OMB Number: 4040-0001
Expiration Date: 06/30/2011

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. * TYPE OF SUBMISSION
☐ Pre-application ☒ Application ☐ Changed/Corrected Application

2. DATE SUBMITTED 02/11/2010

Applicant Identifier

4. a. Federal Identifier

b. Agency Routing Identifier

5. APPLICANT INFORMATION

*** Organizational DUNS:** [redacted]

* Legal Name: Colorado State University
Department:
Division:
* Street1: 601 S. Howes Street
Street2:
* City: Fort Collins
County / Parish:
* State: CO: Colorado
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 80523-2002

Person to be contacted on matters involving this application
Prefix:
* First Name: Christine
Middle Name:
* Last Name: Getzelman
Suffix:
* Phone Number: [redacted]
Fax Number: [redacted]
Email: [redacted]

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):* [redacted]

7. * TYPE OF APPLICANT: H: Public/State Controlled Institution of Higher Education
Other (Specify):
Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:
☒ New ☐ Resubmission
☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).
☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration
☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY: National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:
TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
Mechanisms of Enteric Burkholderia psuedomallei infection

12. PROPOSED PROJECT:
* Start Date: 08/01/2010
* Ending Date: 07/31/2012

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT** CO-004

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION
Prefix: Dr.
* First Name: Steven
Middle Name: W
* Last Name: Dow
Suffix:
Position/Title: Professor
* Organization Name: Colorado State University
Department: Clinical Sciences
Division: CVMBS
* Street1: 1678 Campus Delivery
Street2:
* City: Fort Collins
County / Parish:
* State: CO: Colorado
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 80523-1678
* Phone Number: [redacted]
Fax Number: [redacted]
* Email: [redacted]

PI: **Dow, Steven W.**	Title: Mechanisms of Enteric Burkholderia psuedomallei infection	
Received: 02/11/2010	FOA: PA10-069	Council: 10/2010
Competition ID: ADOBE-FORMS-B	FOA Title: NIH EXPLORATORY DEVELOPMENTAL RESEARCH GRANT PROGRAM (PARENT R21)	
1 R21 AI091991-01	Dual:	Accession Number: 3269078
IPF: 1725201	Organization: COLORADO STATE UNIVERSITY-FORT COLLINS	
Former Number:	Department: Clinical Sciences	
IRG/SRG: ZRG1 IDM-A (80)S	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted] Year 2: [redacted]	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Steven Dow	Colorado State University	PD/PI
Mercedes Gonzalez-Juarrero Ph.D	Colorado State University	Other Professional-Co-Investigator
Herbert Schweizer	Colorado State University	Other Professional-Consultant

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	398,174.00
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	398,174.00
d. Estimated Program Income	0.00

16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalities. (U.S. Code, Title 18, Section 1001)

☒ *** I agree**

**** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.***

18. SFLLL or other Explanatory Documentation

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix: * First Name: Linda Middle Name:

* Last Name: Monum Suffix:

* Position/Title: Research Administrator

* Organization: Colorado State University

Department: Sponsored Programs Division:

* Street1: 601 S. Howes Street

Street2:

* City: Fort Collins County / Parish:

* State: CO: Colorado Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 80526-1678

* Phone Number: [redacted] Fax Number: [redacted]

* Email: [redacted]

*** Signature of Authorized Representative**

Linda Monum

*** Date Signed**

02/11/2010

20. Pre-application

Add Attachment | Delete Attachment | View Attachment

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 08/31/2011

Project/Performance Site Location(s)

Project/Performance Site Primary Location ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Colorado State University

DUNS Number: [redacted]

* Street1: 300 W. Drake Road

Street2:

* City: Fort Collins County:

* State: CO: Colorado

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 80523-1678 * Project/ Performance Site Congressional District: CO-004

Project/Performance Site Location 1 ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:

DUNS Number:

* Street1:

Street2:

* City: County:

* State:

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: * Project/ Performance Site Congressional District:

Additional Location(s) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☐ Yes ☒ No

1.a If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☐ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

If no, is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Human Subject Assurance Number:

2. * Are Vertebrate Animals Used? ☒ Yes ☐ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☒ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number [redacted]

3. * Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. * Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. * Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. * Project Summary/Abstract 1234-Abstract.pdf Add Attachment | Delete Attachment | View Attachment

8. * Project Narrative 1235-Relevance statement.pdf Add Attachment | Delete Attachment | View Attachment

9. Bibliography & References Cited 1236-Literature cited.pdf Add Attachment | Delete Attachment | View Attachment

10. Facilities & Other Resources 1237-2.10 FACILITIES.pdf Add Attachment | Delete Attachment | View Attachment

11. Equipment 1238-2.10 MAJOR EQUIPMENT.pdf Add Attachment | Delete Attachment | View Attachment

12. Other Attachments Add Attachments | Delete Attachments | View Attachments ☐

Tracking N er:GRANT10529698 Funding O uni y Number:PA-10-069 Received Da e:2010-02-11T17:02:50-04:00

Burkholderia pseudomallei (Bp) is a Gram-negative bacterial pathogen that can cause a variety of difficult-to-treat infections in humans ranging from acute sepsis to chronic abscesses. While *Bp* is endemic in southeast Asia and northern Australia, infections are now being diagnosed with increasing frequency around the world, including in Central and South America. Therefore, it is likely that *Bp* infections will soon be identified in the U.S. *Though infection with Bp was previously thought to occur by inhalation or skin inoculation, our new studies indicate that Bp is actually a primary enteric pathogen, which can readily establish acute or persistent GI tract infection following oral inoculation in mouse models*. However, at present essentially nothing is known regarding the pathogenesis of enteric infection with *Bp*. Therefore, the studies proposed here are intended to fill a critical void in our understanding of pathogenesis of infection with this important and emerging bacterial pathogen. First, we will use the mouse infection model of Bp infection to determine whether most or all strains of Bp can establish enteric infection and to identify virulent and avirulent isolates. Second, we will use the model to define the role of the intestine as a reservoir for Bp infection and to identify cells in the GI tract where the organism is maintained during chronic infection. Last, we will investigate how Bp is disseminated to other organs during chronic enteric infection. The information generated in these studies will substantially alter our view of Bp as a pathogen and also lead to a reassessment of the risks posed by oral Bp infection.

Burkholderia pseudomallei is an important and dangerous bacterial pathogen that appears in recent years to be spreading around the world, including Central and South America. This organism is particularly dangerous because it is able to survive for years in soil and water, is very resistant to most antibiotics, and can cause rapidly fatal infections in humans. Previously it was assumed that the organism was contracted only by inhalation or skin injury, but our new data indicate that *B. pseudomallei* is also very infectious orally and causes chronic intestinal infection with fecal shedding. We will therefore study the mechanisms that allow *B. pseudomallei* to infect the intestinal tract, using mouse models of infection.

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

The Dow lab in the Infectious Disease Annex occupies a 900 sq BSL-2 lab that is equipped with 2 tissue culture hoods, tow double water-jacketed CO2 incubators, an IEC PR-7000 centrifuge, a refrigerated microcentrifuge, 2 freezer (-20C and -80C), two refrigerators, a Dynatech ELISA reader and plate washer, and a Leica inverted microscope and a DSML direct microscopy with digital camera attachment. Smaller equipment includes 2 electronic balances, 2 water baths, heating blocks, BioRad power supplies (2) and gel boxes (2 each), and blotting apparatus, and vortexers, and PX2 Hybaid PCR machine. Share facilities and equipment include a high-speed centrifuge, an ice machine, and gel scanner and analysis system. The lab also has access to a Beckman Coulter Cyan ADP multicolor flow cytometer in the adjacent laboratories.

The Regional Biocontainment Laboratory (RBL) immediately adjacent to the Infectious Disease Annex houses 3 large suites of BSL-3 containment facilities. The Dow laboratory currently occupies one suite, along with the Schweizer lab, where the Burkholderia pseudomallei animal challenge studies and tissue culture work is done.

Clinical:

The Veterinary Teaching Hospital occupies approx 80,000 sq ft of space and is fully equipped for clinical management of diseases of companion animals and livestock. Equipment includes in-house laboratory, CT, MRI, radiation therapy, surgery suites and nuclear medicine.

Animal:

Laboratory animals are housed in a 20,000 sq ft Biological Resources Building on campus. This facility is AALAC accredited and currently houses mice, rats, rabbits and dogs. Within the RBL building, there is up to 8,000 sq ft of space for housing rodents under BSL3 and BSL2 containment conditions.

