APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED	Applicant Identifier
3. DATE RECEIVED BY STATE	**State Application Identifier**

1. * TYPE OF SUBMISSION

☐ Pre-application ☒ Application ☐ Changed/Corrected Application

4. Federal Identifier

5. APPLICANT INFORMATION

*** Organizational DUNS:** 168559177

* Legal Name: UNIVERSITY OF NEBRASKA MEDICAL CENTER

Department: | Division:

* Street1: 987835 NEBRASKA MEDICAL CENTER

Street2: N/A

* City: OMAHA | County: Douglas

* State: NE: Nebraska | Province:

* Country: USA: UNITED STATES | * ZIP / Postal Code: 68198-7835

Person to be contacted on matters involving this application

Prefix: Ms. | * First Name: Deborah | Middle Name: K

* Last Name: Vetter | Suffix:

* Phone Number: 402-559-7456 | Fax Number: 402-559-2957

Email: spadmin@unmc.edu

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):* 47-0049123

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: DHHS/NIH

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Characterization of a novel murine model of central nervous system catheter infection

12. * AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)*

Nebraska

13. PROPOSED PROJECT:

* Start Date: 04/01/2010 | * Ending Date: 03/31/2015

14. CONGRESSIONAL DISTRICTS OF:

a. * Applicant: NE-002 | b. * Project: NE-002

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. | * First Name: Jessica | Middle Name: R

* Last Name: Nichols | Suffix: MD

Position/Title: Asst Professor

* Organization Name: UNIVERSITY OF NEBRASKA MEDICAL CENTER

Department: PEDIATRICS | Division:

* Street1: 982162 Nebraska Medical Center

Street2:

* City: Omaha | County: Douglas

* State: NE: Nebraska | Province:

* Country: USA: UNITED STATES | * ZIP / Postal Code: 68198-2162

* Phone Number: 402-559-6400 | Fax Number: 402-559-4077

* Email: jrnichols@unmc.edu

OMB Number: 4040-0001
Expiration Date: 04/30/2008

16. ESTIMATED PROJECT FUNDING

a. * Total Estimated Project Funding	768,960.00
b. * Total Federal & Non-Federal Funds	768,960.00
c. * Estimated Program Income	0.00

17. * 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

18. 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 penalties. (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.***

19. Authorized Representative

Prefix: Ms. * First Name: Deborah Middle Name: K

* Last Name: Vetter Suffix:

* Position/Title: Director

* Organization: UNIVERSITY OF NEBRASKA MEDICAL CENTER

Department: Division:

* Street1: 987835 NEBRASKA MEDICAL CENTER

Street2: N/A

* City: OMAHA County: Douglas

* State: NE: Nebraska Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 68198-7835

* Phone Number: 402-559-7456 Fax Number: 402-559-2957

* Email: spadmin@unmc.edu

*** Signature of Authorized Representative**: Deborah K. Vetter

*** Date Signed**: 06/11/2009

20. Pre-application Add Attachment | Delete Attachment | View Attachment

21. Attach an additional list of Project Congressional Districts if needed.

Add Attachment | Delete Attachment | View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

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

RESEARCH & RELATED Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name: UNIVERSITY OF NEBRASKA MEDICAL CENTER

* Street1: 987835 NEBRASKA MEDICAL CENTER

Street2: N/A

* City: OMAHA County: Douglas

* State: NE: Nebraska Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 68198-7835

Project/Performance Site Location 1

Organization Name:

* Street1:

Street2:

* City: County:

* State: Province:

* Country: USA: UNITED STATES * ZIP / Postal Code:

Additional Location(s) Add Attachment Delete Attachment View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

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RESEARCH & RELATED Other Project Information

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

1.a If YES to Human Subjects

Is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Exemption Number: ☐1 ☐2 ☐3 ☐4 ☐5 ☐6

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 A3294-01

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.a. * Does this project involve activities outside the U.S. or partnership with International Collaborators? ☐ Yes ☒ No

5.b. If yes, identify countries:

5.c. Optional Explanation:

6. * Project Summary/Abstract 18555_abstract.pdf | Add Attachment | Delete Attachment | View Attachment

7. * Project Narrative 18632_description.pdf | Add Attachment | Delete Attachment | View Attachment

