rabies cases in and around the vaccine area by collecting euthanized sick wildlife, roadkills and analyzing MDPH data. Recently, we have discovered that skunks are now carrying the raccoon rabies strain and are able to transmit the disease. Across the United States, oral rabies vaccination campaigns are preventing the spread of raccoon rabies to the Western US. Many millions of federal dollars are being spent in this rabies containment effort. Skunk to skunk transmission has serious implications for the national rabies control efforts mainly because the vaccine does not work in this species. I have two areas of research for students:

- 1) Field work with wildlife. In this research position, the student will spend most of his or her time live-trapping wildlife within the vaccination zone. Students will collect serum samples for determination of vaccination rate in raccoons, and collect euthanized wildlife for rabies testing. The student will be involved in all aspects of running an oral vaccine program and the public health aspects of rabies control. A large camping trailer (with kitchen and bath) will be available for housing within the vaccination area. Research will be conducted into the impact of skunk to skunk transmission within a vaccination zone.
- 2) Epidemiology Research: In this research position, the student will analyze data on rabies positive cases throughout Massachusetts. The aim is to conduct retrospective research into the geographic origin of the new skunk strain, and try to determine location and rate of strain spread throughout the state and region. This is cutting edge research has national implications as mentioned above.

Area of Research: **Infectious Diseases**
Mentor: **Abhineet Sheoran**

Project Description

The research in my laboratory focuses on microbial pathogenesis, immune response against microbial pathogens, and monoclonal antibody-based therapies. We are investigating pathogenesis and immunity of *Cryptosporidium parvum*, which causes chronic diarrhea in HIV patients. We are studying systemic as well as local cellular and humoral immune responses against *C. parvum* types 1 and 2 and *C. meleagridis* in the gnotobiotic piglet (GB) model, and the extent of cross-protection between *C. parvum* types 1 and 2 and *C. meleagridis* in the GB piglet model. We are also identifying immunodominant molecules that are unique to each *C. parvum* type and *C. meleagridis*, and also that are common among *C. parvum* types and *C. meleagridis*, which will help in identification of key specific- and cross-protective parasite antigens.

We are investigating efficacy of Shiga toxin (Stx)-specific human monoclonal antibodies (HuMAbs) for therapy of hemolytic uremic syndrome. The project currently focuses on defining the structural and functional characteristics which facilitate protective efficacy of Stx1 and Stx2-specific HuMAbs, characterizing the mechanisms of neutralization of Stx-mediated cytotoxicity by HuMAbs, and testing protective efficacy of each HuMAb in animal models when administered following oral infection with Stx producing *E. coli*.

The research project on microsporidia focuses on establishing a purification procedure for *Enterocytozoon bieneusi* from feces of AIDS patients, characterizing antigens, and developing in vitro models of *E. bieneusi* infection and also immunoassays for diagnosis.