Co

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Cyan ADP multicolor flow cytometer (Infectious Disease Annex lab)

Confocal Core Unit with a Zeiss LSM 510 META laser scanning confocal microscope equipped with 4 lasers (Infectious Disease Annex)

IVIS in vivo animal imaging system (RBL building)

Typhoon image analysis system (Animal Cancer Center, Vet Teaching Hosp)

CyAn MLE Flow Cytometer (Animal Cancer Center, VTH)

Olympus Fluoview Lasar Scanner confocal microscope (VTH)

Leica cryostat (Clinical Sciences laboratory, VTH)

Leica direct microscopes and digital camera (Infectious Disease Annex)

Mo-Flo high speed cell sorter (Pathology Bldg, CSU)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. * First Name: Steven Middle Name: W
* Last Name: Dow Suffix:
Position/Title: Professor Department: Clinical Sciences
Organization Name: Colorado State University Division: CVMBS
* Street1: 1678 Campus Delivery
Street2:
* City: Fort Collins County/ Parish:
* State: CO: Colorado Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 80523-1678
* Phone Number: [redacted] Fax Number: [redacted]
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** PD/PI **Other Project Role Category:**

Degree Type: BA, DVM, PhD
Degree Year: 1978, 1982, 1992

***Attach Biographical Sketch** 1242-2.10 Dow Burk Biosketch [Add Attachment] [Delete Attachment] [View Attachment]
Attach Current & Pending Support [Add Attachment] [Delete Attachment] [View Attachment]

PROFILE - Senior/Key Person 1

Prefix: * First Name: Mercedes Middle Name:
* Last Name: Gonzalez-Juarrero Suffix: Ph.D
Position/Title: Associate Professor Department: Microbiology, Immunology & Pat
Organization Name: Colorado State University Division:
* Street1: 1682 Campus Delivery
Street2:
* City: Fort Collins County/ Parish:
* State: CO: Colorado Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 80523-1682
* Phone Number: [redacted] Fax Number: [redacted]
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** Other Professional **Other Project Role Category:** Co-Investigator

Degree Type: BS, PhD
Degree Year: 1982, 1990

***Attach Biographical Sketch** 1243-Gonzalez Juarrero bioske [Add Attachment] [Delete Attachment] [View Attachment]
Attach Current & Pending Support [Add Attachment] [Delete Attachment] [View Attachment]

Tracking N er:GRANT10529698 Funding O uni y Number:PA-10-069 Received Da e:2010-02-11T17:02:50-04:00

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2

Prefix: | * First Name: Herbert | Middle Name:

* Last Name: Schweizer | Suffix:

Position/Title: Professor | Department: Microbiology, Immunology, Path

Organization Name: Colorado State University | Division: CVMBS

* Street1: 1682 Campus Delivery

Street2:

* City: Fort Collins | County/ Parish:

* State: CO: Colorado | Province:

* Country: USA: UNITED STATES | * Zip / Postal Code: 80523-1682

* Phone Number: [redacted] | Fax Number: [redacted]

* E-Mail: [redacted]

Credential, e.g., agency login:

*** Project Role:** Other Professional | **Other Project Role Category:** Consultant

Degree Type:

Degree Year:

***Attach Biographical Sketch**	1244-schweizer_biosketch_2010	Add Attachment	Delete Attachment	View Attachment
Attach Current & Pending Support		Add Attachment	Delete Attachment	View Attachment

Tracking N er:GRANT10529698 Funding O uni y Number:PA-10-069 Received Da e:2010-02-11T17:02:50-04:00

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Dow, Steven W.	Professor of Immunology
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Virginia	BA	1978	Biology
University of Georgia	DVM	1982	Veterinary Medicine
Colorado State University	PhD	1992	Comparative Pathology

A. Personal Statement. The goal of our research program is to better understand the pathogenesis of *Burkholderia pseudomallei* infection, using animal models of infection. These studies stem from our ongoing work in developing new immunotherapeutics for vaccines and treatment of bacterial infectious diseases. My background in comparative pathology and immunology provides a strong basis for designing and directing the proposed studies. In addition, over the past 5 years I have gained considerable experience working with animal models of Burkholderia infection, including models of both *B. mallei* and *B. pseudomallei*. For example, I have been involved for several years in the development of inhalational animal models of infection with several BSL3 level pathogens, including *Francisella tularensis*, *Yersinia pestis*, and the *Burkholderias*. The studies of acute and chronic enteric infection with *Burkholderia pseudomallei* are a natural extension of our work with mucosal infection and immunity to bacterial pathogens. Our group has the necessary expertise and containment facilities to conduct the proposed studies and our recent publication record demonstrates our emerging expertise and collaborations in the Burkholderia field. I have also enlisted the help of several key collaborators and co-investigators (Schweizer, Gonzalez) whose expertise will strengthen the project team.

B. Positions and Honors.

Positions and Employment

2009-present Assistant Dept. Chairman for Graduate Studies, Dept of Clinical Sciences
2007-present: Professor, Dept of Microbiology, Immunology, and Pathology and Dept of Clinical Sciences, Colorado State University, Ft. Collins, CO
2001-2006 Associate Professor, Dept of Microbiology, Immunology, and Pathology and Dept of Clinical Sciences, Colorado State University, Ft. Collins, CO
1995-2001 Instructor, National Jewish Medical and Research Center, Denver, CO and the University of Colorado Health Sciences Center, Denver, CO
1993-1995 Post-Doctoral Fellow, National Jewish Medical and Research Center, Denver, CO
1987-1992 Graduate Student, Department of Pathology, Colorado State University, Ft. Collins, CO
1984-1987 Residency in Small Animal Medicine, Dept Clinical Sciences, Colorado State University

Other Experience and Professional Memberships

Ad hoc study section member, Microbiology Infectious Disease RC, National Institutes of Health, Feb. 2005
Ad hoc study section member, Innate Host Defense IRG, National Institutes of Health, Oct. 2005
Ad hoc study section member, Immune Mechanisms SEP, November, 2009
Current Study section member, Topics in Bacterial Pathogenesis, NIH/NIAID, Oct. 2006; Feb, 2007, June 2008
Member, American Association of Immunologists, American Society for Microbiology

Honors

Phi Beta Kappa, University of Virginia, 1978
Summa cum laude graduate, University of Georgia, 1982
Pfizer Animal Health Award for Research Excellence, Colorado State University, 2004

C. Selected Peer-reviewed Publications.

Most relevant to the current application

1. Goodyear A, Troyer R, Jones A, and Dow S. (2009) Protection from Burkholderia infection by inhalational immunotherapy. *Infection and Immunity* 77:1759-1765. PMID 19179415
2. Troyer R, Propst K, Fairman, J, Bosio K, and Dow S. (2009) Mucosal immunotherapy for protection from pneumonic Francisella infection. *Vaccine* 27:4424-4433 PMID 19490961
3. [redacted]
4. [redacted]
5. Trunck LA, Propst KL, Wuthiekanun V, Tuanyok A, Beckstrom-Sternberg SM, Beckstrom-Sternberg JS, Peacock SJ, Keim P, Dow SW, Schweizer HP. (2009) Molecular Basis of Rare Aminoglycoside Susceptibility and Pathogenesis of Burkholderia pseudomallei Clinical Isolates from Thailand. *PLoS Negl Trop Dis*.519. PMMID 19771149
6. [redacted]

Additional recent publications of importance to the field (in chronological order)

1. [redacted]
2. Irwin, SM, Izzo AA, Dow SW, Skeiky YA, Reed SG, Alderson MR, and Orme IM. (2005). Tracking antigen-specific CD8 T lymphocytes in the lungs of mice vaccinated with the Mtb72F polyprotein. *Infect Immun*.73:5809-16. PMID 16113299
3. Bosio C, Goodyear A, and Dow S. (2005) Early interaction of *Yersinia pestis* with APCs in the lungs. *J Immunol* 175:6750-6756. PMID 16272331
4. Bosio C and Dow S. (2005) Aberrant activation of pulmonary dendritic cells by *Francisella tularensis*. *J Immunol* 175:6792-6801. PMID 16272336
5. Zaks K, Jordan M, Guth A, Sellins K, Kedl R, Izzo A, Bosio C, and Dow S. (2006) Efficient immunization and cross-priming by vaccine adjuvants containing TLR3 and TLR9 agonists complexed to cationic liposomes. *J Immunol* 176:7335-7345.
6. Guth A, Bosio C, Janssen W, Crouch E, Henson P, and Dow S. (2009) Lung environment determines unique phenotype of alveolar macrophages. *J. Physiol Lung Cell Mol Biol*; 296:L936-946. PMID 19304907

D. Research Support

Ongoing Research Support

U54 AI065357-01 RP1.2 (RCE). Dow (P.I.; 15% effort) 5/1/09-5/1/14
Immuno-Antimicrobial Therapy for Pneumonic Burkholderia Infection
This project will investigate the role of the innate immune system in controlling *Burkolderia mallei* infection of the lungs, using mouse models. These studies will also investigate the ability of immunotherapy to generate protective immunity to pneumonic *Burkholderia* infection.

U54 AI065357-02 (Developmental Project) Dow (PI) 9/1/08 - 9/1/09
Inhalational delivery of antibiotic nanoparticles for rapid protection from pneumonic Burkholderia infection
Role: PI (5% effort)
These studies will investigate the effectiveness of inhaled delivery of sustained release antibiotic nanoparticles in mouse inhalational challenge models with *B. pseudomallei.*

[redacted]

[redacted]

Completed Research Support

SBIR NIH/NIAID Fairman (PI) 6/1/06 - 2/31/09
"Innate Immune Stimulation as a Pathogen Countermeasure."
Role: PI of CSU subcontract (18% effort)
These studies are investigating the effectiveness of activating innate immune responses using parenterally and mucosally administered liposome-DNA complexes for eliciting protection from pulmonary *Francisella tularensis* infection.

U01 AI056487-01 Dow (P.I.; 20% effort) 9/29/03-1/31/08
Antigen Presentation And Pulmonary Immunity To Yersinia Pestis
These studies will assess innate and adaptive immune responses to a novel liposome-nucleic acid vaccine adjuvant and to assess the ability of this adjuvant to elicit protective mucosal immunity against inhaled *Yersinia pestis* infection.