8. Bibliography & References Cited 18641_References.pdf | Add Attachment | Delete Attachment | View Attachment

9. Facilities & Other Resources 18633_Resources.pdf | Add Attachment | Delete Attachment | View Attachment

10. Equipment 18634_Major Equipment .pdf | Add Attachment | Delete Attachment | View Attachment

11. Other Attachments Add Attachments | Delete Attachments | View Attachments ☒

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Cerebrospinal fluid (CSF) shunt infections are a frequent and serious complication in the treatment of hydrocephalus in the pediatric population, with a reported incidence of 5-15%[1]. The most common organisms responsible for these central nervous system (CNS) catheter infections, *Staphylococcus epidermidis* and *Staphylococcus aureus,* are both known to form biofilms[2,3]. These biofilms are organized communities of bacterial cells that aggregate on the catheter surface, enclosed in a self-produced matrix that protects the organisms. The biofilm's ability to evade the host immune response and antimicrobial agents makes it difficult to manage CNS catheter infections non-surgically, such that catheter removal is currently required to effectively treat these infections. **While the growth characteristics and other adaptations of the bacteria required for biofilm formation are being extensively investigated by microbiologists, very little is known about the host interaction with the biofilm, particularly with regard to the immune response to catheter biofilm infections.**

To explore the neuroimmune response to CNS catheter infections, I have developed a novel model of CNS catheter infection in the mouse. This technique results in a consistent catheter-associated infection with *S. aureus* and ventriculitis, similar to the sequelae seen in humans with ventricular shunt infections. Establishment of this model provides a powerful tool to identify important factors in the host immune response to CNS biofilms through the use of genetically engineered knockout or transgenic mouse strains. The ***objective*** of this study is to utilize this model of CNS catheter infection to characterize the host immune response to a CNS biofilm infection with *S. aureus* by investigating the kinetics of bacterial growth and the host innate immune response in this setting. **Understanding the interactions between the neuroimmune system and the biofilms that form on infected catheters will allow us to explore novel management strategies for these infections in future studies.**

The overall ***hypothesis*** of this K08 proposal is that the host innate immune response in the brain is actively attenuated in response to biofilm colonization of a CNS catheter**.** To test this hypothesis, we will perform experiments outlined in two specific aims. In **Aim 1**, we will characterize the bacterial growth kinetics and innate immune response in a murine model of CNS catheter infection. In **Aim 2**, we will define the role of bacterial regulatory factors in the development of CNS catheter infection by using an isogenic mutant *S. aureus* strain, deficient in *sarA* expression, which is known to play a role in biofilm formation.

Finally, the candidate is a pediatric infectious disease specialist with a long-standing interest in CNS infections and the role of the host response in pediatric infections. She is a well-supported candidate with an avid interest in becoming a physician scientist who will benefit highly from a Clinical Scientist Development Award.

Cerebrospinal fluid shunt infections are a frequent and serious complication in the treatment of hydrocephalus in the pediatric population. In this proposal, we will study the interactions between the immune system and the biofilms that form on these catheters within the central nervous system. These studies will provide valuable information about the immune response to this biofilm infection within the CNS, potentially leading to novel diagnostic and therapeutic tools for use in management of these infections.