RO1 CA86224-01 Dow (PI, 50% effort) 9/1/99-9/1/2007
Systemic Gene Therapy for Inhibiting Tumor Angiogenesis
These studies investigated the ability of intravenous delivery of cationic liposome-DNA complexes to inhibit tumor angiogenesis and deliver anti-angiogenic genes, using both mouse models and spontaneous tumor models in dogs.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Mercedes Gonzalez Juarrero	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Universidad Complutense de Madrid, Spain	Bachelor	1977-1982	Biology
Plum Island Animal Disease Centre, USDA-ARS, NY, USA /Universidad Autónoma de Madrid, Spain	PhD	1985-1990	Immunology, Virology
International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya	Postdoctoral	1990-1993	Immunology Parasitology
Department of Microbiology, Immunology and Pathology, Colorado State University, CO, USA	Postdoctoral	1999-2003	Immunology Bacteriology

A. Personal Statement

The goal of this proposal is to understand the mechanisms by which *Burkholderia pseudomallei (Bp)*, establishes and maintains persistent enteric infections. Among other goals of this proposal is to identify the cells in the GI tract where the organism is maintained during chronic infection. My role as a collaborator in these studies is derived from my previous experience in the field of immunopathology My experience in the field of infectious diseases, immunology, pathology and inflammatory responses entails studies in viral, parasites or bacterial infections (e.g *African swine fever* virus, HIV, *Theileria Parva* and *Mycobacterium tuberculosis*, *Mycobacterium leprae*) in pigs, cattle, mice or human. . More specifically, during the last ten years I have worked in the tuberculosis field revealing the nature of immune and inflammatory host responses to mycobacterial infections. This work has provided important information about the ability of the infecting bacilli to establish persistence and potentially latent disease as well as practical ways of using targeted lung therapies to the benefit of the host. During the last five years I have also been working in studies involving the immune responses to the chronic inflammatory responses developed after implantation of medical devices. Finally, most recently I am a collaborator in a project funded by the Rocky Mountain Regional Center for Biodefense and Emerging Infectious Diseases granted to Drs. John T. Belisle and E. Torsten. The aim of this study is

Burkholderia pseudomallei

immunological tools and assays aiming to define the Immunolipidome of *Burkholderia pseudomallei*. Thus, while developing the above mentioned research I have acquired significant expertise in *in vivo* and *in vitro* models of infection as well as in flow cytometry, immunocytochesmistry and confocal microscopy in tissue sections. In addition, my studies have involved the design of vaccines or immunotherapies using molecular biology and immunological tools aiming to promote protection of the host against pathogen infection

B. Positions and Honors

1985- 1987	Research scholarship from USDA/OICD/International Research Division, program #G-5-272
1987- 1989	Research Scholarship from US-Spain Joint Committee for Scientific and Technology Cooperation, Program #G11
1998	Volunteer researcher at the Ethiopian-Netherlands AIDS Research Project (ENARP), Addis Ababa, Ethiopia.
1999- 2003	Awarded grant ROI AI-44072 Supplement to promote Reentry into Biomedical and Behavioral Research Careers
2003- 2009	Assistant Professor at the Department of Microbiology, Immunology and Pathology, Colorado State University, CO, USA
2005 present	Affiliate Faculty Cell and Molecular Biology Department, Colorado State University.
2007 -present	Philippines
July 2009-	Associate Professor at the Department of Microbiology, Immunology and Pathology, Colorado State University, CO, USA

Professional Memberships

Memberships

American Society of Microbiology
American Association of Immunology
Society of Leukocyte Biology

Editorial boards

Member of the Editorial Advisory Board of Tuberculosis. July 2007 to present
Member of the Editorial Board FEMS Immunology and Medical Microbiology. May 2008 to present.

C. Selected Peer-reviewed Publications (Selected from 30 peer-reviewed publications)

Most relevant to the current application

1. Higgins DM, Basaraba RJ, Hohnbaum AC, Lee EJ, Grainger DW, Gonzalez-Juarrero Localized immunosuppressive environment in the foreign body response to implanted biomaterials. M.Am J Pathol. 2009 Jul;175(1):161-70. Epub 2009 Jun 15.PMID: 19528351 [PubMed - indexed for MEDLINE
2. [redacted]
3. Ordway D, Henao-Tamayo M, Orme IM, Gonzalez-Juarrero M Foamy macrophages within lung granulomas of mice infected with Mycobacterium tuberculosis express molecules characteristic of dendritic cells and antiapoptotic markers of the TNF receptor-associated factor family.. J Immunol. 2005 Sep 15;175(6):3873-81.PMID: 16148133 [PubMed - indexed for MEDLINE]
4. Gonzalez-Juarrero M, Hattle JM, Izzo A, Junqueira-Kipnis AP, Shim TS, Trapnell BC, Cooper AM, Orme IM Disruption of granulocyte macrophage-colony stimulating factor production in the lungs severely affects the ability of mice to control Mycobacterium tuberculosis infection .. J Leukoc Biol. 2005 Jun;77(6):914-22. Epub 2005 Mar 14.PMID: 15767289 [PubMed - indexed for MEDLINE]
5. Rosas-Taraco AG, Higgins DM, Sánchez-Campillo J, Lee EJ, Orme IM, González-Juarrero Intrapulmonary delivery of XCL1-targeting small interfering RNA in mice chronically infected with Mycobacterium tuberculosis . M. Am J Respir Cell Mol Biol. 2009 Aug;41(2):136-45. Epub 2008 Dec 18.PMID: 19097989 [PubMed - indexed for MEDLINE]

Additional recent publications of importance to the field (in chronological order)

1. Gonzalez-Juarrero M, Kingry LC, Ordway DJ, Henao-Tamayo M, Harton M, Basaraba RJ, Hanneman WH, Orme IM, Slayden RA Immune response to Mycobacterium tuberculosis and identification of molecular markers of disease.. Am J Respir Cell Mol Biol. 2009 Apr;40(4):398-409. Epub 2008 Sep 11.PMID: 18787176 [PubMed - indexed for MEDLINE]
2. Orme I, Gonzalez-Juarrero Animal models of M. tuberculosis Infection. M. Curr Protoc Microbiol. 2007 Nov;Chapter 10:Unit 10A.5.PMID: 18770606 [PubMed - indexed for MEDLINE]
3. Chamberlain LM, Godek ML, Gonzalez-Juarrero M, Grainger DW Phenotypic non-equivalence of murine (monocyte-) macrophage cells in biomaterial and inflammatory models. .J Biomed Mater Res A. 2009 Mar 15;88(4):858-71.PMID: 18357567 [PubMed - indexed for MEDLINE]
4. Higgins DM, Sanchez-Campillo J, Rosas-Taraco AG, Higgins JR, Lee EJ, Orme IM, Gonzalez-Juarrero Relative levels of M-CSF and GM-CSF influence the specific generation of macrophage populations during infection with Mycobacterium tuberculosis M. J Immunol. 2008 Apr 1;180(7):4892-900.PMID: 18354213 [PubMed - indexed for MEDLINE
5. Ordway D, Higgins DM, Sanchez-Campillo J, Spencer JS, Henao-Tamayo M, Harton M, Orme IM, Gonzalez Juarrero M XCL1 (lymphotactin) chemokine produced by activated CD8 T cells during the chronic stage of infection with Mycobacterium tuberculosis negatively affects production of IFN-gamma by CD4 T cells and participates in granuloma stability..J Leukoc Biol. 2007 Nov;82(5):1221-9. Epub 2007 Aug 15.PMID: 17699612 [PubMed - indexed for MEDLINE]
6. Ordway D, Harton M, Henao-Tamayo M, Montoya R, Orme IM, Gonzalez-Juarrero M Enhanced macrophage activity in granulomatous lesions of immune mice challenged with Mycobacterium tuberculosis.. J Immunol. 2006 Apr 15;176(8):4931-9.PMID: 16585589 [PubMed - indexed for MEDLINE]
7. Ordway D, Henao-Tamayo M, Orme IM, Gonzalez-Juarrero M Foamy macrophages within lung granulomas of mice infected with Mycobacterium tuberculosis express molecules characteristic of dendritic cells and antiapoptotic markers of the TNF receptor-associated factor family.. J Immunol. 2005 Sep 15;175(6):3873-81.PMID: 16148133 [PubMed - indexed for MEDLINE]
8. Taylor JL, Ordway DJ, Troudt J, Gonzalez-Juarrero M, Basaraba RJ, Orme IM Factors associated with severe granulomatous pneumonia in Mycobacterium tuberculosis-infected mice vaccinated therapeutically with hsp65 DNA.. Infect Immun. 2005 Aug;73(8):5189-93.PMID: 16041037 [PubMed - indexed for MEDLINE]
9. Gonzalez-Juarrero M, Shim TS, Kipnis A, Junqueira-Kipnis AP, Orme IM Dynamics of macrophage cell populations during murine pulmonary tuberculosis.. J Immunol. 2003 Sep 15;171(6):3128-35.PMID: 12960339 [PubMed - indexed for MEDLINE
10. González-Juarrero M, Turner J, Basaraba RJ, Belisle JT, Orme IM Florid pulmonary inflammatory responses in mice vaccinated with Antigen-85 pulsed dendritic cells and challenged by aerosol with Mycobacterium tuberculosis. Cell Immunol. 2002 Nov;220(1):13-9.PMID: 12718935 [PubMed - indexed for MEDLINE]

C.-Research Support.

Department of Microbiology, Immunology and Pathology, Bridge Funds. 07/01/09-12/31/09

-10 against *Mycobaterium tuberculosis".*

Role PI
Co: Peter Murray.

[redacted]

RO1 AI-45707 (I. M. Orme, PI) 12/10/1998-3/31/2009
NIH/NIAID
Chronic Tuberculosis: Latent or Dynamic
The major goal of this project is to determine the immunological mechanisms underlying chronic TB and its reactivation
Role: CO-PI

2RO1 EB000894-06A1 09/01/2007-06/31/2008
NIBIB/NIH
Molecular comparison of macrophage foreign body responses
The major goal is the definition of macrophage models to study the inflammatory responses originated by surgically implanted biomaterials
Role: PI (CSU Subcontract EB 00894)

1S10RR023735-01 10/01/2006- 01/10/2008
National Center For Research Resources
Purchase, installation of Zeiss LSM 510 Laser Scanning Microscope. Creation of a confocal core unit for the MIP
Role: PI

ROI AI-063457 (V. D. Vissa, PI) 05/15/2005-01/31/2010
NIH/NIAID
Molecular Epidemiology of Leprosy
The major role of this grant is to determine the epidemiological changes of different isolates of *M. leprae*.
Role: Consultant

ROI AI-44072 (I. M. Orme) 08/01/2000- 08/01/2003
NIH/NIAID
Chronic Tuberculosis: Latent or Dynamic
The major goal of this project is to understand the basis of latent tuberculosis
Role: PI of Supplement to promote Reentry into Biomedical and Behavioral Research Careers

Program Director/Principal Investigator (Last, First, Middle):

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Schweizer, Herbert Paul	Professor of Microbiology
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Konstanz	Diplome	09/80	Microbiology
University of Konstanz	Ph.D.	07/83	Microbiology
Postdoctoral Training (see Positions & Empl.)			

A. Personal Statement. For over two decades my research group has studied various aspects of bacterial physiology and genetics, most notably mechanisms of *Pseudomonas aeruginosa* fatty acid biosynthesis and antimicrobial resistance mechanisms. About 5 years ago these studies were extended to *Burkholderia pseudomallei*. In this short period of time, my research group developed a nationally and internationally recognized *B. pseudomallei* research program. We constructed state-of-the-art select agent compliant genetic tools, procured a large collection of clinical and environmental *B. pseudomallei* strains from diverse geographical sources, contributed to the understanding of mechanisms of resistance to clinically significant antibiotics, tested novel compounds for anti-*B. pseudomallei* activity, and co-authored policy papers on working with this biodefense pathogen. I am serving as a subject matter expert on *B. pseudomallei* with the Dept. of Homeland Security and the Centers for Disease Control and Prevention. I am therefore well qualified to contribute to the proposed studies.

B. Positions and Honors.

Positions and Employment

1983-1984 Postdoctoral Fellow, Division of Microbiology, Univ. of Konstanz, Faculty for Biology
1984-1986 Postdoctoral Fellow, Dept. of Biochemistry & Molecular Biology, Univ. of North Dakota Medical School, and Dept. of Biochemistry, Virginia Polytechnic Inst. & State Univ.
1986-1989 Research Assistant Professor, Dept. of Biological Chemistry, Univ. of Michigan Medical School
1989-1992 Assistant Professor, Dept. of Microbiology & Infectious Diseases, Univ. of Calgary Health Sciences Center
1992-1995 Associate Professor, Dept. of Microbiology & Infectious Diseases, Univ. of Calgary Health Sciences Center
1995-2001 Associate Professor, Dept. of Microbiology, Colorado State University
2001-2002 Professor, Dept. of Microbiology, Colorado State University
2002-2008 Professor, Associate Dept. Head for Graduate Studies and Research, Dept. of Microbiology, Immunology and Pathology, Colorado State University
2007- Associate Director, Rocky Mountain Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research
2008- Professor, Associate Dept. Head, Dept. of Microbiology, Immunology and Pathology, Colorado State University

Other Experience and Professional Memberships

1996- Editorial Board, Biotechniques
2001 National Institutes of Health, SBIR Study Section, ad hoc
2001,2003 National Institutes of Health, Bacteriology and Mycology 1 and 2 Study Sections, ad hoc
2004-2009 National Institutes of Health, IDM Study Section, member
2006 National Institutes of Health, DDR Study Section, ad hoc

2006	USDA grant review panel member
2006	BEI Repository, NIAID Scientific Review Committee
2007-2009	National Institutes of Health, IDM Study Section, Chair
2009	NIH ARRA SBIR/STTR review panel, Chair
2009-	Scientific Advisory Board, Great Lakes RCE
2010-	NIH Center for Scientific Review College of Reviewers, member
Ongoing	Consulting for private companies and government agencies
Ongoing	Peer review of journal articles, and proposal reviews and site visits for other national and international granting agencies

Honors

1983	Ph.D. Summa Cum Laude, University of Konstanz
1984	Feodor Lynen Postdoctoral Fellowship, Alexander von Humboldt Foundation
1995	Scholarship, Medical Research Council of Canada
1992-1995	Member of the Centers for Excellence of the Canadian Bacterial Diseases Network
2006	Elected to American Academy of Microbiology

C. Selected peer-reviewed publications (in chronological order from 122 total).

1. **Chuanchuen, R., T. Murata, N. Gotoh and H.P. Schweizer**. 2005. Substrate-dependent utilization of OprM or OpmH by the *Pseudomonas aeruginosa* MexJK efflux pump. Antimicrob. Agents. Chemother. 49:2133-2136.
2. **Kumar, A. and H.P. Schweizer.** 2005. Bacterial resistance to antibiotics: active efflux and reduced uptake. Adv. Drug Del. Rev. 57:1486-1513.
3. **Kumar, A. K.-L. Chua, H.P. Schweizer**. 2006. Method for regulated expression of single-copy efflux pump genes in a surrogate *Pseudomonas aeruginosa* strain: identification of the BpeEF-OprC chloramphenicol and trimethoprim efflux pump of *Burkholderia pseudomallei* 1026b. Antimicrob. Agents Chemother. 50:3460-3463.
4. **Schweizer, H.P.** 2007. Understanding efflux: an asset for drug discovery. 2007. Am. Drug Disc. 2:12-17.
5. **Moir, D.T., T. Opperman, M. Di, H.P. Schweizer,** T.L. Bowlin. 2007. A high-throughput, homogeneous, bioluminescent assay for *Pseudomonas aeruginosa* gyrase inhibitors and other DNA damaging agents. J. Biomolec. Scr. 12:855-864.
6. **Mima, T., S. Joshi, M. Gomez-Escalada, H.P. Schweizer**. 2007. Identification and characterization of TriABC-OpmH, a triclosan efflux pump of *Pseudomonas aeruginosa* requiring two membrane fusion proteins. J. Bacteriol. 189:7600-7609.
7. **Choi, K.-H., T. Mima, Y. Casart, D. Rholl, A. Kumar, I. Beacham, H.P. Schweizer**. 2008. Genetic tools for select agent compliant manipulation of *Burkholderia pseudomallei.* Appl. Env. Microbiol. 74:1064-1075. PMCID: PMC2258562
8. **Peacock, S.J. H.P. Schweizer, D.A.B. Dance, T.L. Smith, J.E. Gee, V. Wuthiekanun, D. DeShazer, I. Steinmetz, P. Tan, B.J. Currie**. 2008. Consensus guidelines on the management of accidental laboratory exposure to *Burkholderia pseudomallei* and *Burkholderia mallei*. Emerg. Infect. Dis. 14(7):e2. PMCID: PMC2600349
9. **Chuanchuen, R., W. Wannaprasat, K. Ajariyakhajorna, H.P. Schweizer**. 2008. Role of the MexXY multidrug efflux pump in aminoglycoside resistance in *Pseudomonas aeruginosa* isolates from *Pseudomonas* mastitis. Microbiol. Immunol. 52:392-398. www3.interscience.wiley.com/journal/120841054/abstract?CRETRY=1&SRETRY+0
10. **Qiu, D., F.H. Damron, T. Mima, H.P. Schweizer,** H.D. Yu. 2008. A series of P_{BAD}-based shuttle vectors for functional analysis of toxic and highly-regulated genes in *Pseudomonas* and *Burkholderia* species, and other bacteria. Appl. Env. Microbiol. 74:7422-7426. PMCID: PMC2592904
11. **Kumar, A., M. Mayo, L. A. Trunck, A. C. Cheng, B.J. Currie, H.P. Schweizer.** 2008. Expression of resistance-nodulation-cell division efflux pumps in commonly used *Burkholderia pseudomallei* strains and clinical isolates from northern Australia. Trans. Royal Soc. Trop. Med. Hyg. 102/S1:S145-S151. http://linkinghub.elsevier.com/retrieve/pii/S0035920308700324

12. **Moir, D.T., M. Di, R. A. Moore, H. P. Schweizer, D. E. Woods.** 2008. Cellular reporter screens for inhibitors of *Burkholderia pseudomallei* targets in *Pseudomonas aeruginosa*. Trans. Royal Soc. Trop. Med. Hyg. 102/S1:S152-S162. http://linkinghub.elsevier.com/retrieve/pii/S0035920308700336
13. **Trunck, L.A., K.L. Propst, V. Wuthiekanun, A. Tuanyok, S.M. Beckstrom-Sternberg, J.S. Beckstrom-Sternberg, S.J. Peacock, P. Keim, S.W. Dow, H. P. Schweizer**. 2009. Molecular Basis of Rare Aminoglycoside Susceptibility and Pathogenesis of *Burkholderia pseudomallei* Clinical Isolates from Thailand, PLoS Negl. Trop. Dis. 3(9): e519. doi:10.1371/journal.pntd.0000519. PMCID: PMC2737630
14. **Lopez, C.M, D.A. Rholl, L.A. Trunck, H.P. Schweizer**. 2009. Versatile dual-technology system for markerless allele replacement in *Burkholderia pseudomallei*. Appl. Env. Microbiol. 75:6496-6503. PMCID: PMC2782473
15. [redacted]

D. Research Support.

ACTIVE

NIH U54 AI065357 Belisle (PI); Schweizer PI of subproject 5/1/09-4/30/14
Title: *Burkholderia pseudomallei* antibiotic resistance mechanisms
The major goals of this project are to identify resistance mechanisms for clinically significant antibiotics and to generate knowledge and tools for rapid identification of resistance mechanisms.

NIH U54 AI065357 Belisle (PI); Schweizer PI of subproject 5/1/09-4/30/14
Title: RMRCE Developmental proposals
The goals of this project are to administer the Developmental Research Project aspects of the RMRCE.

NIH UO1 AI082052 Bowlin (PI); Schweizer (PI of subcontract) 6/1/09-5/31/11
Title: Development of a novel lead series against category A & B bacterial pathogens
The major goals of this project are to use rational drug design strategies to further develop a lead series of antibacterial compounds. Efforts in Dr. Schweizer's laboratory will be directed towards assessing *in vitro* and *in vivo* efficacies against *Burkholderia pseudomallei*.