References

1. Nichols JR, Aldrich AL, Mariani MM, Vidlak D, Esen N, Kielian T. TLR2 deficiency leads to increased Th17 infiltrates in experimental brain abscesses. Journal of Immunology 2009; 182:7119-7130.
2. Garg S, Nichols J, Esen N, Liu S, Phulwani N, Syed M, Wood W, Zhang Y, Becker K, Aldrich A, Kielian T. MyD88 expression in the CNS is pivotal for eliciting protective immunity in brain abscesses. *ASN Neuro* 2009; 1:art:e00007.
3. McGirt MJ, Zaas A, Fuchs HE, George TM, Kaye K, Sexton DJ. Risk factors for pediatric ventriculoperitoneal shunt infections and predictors of infectious pathogens. Clinical Infectious Diseases 2003; 36:858-62.
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9. Hampl JA, Weitzel A, Bonk C, Kohnen W, Roesner D, Jansen B. Rifampin-Impregnated silicone catheters: A potential tool for prevention and treatment of CSF infections. Infection 2003; 31:109-111.
10. Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. British Journal of Pharmacology 2006; 147:S232-240.
11. Kielian T, Phulwani NK, Esen N, Syed MM, Haney AC, McCastlain K, Johnson J. MyD88-Dependent signals are essential for the host immune response in experimental brain abscess. Journal of Immunology 2007; 178:4528-4537.
12. Kielian T and Hickey WF. Proinflammatory cytokine, chemokine and cellular adhesion molecule expression during the acute phase of experimental brain abscess development. American Journal of Pathology 2000; 157:647-658.
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14. Texereau J, Chiche JD, Taylor W, Choukroun G, Comba B, Mira JP. The importance of Toll-like receptor 2 polymorphisms in severe infections. Clinical Infectious Diseases 2005; 41:S408-415.
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16. Kadurugamuwa JL, Sin L, Albert E, Yu J, Francis K, DeBoer M, Rubin M, Bellinger-Kawahara C, Parr TR, Contag PR. Direct continuous method for monitoring biofilm infection in a mouse model. Infection and Immunity 2003; 71:882-890.
17. Kuklin NA, Pancari GD, Tobery TW, Cope L, Jackson J, Gill C, Overbye K, Francis FP, Yu J, Montgomery D, Anderson AS, McClements W, Jansen KU. Real-time monitoring of bacterial infection in vivo: development of bioluminescent staphylococcal foreign-body and deep-thigh-wound mouse infection models. Antimicrobial Agents and Chemotherapy 2003; 47:2740-2748.
18. Singh PK, Parsek MR, Greenberg EP, Welsh MJ. A component of innate immunity prevents bacterial biofilm development. Nature 2002; 417:552-555.
19. Reichel CA, Khandoga A, Anders HJ, Schlöndorff D, Luckow B, Kromback F. Chemokine receptors Ccr1, Ccr2 and Ccr5 mediate neutrophil migration to postischemic tissue. Journal of Leukocyte Biology 2006; 79:114-122.
20. Assenmacher M, Lohning M, Scheffold A, Manz RA, Schmitz J, Radbruch A. Sequential production of IL-2, IFN-gamma and IL-10 by individual staphylococcal enterotoxin-B activated T helper lymphocytes. European Journal of Immunology 1998; 28:1534-1543.
21. Sholl-Franco A, da Silva AGLS, Adão-Novaes J. Interleukin-4 as a neuromodulatory cytokine. Annals of the New York Academy of Sciences 2009; 1153:65-75.

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23. Beenken KE, Dunman PM, McAleese F, Macapagal D, Murphy E, Projan SJ, Blevins JS, Smeltzer MS. Global gene expression in *Staphylococcus aureus* biofilms. Journal of Bacteriology 2004; 186:4665-4684.
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26. Cerca N, Brooks JL, Jefferson KK. Regulation of the intercellular adhesion locus regulator (*icaR*) by SarA, σ^B and IcaR in *Staphylococcus aureus*. Journal of Bacteriology 2008; 190:6530-6533.
27. Kielian T, Haney A, Mayes PM, Garg S, Esen N. Toll-like receptor 2 modulates the proinflammatory milieu in *Staphylococcus aureus*-induced brain abscess. Infection and Immunity 2005; 73:7428-7435.
28. Kielian T, Barry B, Hickey WF. CXC chemokine receptor-2 ligands are required for neutrophil-mediated host defense in experimental brain abscesses. Journal of Immunology 2001; 166:4634-4643.
29. Kielian T, Bearden ED, Baldwin AC, Esen N. IL-1 and TNF-α play a pivotal role in the host immune response in a mouse model of *Staphylococcus aureus*-induced experimental brain abscess. Journal of Neuropathology and Experimental Neurology 2004; 63:381-396.

Resources:

Laboratory:
The proposed work will be done in the PI's laboratory in the newly opened Durham Research Center II at the University of Nebraska Medical Center. The PI is assigned approximately 500 square feet of wet lab space on the 7th floor of DRCII (Room 7016), immediately adjacent to Dr. Kielian's laboratory space. There will be shared equipment with Dr. Kielian's space, inlcuding tissue culture facilities.

Animal:
Dr. Nichols' mice will be housed in an AAALAC-approved vivarium located in the DRC II and serviced by staff of the Department of Comparative Medicine, which is directed by licensed veterinarians. The mice will be housed in a restricted-access room, shared with Dr. Kielian. The University of Nebraska Medical Center Institutional Animal Care and Use Committee must approve all animal protocols prior to implementation.

Computer:
Dr. Nichols is supplied with 2 PCs and a color laser printer. In the lab and office, she has access to the internet and the local area network. All data saved to the server is backed up nightly at an off-site secure location. Dedicated technical support is available for all hardware and software problems or concerns.

Office:
Dr. Nichols has approximately 200 sq ft of office space on the 7th floor of DRCII, with computer equipment as above. She also has secretarial support and access to all other pertinent office equipment.

Resources:

Laboratory:
Dr. Kielian has approximately 2,500 sq. ft. of laboratory space located on the 7th floor of the new Durham Research Center II (DRCII), which opened in May 2009. Dr. Kielian's mice are housed in a dedicated BSL2 suite containing a procedure room adjacent to the BSL2 animal housing room, located on the ground floor of DRCII. Dedicated areas in Dr. Kielian's laboratory exist for tissue culture, molecular biology studies, immunofluorescence staining, ELISAs, microbiological techniques, electrophysiology, and confocal microscopy.