NIH R43 AI79986 Moir (PI); Schweizer (PI of subcontract) 6/15/08-6/14/10
Title: Therapeutics targeting fatty acid synthesis in *Pseudomonas aeruginosa*
The major goals of this project are to identify novel fatty acid synthesis inhibitors and to evaluate their *in vitro* efficacies.

NIH U54 AI065357 Belisle (PI); Schweizer PI of subproject 6/1/05-4/30/10
Title: *Burkholderia* spp.: novel therapeutic approaches
The goals of this project are to identify and evaluate efflux pump inhibitors as therapeutics for *Burkholderia pseudomallei* and related species.

NIH U54 AI065357 Supplement Belisle (PI); Schweizer (PI of subproject) 5/1/08-4/30/10
Title: Evaluation of Novel Melioidosis Therapeutics
The goals of this project are to evaluate several investigational drugs as novel melioidosis therapeutics.

COMPLETED

HDTRA1-08-C-0049 Flavin (PI); Schweizer (PI of subcontract) 10/15/08-12/30/09
Title: Development of cethromycin, a novel antibiotic
The major goal of this project is to evaluate the efficacy of cethromycin as an antibiotic against various biodefense pathiogens. Efforts in Dr. Schweizer's laboratory are aimed at defining *in vitro* efficacy against *Burkholderia pseudomallei* and to assess potential resistance mechanisms.

Program Director/Principal Investigator (Last, First, Middle):

[redacted]

NIH U54 AI065357 Supplement Belisle (PI); Schweizer PI of subproject 12/1/06-4/30/09
Title: Attenuated BSL2-Compatible *Burkholderia mallei* and *B. pseudomallei* Strains
The major goals of this project are to engineer avirulent *Burkholderia* strains for which reagent exempt status can be sought through CDC's Select Agent program.

NIH U54 AI065357 Supplement Belisle (PI); Eckstein (PI) and Schweizer 5/1/07-4/30/09
(co-investigator on sub-sproject)
Title: Defining the Immunolipidome of *Burkholderia pseudomallei*
The goals of this project are to identify *B. pseudomallei*-specific immunogenic lipids with the ultimate purpose of developing species-specific diagnostics. Dr. Schweizer is providing *B. pseudomallei* extracts and expertise.

[redacted]

RO3 AI058141 Schweizer (PI) 7/1/04-6/30/07
Title: Genetic tools for pathogenic bacteria
The goals of this project were to develop new genetic tools for pathogenic bacteria, specifically novel Tn*7*-based gene integration vectors.

PENDING

[redacted]

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
* First Name:
Middle Name: W
* Last Name: Dow
Suffix:

2. Human Subjects

Clinical Trial? ☒ No ☐ Yes

* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix:
* First Name: Christine
Middle Name:
* Last Name: Getzelman
Suffix:

* Phone Number: [redacted]
Fax Number: [redacted]
Email: [redacted]

* Title: Senior Research Administrator

* Street1: 601 S. Howes
Street2:
* City: Fort Collins
County/Parish:
* State: CO: Colorado
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 80523-2002

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Tracking N er:GRANT10529698 Funding O uni y Number:PA-10-069 Received Da e:2010-02-11T17:02:50-04:00

PHS 398 Modular Budget, Periods 1 and 2

OMB Number: 0925-0001

Budget Period: 1

Start Date: 08/01/2010 End Date: 07/31/2011

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	[redacted]
Consortium F&A	
* Total Direct Costs	[redacted]

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs (MTDC)	47	[redacted]	[redacted]
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) DHHS, Wallace Chan, [redacted]

Indirect Cost Rate Agreement Date 06/26/2009

Total Indirect Costs [redacted]

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) [redacted]

Budget Period: 2

Start Date: 08/01/2011 End Date: 07/31/2012

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	[redacted]
Consortium F&A	
* Total Direct Costs	[redacted]

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs (MTDC)	[redacted]	[redacted]	[redacted]
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) DHHS, Wallace Chan, [redacted]

Indirect Cost Rate Agreement Date 06/26/2009

Total Indirect Costs [redacted]

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) [redacted]

PHS 398 Modular Budget, Periods 3 and 4

Budget Period: 3

Start Date: | End Date:

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	
Consortium F&A	
* Total Direct Costs	

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.				
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number)

Indirect Cost Rate Agreement Date | Total Indirect Costs

C. Total Direct and Indirect Costs (A + B) Funds Requested ($)

Budget Period: 4

Start Date: | End Date:

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	
Consortium F&A	
* Total Direct Costs	

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.				
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number)

Indirect Cost Rate Agreement Date | Total Indirect Costs

C. Total Direct and Indirect Costs (A + B) Funds Requested ($)

Tracking N er:GRANT10529698 Funding O uni y Number:PA-10-069 Received Da e:2010-02-11T17:02:50-04:00

PHS 398 Modular Budget, Periods 5 and Cumulative

Budget Period: 5

Start Date: End Date:

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	
Consortium F&A	
* Total Direct Costs	

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.				
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number)

Indirect Cost Rate Agreement Date

Total Indirect Costs

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($)

Cumulative Budget Information

1. Total Costs, Entire Project Period

*Section A, Total Direct Cost less Consortium F&A for Entire Project Period	$ [redacted]
Section A, Total Consortium F&A for Entire Project Period	$
*Section A, Total Direct Costs for Entire Project Period	$ [redacted]
*Section B, Total Indirect Costs for Entire Project Period	$ [redacted]
*Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period	$ [redacted]

2. Budget Justifications

Personnel Justification	1245-DowBdgtJust.pdf	Add Attachment	Delete Attachment	View Attachment
Consortium Justification		Add Attachment	Delete Attachment	View Attachment
Additional Narrative Justification		Add Attachment	Delete Attachment	View Attachment

Tracking N er:GRANT10529698 Funding O uni y Number:PA-10-069 Received Da e:2010-02-11T17:02:50-04:00

Budget justification:

Personnel:

Steve Dow: PI: 1.2 Calendar months effort; duties will include overseeing entire project, plus experimental design and data analysis and interpretation; manuscript preparation

Mercedes Gonzalez-Juarerro: Co-I; 3.6 Calendar months effort; her duties will include conducting some of the animal infection experiments as well as bacterium localization experiments, using immunohistochemistry and laser confocal microscopy

Andrew Goodyear (Grad student): 6 Calendar months effort; his duties will include conducting animal challenge experiments as well as quantitation of bacteria in the gut and other tissues; also screening of new isolates of B. pseudomallei in vivo and in vitro

OMB Number: 0925-0001

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)		Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	1240-Specific Aims.pdf	Add Attachment	Delete Attachment	View Attachment
3. *Research Strategy	1241-Research Strategy.pdf	Add Attachment	Delete Attachment	View Attachment
4. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
5. Progress Report Publication List		Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

6. Protection of Human Subjects		Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
8. Targeted/Planned Enrollment Table		Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

10. Vertebrate Animals	1246-Vertebrate animals.pdf	Add Attachment	Delete Attachment	View Attachment
11. Select Agent Research	1247-Select Agent research.p	Add Attachment	Delete Attachment	View Attachment
12. Multiple PD/PI Leadership Plan		Add Attachment	Delete Attachment	View Attachment
13. Consortium/Contractual Arrangements		Add Attachment	Delete Attachment	View Attachment
14. Letters of Support	1248-Dow Letters of Support	Add Attachment	Delete Attachment	View Attachment
15. Resource Sharing Plan(s)	1249-Resource sharing plan.p	Add Attachment	Delete Attachment	View Attachment

16. Appendix Add Attachments | Remove Attachments | View Attachments

Tracking N er:GRANT10529698 Funding O uni y Number:PA-10-069 Received Da e:2010-02-11T17:02:50-04:00

2. Specific Aims. The overall goal of this proposal is to better understand how the Gram-negative bacterial pathogen *Burkholderia pseudomallei (Bp)* initiates and sustains infections in the GI tract. The *Bp* bacterium is normally found in soil and water, but is also a deadly pathogen in humans, where it can cause a variety of difficult-to-treat infections ranging from acute sepsis to chronic abscesses. While *Bp* is endemic in southeast Asia and northern Australia, infections are now being diagnosed with increasing frequency around the world, including in Central and South America. Therefore, it is likely that *Bp* infections will soon be identified in the U.S., as the result of either deliberate or accidental introduction. Thus, the proposed studies to gain a better understanding of the pathogenesis of *Bp* infection can be justified based on both national and international health concerns.

Little is known regarding how infection with *Bp* develops, though inhalation or cutaneous inoculation are currently considered the most likely routes of infection. *However, our new studies indicate that Bp is actually a primary enteric pathogen, which can readily establish acute or persistent GI tract infection following oral inoculation in mouse models. Furthermore, our findings also suggest that GI tract is the primary reservoir for maintenance and dissemination of Bp during chronic infection*. Thus, re-defining *Bp* as a primary enteric pathogen will have major implications for understanding how humans are infected with *Bp* and the risks posed by *Bp* contaminated food, soil and water. However, at present essentially nothing is known regarding the pathogenesis of enteric infection with *Bp*.

Therefore, the studies proposed here are intended to fill a critical void in our understanding of pathogenesis of infection with this important and emerging bacterial pathogen. To address these knowledge gaps, we will use a mouse model of enteric *Bp* infection developed in our lab to answer three key questions. First, is enteropathogenicity a property of all isolates of *Bp*, or are only certain isolates virulent after oral inoculation? If highly virulent enteric strains of *Bp* are identified, will *in vitro* assays of invasion correlate with the virulence phenotype? Secondly, what are the target cells for *Bp* infection in the intestine during acute and chronic infection? This information could be very important for developing new vaccination or treatment strategies. Third, how does *Bp* disseminate from the intestine to other organs following enteric infection, since widely disseminated infections are a key feature of *Bp* infection? For example, if dissemination were found to be primarily cell-associated, then different classes of antimicrobials could be used for treatment of chronic infection as opposed to acute infection. The information generated from these studies may substantially alter our view of *Bp* as a pathogen and lead to a reassessment of the risks posed by oral *Bp* infection. The questions raised above will be addressed by means of 3 Specific Aims.

Aim 1. Determine whether enteropathogenicity is a general feature of all or only some *B. pseudomallei* isolates.

Aim 2. Identify intestinal target cells for *B. pseudomallei* during acute and chronic enteric infection.

Aim 3. Determine how *B. pseudomallei* disseminates from the GI tract following oral inoculation.

3. Research Strategy

(a) Significance. *Burkholderia pseudomallei* (*Bp*) infection is a Gram-negative bacterial pathogen that normally survives as a saprophyte in soil and water, but is also capable of infecting most mammals and causing serious infections (1-5). *Bp* infection is a major cause of bacterial sepsis and chronic disseminated infections (meliodosis) in humans in Thailand and northern Australia (4-8). The fatality rate for patients with *Bp* infection, even with prompt and aggressive treatment, still ranges from 20% to over 50%. Moreover, *Bp* is an emerging pathogen and infections have been increasingly reported in many regions of the world, including Central and South America (9-13). In fact, *Bp* infection is now considered endemic in regions of China and India, and in Brazil (11, 12, 14). Infections with *Bp* are particularly dangerous because the organism is intrinsically resistant to many antimicrobials, can persist for years in the soil and in water, and can cause a wide array of clinical symptoms, ranging from acute sepsis, to chronic recurrent infection, to clinically silent infection (5-8, 15-17). Meliodosis is also an increasing problem in travelers who have visited regions of the world where *Bp* is endemic(18). Thus, *Bp* is dangerous bacterial pathogen with high potential for spread into new regions of the world including the U.S. via deliberate or accidental introduction in soil, food, or water.

Currently *Bp* is not considered a primary enteric pathogen for infection of humans. At present, infection with *Bp* is presumed to occur following inhalation or cutaneous inoculation, though the actual link between cutaneous exposure and infection is weak (Dr. Sharon Peacock, see Letter of Support). Thus, current treatment and prevention efforts for human meliodosis do not consider the impact of oral infection or persistent fecal carriage and shedding of the organism (5, 7, 8). There is however epidemiological evidence to suggest that oral infection with *Bp* does occur in humans. For example, outbreaks of meliodosis in villages in Indonesia have been linked directly to drinking water supplies contaminated with *Bp*, which can survive for years in water(19). Infections with *Bp* increase significantly during times of greater exposure to very wet conditions (eg, rice farming during the monsoon season), which would be consistent with oral exposure to a water borne agent (20-22). Outbreaks of meliodosis have also been associated with tsunami events (23, 24). In addition, patients with meliodosis have been misdiagnosed as having typhoid (enteric fever)(25). In fact, clinical observations (Dr. Peacock, personal communication) suggest that oral infection may be a much more important route of infection with *Bp* in humans than previously assumed. Since *Bp* can persist in water or soil for years, enteric infection of humans with *Bp* would have major public health consequences(26, 27).

Virtually nothing is known regarding enteric infection with *Bp*. Development of a new animal model of enteric *Bp* infection would therefore be valuable for several reasons. For one, a mouse enteric *Bp* model would be essential for helping understand the pathogenesis of enteric meliodosis in humans. A new mouse model of an enteric *Bp* infection would also add an important new animal model for study of enteric pathogens in general. From a clinical perspective, an enteric *Bp* infection model in mice would also be critical for development of new vaccines for *Bp* and for development of new antimicrobial treatment and prevention strategies.

(b) Innovation. We have spent several years developing mouse models of *Burkholderi*a infection for evaluating new immunotherapeutic approaches to treatment and for investigating the immunopathogenesis of pulmonary infection with *B. mallei* and *B. pseudomallei* (28-30). In the course of these studies, we made the unexpected observation that mice could be easily infected with *Bp* following oral inoculation and remain persistently infected and undergo persistent fecal shedding of *Bp*. We have determined mouse strain differences in susceptibility to oral infection with *Bp*. It also became clear from our studies that the ability to cause enteric infection was not only a property of laboratory adapted strains of *Bp*. These preliminary data therefore provide compelling new evidence that *Bp* is an enteric pathogen, a fact not previously appreciated by others in the field. Thus, we have now developed a new mouse model of enteric infection with *Bp* that has the potential to substantially alter the current *Bp* infection paradigm.

Our access to a collection of over 30 clinical isolates of *Bp* obtained from patients in Thailand and Australia provides a key resource for these studies. This panel of *Bp* isolates, provided by our

collaborator Dr. Herbert Schweizer (CSU), has proven very useful previously for investigating *Bp* antibiotic resistance mechanisms (31-34). Studies with these *Bp* isolates have led to a productive and ongoing collaboration between the Dow and Schweizer labs, as evidenced by several publications in press or recently published(30). Moreover, this panel of isolates has allowed us to determine in initial oral animal challenge studies that enteric virulence is likely a general property of nearly all *Bp* stains, and not just the laboratory-adapted 1026b strain.

Our studies have also benefitted from a key technical innovation, namely the development by our lab of a new selective medium for culture of *Bp* from intestinal contents and feces (35, 36). This new modified Ashdown's medium has been extremely useful for identifying and quantifying *Bp* in the intestines and feces of infected mice. The new medium suppresses the growth of all normal commensal enteric bacteria found in mice, while selectively allowing the growth of *Bp* from intestinal contents and feces, including all clinical *Bp* isolates tested to date. Currently, this medium is being evaluated for use with clinical specimens from human patients in Thailand, in a collaborative effort with Dr. Peacock.

A number of studies have investigated animal models for meliodosis, primarily in the context of pneumonic infection, and to date none have investigated oral challenge models (37-43). Therefore, we believe the enteric meliodosis infection model we have developed is unique. In addition, the *Bp* oral infection model has several unique features compared to other enteric bacterial infection models in mice (44-47). For example, in mice with enteric *Bp* infection, intestinal lesions are very mild and consist primarily of scattered mononuclear cell infiltrates. Mice with enteric *Bp* infection exhibit few overt signs of infection and do not develop diarrhea. Most mice that develop chronic enteric infection following low-dose inoculation with *Bp* go on to develop disseminated infection to the spleen and CNS over a 45-90 day period. Thus, enteric infection with *Bp* resembles in many respects typhoid fever caused by *Salmonella typhi* in humans and in mouse models, but with several key differences, including 1) greater susceptibility to low-dose oral challenge with *B*p; 2) lack of neutrophilic inflammation in *Bp*-infected mice; and 3) the relatively high prevalence (15-20%) of CNS infection following oral *Bp* infection. Thus, the mouse model of enteric *Bp* infection is novel and should prove useful for generating insights into general mechanisms of enteric bacterial infection.

(c) Approach

Preliminary studies. During the course of developing new animal models of chronic *Bp* infection, we discovered that most chronically infected mice actually harbored substantial numbers of *Bp* in their intestinal tract. This finding prompted us to investigate in greater detail how susceptible mice were to oral inoculation with *Bp*. First, we determined LD_{50} doses for *Bp* strain 1026b when inoculated orally in 3 inbred strains of mice (**Table 1**). Importantly, the LD_{50} doses determined for *Bp* 1026b were much lower than those typically reported for *Salmonella* and *Shigella* infection in mouse models (44, 47, 48). In addition, pre-treatment with antimicrobials or fasting of the mice was not required for succesesful infection. All 3 strains of mice tested were readily infected orally with *Bp*, with 129 Sv/Ev strain mice being extremely susceptible to oral inoculation. We also wished to determine whether oral susceptibility was a unique

Strain	LD_{50} (cfu)
129 Sv/Ev	1.9×10^3
BALB/c	1.0×10^7
C57Bl/6	7.1×10^6

Table 1. LD50 doses for Bp for oral inoculation

To accurately quantitate *Bp* bacterial burdens in the GI tract, we developed a modified Ashdown's selective agar for culture of *Bp* from intestinal contents of mice. We found that standard Ashdown agar did not effectively suppress the growth of normal enteric bacteria from mice (**Figure 1**). In contrast, the new medium completely suppressed the growth of commensal bacteria from the gut and feces of mice infected with *Bp*, while at the same time allowing for selective growth of *Bp*. Using the modified Ashdown agar, we quantitated *Bp* numbers in the gut of chronically infected mice (**Figure 2**). In all mice infected with *Bp* strain 1026b, we found that bacterial numbers remained relatively constant over at least a 30-day period at a level of 10^3 to 10^4 CFU in the small intestine, cecum and colon tissues. Moreover, infected animals also persistently shed *Bp* in their feces, at concentrations of approximately 10^3 CFU per gm of feces. Thus, *Bp* readily establishes persistent and chronic enteric infection with fecal shedding in mice.

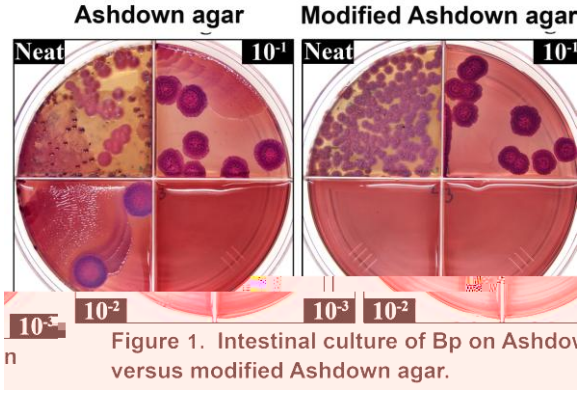


Figure 1. Intestinal culture of Bp on Ashdown versus modified Ashdown agar.

The localization and dissemination studies proposed here will be done using fluorescent GFP and RFP plasmids that can be used to readily produce stable, chromosomally integrated transfectants in nearly all strains of *Bp*. As an example of intracellular expression, macrophages were infected *in vitro* at an MOI of 10 with RFP expressing Bp at 2h and 24h after inoculation, and infected cells readily visualized by fluorescence microscopy (**Figure 3**). These constructs will be provided in collaboration with Dr. Tung Huang (University of Hawaii, see Letter of Collaboration). Cells containing GFP^+ and RFP^+ bacteria will be visualized using confocal microscopy and flow cytometry. Thus, we have the necessary expertise, reagents, bacterial strains, and research infrastructure to complete the proposed studies.

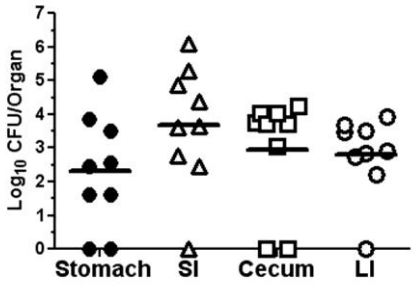
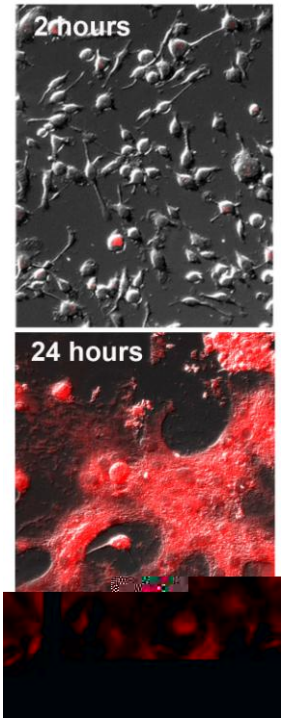


Fig 2. Intestinal colonization with Bp after oral inoculation



Aim 1. Determine whether enteropathogenicity is a general feature of all or only certain *B. pseudomallei* isolates. *Rationale and Hypothesis*. We have observed that the 1026b strain of *Bp* and at least 3 clinical *Bp* isolates efficiently infect the GI tract of adult mice following low-dose oral inoculation, and then disseminate to multiple different organs over a several-month period. However, it is not known if all strains of *Bp* can cause enteric infection, or whether certain strains are particularly virulent following oral inoculation. Nor is it known whether certain *in vitro* properties such as cell invasion and replication can be correlated with *in vivo* virulence, which is an essential step in developing assays for identification of enteric virulence determinants. To address these questions, a low-dose oral challenge model in BALB/c mice will be used to screen a panel of 30 *Bp* isolates for enteric tropism and for virulence. Selected high and low virulence strains of *Bp* will then be further evaluated *in vitro* to assess their ability to invade and replicate in intestinal epithelial cells and macrophages. *We hypothesize that most or all Bp strains can cause enteric infection and that enteric virulence will correlate with increased invasion and replication in intestinal epithelial cells and macrophages*.

Objective 1.1. Screen new *Bp* isolates for enteric infection and virulence.

Experimental Approach. A mouse low-dose oral challenge model will be used to screen a collection of 30 different *Bp* clinical isolates obtained from patients in Thailand and northern Australia by Dr. Herbert Schweizer (see Letter of Collaboration). Prior experience with the *Bp* oral infection model indicates that an oral challenge dose of 10^3 CFU will provide a useful screen for distinguishing enteric from non-enteric isolates. BALB/c mice (n = 4 per group) will be inoculated orally with *Bp* stocks of known titer diluted to deliver 10^3 CFU in an inoculation volume of 200 ul, which will be administered to non-anesthetized mice using a gavage needle to assure delivery deep into the esophagus.

The primary readouts for these challenge experiments will be fecal shedding of *Bp* and survival to day 60 after inoculation. Fresh fecal pellets will be collected from each infected mouse twice weekly and fecal bacterial counts determined from solubilized pellets using modified Ashdown medium. Challenged mice will also be weighed daily and observed for signs of clinical illness. Any ill mice will be euthanized and quantitative bacterial counts determined in GI tissues (small intestine, ileum, cecum, and large intestine) and in the spleen and liver and mesenteric lymph nodes, using culture techniques reported previously (28, 29). Median survival times will be determined using Kaplan-Meier survival curves and compared between *Bp i*solates by log rank analysis, with Bonferroni adjustment for multiple comparisons.

Expected Results, Interpretation, Possible Pitfalls. We expect that at least 3-5 highly pathogenic isolates of *Bp* will be identified, based on evidence of heavy intestinal infection (persistent fecal shedding, high bacterial titers in intestinal cultures), significant weight loss, and short survival times. We also expect to identify 3-5 relatively low virulence *Bp* isolates, which will be defined as those that do not cause death, weight loss, or persistent fecal shedding during the 60 day observation period. For example, based on these criteria, *Bp* strain 1026b would be classified as a low-virulence strain, while *Bp*103 (a new clinical isolate), would be classified as a high-virulence isolate. Therefore, we do not expect major obstacles to using the *in vivo* challenge assay to identify high and low virulence *Bp* isolates. If clearly pathogenic or non-pathogenic *Bp* strains are not identified at the 10^3 CFU oral challenge dose after the first 10 isolates are screened, the challenge dose will be adjusted upward or downward depending on the initial results.

Objective 1.2. Determine whether enteric virulence correlates with increased intestinal epithelial cell or macrophage invasion and replication.

Experimental Approach. Previous studies with *Salmonella* and *Shigella* have shown the enteric virulence correlates with intestinal invasion and cytopathicity(47). Therefore, we will use a mouse primary intestinal epithelial (IE) cell line (mIE2) derived from the Immortomouse® (Robert Whitehead, Vanderbilt University), and a mouse macrophage cell line (RAW267.2) to evaluate invasion and cell killing by *Bp* isolates. These screens will be done using the 3 most and the 3 least virulent enteric *Bp* isolates identified above. For the cell invasion assay, adherent IE or RAW cells in triplicate wells will be infected for 1h with *Bp* at an MOI of 5, then washed and incubated for 1 hour with 10 ug/ml ceftazidime to kill extracellular bacteria. The cells will then be immediately lysed and numbers of intracellular bacteria quantitated, using techniques described previously(28). The ability of different *Bp* strains to invade IE cells will be compared statistically using non-parametric ANOVA, and a similar analysis will be done for macrophage invasion. The cytopathicity assay will be done using the same approach as above, except that cultures will be continued for an additional 24 hours following infection, with 10 ug/ml ceftazidime in the medium to suppress extracellular replication. The number of viable IE or macrophage cells will be determined by MTT assay or by trypan blue exclusion.

Expected Results, Interpretation, Possible Pitfalls. We expect that the more pathogenic *Bp* isolates will invade IE cells and macrophages more efficiently than less pathogenic strains. These results would be important because they would indicate that the ability to invade potential target cells in the intestine correlates with virulence, and this would in turn provide an efficient assay for follow-up studies to identify specific virulence factors. If however invasion is not associated with virulence, this would suggest that intestinal infection may depend on factors other than direct invasion, such as uptake by M cells. Since *Bp* infection causes minimal inflammation in the intestine, we predict that more enteropathogenic strains of *Bp* will paradoxically cause less cell death, despite their ability to infect cells efficiently. Such a result would suggest a mechanism by which *Bp* is able to establish persistent

infection in the GI tract. Since *Bp* is known to be able to infect a number of different cell types, we do not expect problems with either the cell invasion or cytopathicity assays.

Aim 2. Identify intestinal target cells for *B. pseudomallei* during acute and chronic enteric infection. *Rationale and Hypothesis*. Identifying infected cells in the gut is critical to understanding how *Bp* establishes and maintains enteric infection. To address this question, we will use *Bp* strains engineered to stably over-express GFP or RFP, combined with confocal microscopy and flow cytometry to identify *Bp*-infected cells. Examining intestinal tissues over time following infection will allow us to assess early and late targets for *Bp* infection. *We hypothesize that Bp will infect both intestinal epithelial cells and monocytes and macrophages during acute infection, while submucosal macrophages will serve as the primary target cells for chronic infection*.

Objective 2.1. Identify target cells for *Bp* infection during acute and chronic enteric infection.
Experimental Approach. BALB/c mice (n = 4 per group per time point) will be inoculated orally with 5 X 10^5 CFU *Bp* strain 1026b engineered to over-express GFP (see Dr. T. Huang, Letter of Collaboration). Inoculated mice will be euthanized on d1, d3, d7, d14, and d30 after infection. Tissues will be processed for immunohistochemistry (IHC) or flow cytometry, using previously published techniques in our laboratories (49-51). Briefly, sectioned tissues from the GI tract, mesenteric LN, and spleen will be examined using a laser scanning confocal microscope (Zeiss LSM 510 META, 4-laser microscope) available in the laboratory of Dr. Gonzalez-Juarrero (Co-Investigator on this grant). Dual labeling IHC will be utilized to identify cells containing labeled *Bp*, including the following relevant cell markers: F4/80 (mature macrophages); Ly6-G (neutrophils), cytokeratin (epithelial cells); CD11b and Ly6-C (monocytes); CD3 (T cells), and CD11c and DEC-205 (DC). We will also use multicolor flow cytometry to further define the population of infected cells, using techniques reported previously(52).
Expected Results, Interpretation, Possible Pitfalls. We expect that *Bp* will be found primarily within infected intestinal epithelial cells at all levels of the intestine on days 1-3 after inoculation, especially in the ileum and large intestine. This result would be consistent with direct invasion of intestinal epithelium as the primary mechanism of initial enteric infection. By days 7-14, we expect to observe more infected monocytes and macrophages, particularly in the ileum, cecum and large intestine, while infected intestinal epithelial cells will have largely disappeared, consistent with immune elimination or apoptosis. From day 14 onward, we expect that the only *Bp* infected cells in the gut will be macrophages located in submucosal layers of the intestine. We also expect that at these later time points individual infected cells will contain only relatively few (ie, 3-5) bacteria per cell, consistent with a sustained but non-cytopathic and low level infection. We do not expect to find *Bp* associated with M cells or Peyers patches, as we have not been able to culture *Bp* from mesenteric LNs during preliminary studies. If dual-labeling IHC proves problematic, multicolor utilize flow cytometry should prove very useful in helping to conclusively identify *Bp* infected cells. If numbers of infected cells are too low to visualize, we would deliver a higher inoculum of GFP-Bp. We can also employ an anti-Burkholderia capsule antibody obtained from Dr. David Waag (USAMRIID) to detect Bp infected cells, as reported recently for *B. mallei(29)*.

Aim 3. Determine how *B. pseudomallei* disseminates from the GI tract following oral inoculation. *Rationale and Hypothesis*. A major feature of chronic meliodosis in humans is persistent infection and widespread dissemination of infection to various organs. However, a reservoir for persistent infection has not been identified, nor is it known how the bacterium disseminates. Our preliminary studies suggest that in chronic Bp infection, by analogy to *Salmonella typhi* infection, the gut is the primary reservoir persistent infection and that dissemination occurs via infected leukocytes, especially monocytes(48). *We therefore hypothesize that infected monocytes serve as the primary means of disseminating Bp from the gut during enteric infection*.

Objective 3.1. Evaluate entry of Bp into the bloodstream during acute and chronic infection. BALB/c mice (n = 5 per group) will be inoculated orally with GFP-Bp 1026b, then blood samples will be collected for analysis by flow cytometry and blood culture beginning 30 minutes post-inoculation, and continuing at 1h, 3h, 6h, 12h, 24h, 48h, 72h, 7d, 14d, 30d and 60d post-inoculation. The early time

points were selected because extraintestinal *Salmonella typhi* invasion has been shown to occur rapidly (48, 53). The later time points were selected in order to assess the degree to which chronic low level shedding of bacteria is maintained throughout chronic Bp infection. Blood mononuclear cells will be immunostained for flow cytometry and GFP^+ cells will identified using relevant cell surface markers (29, 52, 54). Cytospin preparations of blood cells will also be examined by confocal microscopy. Blood will be cultured after lysing WBC using 0.1% saponin, which we have determined allows efficient detection of *Bp* in blood.

Expected Results, Interpretation, Possible Pitfalls. We expect to observe the rapid appearance of GFP^+ *Bp* in the bloodstream very soon after oral inoculation with *Bp* and predict that the majority of bacteria will be contained within $CD11b^+/Ly6C^+$ inflammatory monocytes. This result would suggest that *Bp* rapidly exits the gut (possibly via infected DC) and enters the bloodstream in a manner similar to that reported for *Salmonella typhimurium* in mice(48). After 24-48 hours, the number of bacteria in the bloodstream should rapidly diminish, while by day 7-14, we expect to observe the reappearance of GFP^+ Bp in monocytes, coincident with an increase of *Bp* numbers in the intestine. Numbers of *Bp* in the bloodstream should then remain relatively constant over the next 30-60 days. One problem in interpretation may be animals that develop disseminated infections in the spleen and become septic. Therefore, in separate studies we will correlate bacterial numbers in the bloodstream with bacterial numbers in the spleen and liver.

Objective 3.2. Determine whether depopulation of gut Bp will reduce bacterial dissemination. The role of the intestine as a chronic reservoir for infection will also be assessed in mice infected with a very low dose (500 CFU) of *Bp* (to avoid rapid dissemination), as this inoculum leads to nearly 100% persistent intestinal colonization within 30d. Beginning 30 days after infection, one group of mice (n = 10 per group) will be treated orally once daily with the non-absorbed antibiotic neomycin (5-10 mg/mouse to depopulate intestinal gram-negative bacteria(55). The incidence of development of disseminated infection will be compared between neomycin treated and untreated mice over the next 60 days.

Expected Results, Interpretation, Possible Pitfalls. Depopulation of gut *B*p bacteria with oral neomycin should reduce the rate of development of disseminated infection if the gut is the primary reservoir of infection. Thus, mice treated with oral neomycin should develop splenic and CNS infections at a significantly reduced rate compared to untreated mice. Interpretation of this result could be confounded if neomycin reaches systemic antibacterial levels (unlikely at the doses proposed here), but we will measure blood levels of neomycin and determine an MIC for *Bp* if positive results are obtained. In the case of negative results, we will culture intestinal contents to assure that Bp is adequately depleted, since resistance to neomycin by *Bp* is also possible.

Objective 3.3. Determine whether dissemination of *Bp* from the gut is reduced in $CD18^{-/-}$ animals. Monocyte and neutrophil migration is severely impaired in mice lacking the integrin CD18 and the $CD18^{-/-}$ mouse model has been used previously to define the role of leukocytes in disseminating *Salmonella* infection(48). Therefore, wild type C57Bl/6 and congenic $CD18^{-/-}$ mice (n = 40 per group) will be infected with a very low dose of *Bp* (500 CFU) and the development of enteric infection and dissemination will be assessed over a 60-day period. At 7 day intervals, groups of mice (n = 5) will be sacrificed and the bacterial burden in spleen, liver, and lung tissues will be determined, as described previously(29).

Expected Results, Interpretation, Possible Pitfalls. We expect that $CD18^{-/-}$ mice will develop disseminated infections in the CNS and spleen at a significantly reduced rate compared to wild type mice, which would suggest that trafficking of the infection via infected monocytes is required for dissemination. If no difference is observed between $CD18^{-/-}$ and WT mice, this would suggest that the bacterium may exist in a non-cell-associated form in the bloodstream. However, it is also possible that $CD18^{-/-}$ mice will succumb at a more rapid rate to *Bp* infection due to their inability to control the primary intestinal infection. This will be assessed using quantitative cultures of feces and intestinal contents in WT and $CD18^{-/-}$ mice.

10. Vertebrate Animals

a. **Mouse studies**. It is estimated that a total of 660 mice will be used for the experiments proposed here. This total will include 500 BALB/c mice, 80 C57Bl/6 mice, and 80 $CD18^{-/-}$ mice on the C57Bl/6 background.

b. **Justification of animal use**. These studies will be done in mice because this is the lowest vertebrate species that can be used to model enteric infection with Burkholderia pseudomallei. In addition, there is already a great deal of prior data generated in this species. For the mouse studies, most experiments will utilize groups of 4 or 5 mice each. Our prior experience with most of the Bp infection models to be employed here indicate that this group size is sufficient to generate statistically significant results, with a power of 80% to detect differences. Comparisons of two groups will be done by non-parametric t-test and between more than two groups will be compared by non-parametric ANOVA and multiple means comparison tests. All in vivo experiments will be repeated at least once to assure reproducibility.

c. **Veterinary care**. Mice will be housed within the rodent holding facilities in the Regional Biocontainment Laboratory at the Foothills Campus at CSU. Animals will be cared for under the direction of Lab Animal Resources, which includes at least one veterinarian on call at all times. The animal facilities at CSU have been ALAAC approved and are inspected annually.

d. **Humane treatment**. For all Bp challenge experiments, mice will be monitored 3 times daily during the first 7 days after infection, then once to twice daily during the chronic phases of infection. Animals that exhibit weight loss > 20%, reluctance to move, inappetance, and/or significant respiratory distress will be immediately euthanized.

e. **Method of euthanasia**. Any mice that require euthanasia will be euthanized by inhalation of CO2, as recommended by the American Veterinary Medical Association.

11. Select Agents

1. The project involves use of *Burkholderia pseudomallei*, which is considered a category B Select Agent

2. The facilities at Colorado State University are registered with CDC under entity number C20070924-0686.

3. Possession and use of Select Agents is monitored by the responsible university biosafety officers. For transfer of Select Agents, Federal guidelines are followed, including filing of USDA import permits (where applicable), CDC/APHIS Form 2 and other permits (where applicable).

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January 20, 2010

Steve Dow, Ph.D.
Department of Microbiology, Immunology and Pathology
College of Veterinary Medicine and Biomedical Sciences
Colorado State University
Fort Collins, CO 80523-1678

Dear Steve,

I am writing this letter to indicate my excitement and willingness to collaborate with you on your grant proposal "Mechanisms of Enteric *Burkholderia pseudomallei* Infection". The studies you propose are very interesting and suggest quite a novel route of infection with this organism. These studies will also help to extend our recent and ongoing collaborations in animal infection models with attenuated strains of *B. pseudomallei*.

As you know, we have successfully produced stably transfected GFP and RFP expressing *B. pseudomallei* strains that can be used for in vitro and in vivo tracking. We will therefore be happy to provide you with our GFP and RFP plasmids that can be used to transform your *B. pseudomallei* isolates for in vivo and in vitro tracking studies. I wish you luck with your proposal and enthusiastically look forward to our ongoing collaborations.

Sincerely and best regards,

Tung T. Hoang, Ph.D.

15. Resource Sharing Plan. Once results of the studies have been published, we will be willing to provide, in conjunction with the Schweizer lab and subject to the approvals of the MTA under which the Thai isolates of B. pseudomallei were obtained, organisms that were identified as valuable in the course of these studies.

PHS 398 Checklist

OMB Number: 0925-0001

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier:

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☐

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ☐ No ☐

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4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. * Disclosure Permission Statement

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

☒ Yes ☐ No

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