Animal:
Animals are housed in an AAALAC-approved vivarium located in the Durham Research Center II. The vivarium is serviced by staff of the Department of Comparative Medicine, which is directed by licensed veterinarians. Mice are housed in restricted-access rooms equipped with ventilated micro-isolator cages and a novel rodent watering system called the Hydropac™ system. Dr. Kielian has access to racks with a 250 cage capacity to house both breeding colonies and mice for experimental studies. The University of Nebraska Medical Center Institutional Animal Care and Use Committee reviews all animal protocols that must be approved prior to implementation.

Computer:
Dr. Kielian has 12 computers, including 9 Dell desktops (one in Dr. Kielian's office, 8 for laboratory personnel), two Dell workstations for electrophysiology studies, a Dell workstation for confocal microscopy image analysis, and a Dell XPS laptop computer. All of these computers are connected to the UNMC mainframe for internet access and have current versions of Microsoft Office, Adobe Photoshop and Acrobat, and Sigma Stat/Sigma Plot.

Office:
Dr. Kielian has 212 sq. ft. of office space located adjacent to her laboratories equipped with a Dell computer, a Hewlett Packard color laser printer, and an Epson scanner for high quality image scanning.

Other:
A Becton Dickinson FACCalibur flow cytometer, one LSRII cytometer capable of 12-color analysis, and two FACSAria sorters are available on a fee-for-use basis through the UNMC Cell Analysis Facility. A Luminex-based microbead suspension array technology workstation (Bio-Rad) is available for use in the Nebraska Department of Public Health Laboratory on the UNMC campus.

MAJOR EQUIPMENT

Equipment available in Dr. Nichols' laboratory for use on this project includes: upright microscope, 25 cu. ft. refrigerator, -20C freezer, -80C freezer, refrigerated tabletop centrifuge and microfuge, Polytron homogenizer, spectrophotometer and incubator. A rodent stereotaxic apparatus will also be available in the animal surgery room, located in the DRC II vivarium. A biological safety cabinet and microplate reader will be shared with Dr. Kielian's laboratory. In addition, Dr. Nichols will have access to an IVIS Spectrum Unit (Caliper Life Sciences) with LivingImage software, BD FACSAria, and Bio-Plex workstation.

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

Dr. Kielian's laboratory space contains two 4 ft. Class II biological safety cabinets and two stacking CO_2 incubator units for tissue culture. Several Zeiss microscopes are housed in Dr. Kielian's laboratory including a 510 META laser scanning confocal microscope, two AxioExaminer fixed-stage microscopes controlled by Gibraltar stages and housed in Faraday cages for electrophysiology studies, one AxioObserver A1 inverted fluorescence microscope, one Axioskop 40 FL upright microscope, and one Axiovert 40C inverted microscope for viewing cultured cells. All microscopes are equipped with high-resolution, high-sensitivity monochrome cameras. Additional equipment includes a microplate reader, cryostat, 2, 25 cu. ft. refrigerators and -20C freezer, 23 cu. ft. double door -80C freezer, Eppendorf vacuum microfuge, two refrigerated table-top and three microfuges, Polytron homogenizer, and Bio-Rad Criterion and Mini-Protean 3 electrophoresis cells and semi-dry transfer apparatus, a GentleMACS tissue dissociator, an Eppendorf EPMotion 5070 fluid workstation, Eppendorf MCEP RealPlex 2 system w/laptop, Agilent Bioanalyzer 2100 system, and a Alpha Innotech gel documentation system.

List of Referees:

Toni Darville MD
Professor of Pediatrics
Chief, Pediatric Infectious Disease
Department of Pediatrics
Children's Hospital of Pittsburgh
University of Pittsburgh Medical Center
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J. Gary Wheeler MD
Professor of Pediatrics
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Paul Drew PhD
Professor of Neurobiology
Department of Neurobiology and Developmental Sciences
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RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. * First Name: Jessica Middle Name: R
* Last Name: Nichols Suffix: MD
Position/Title: Asst Professor Department: PEDIATRICS
Organization Name: UNIVERSITY OF NEBRASKA MEDICAL CENTER Division:
* Street1: 982162 Nebraska Medical Center
Street2:
* City: Omaha County: Douglas
* State: NE: Nebraska Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 68198-2162
* Phone Number: 402-559-4116 Fax Number: 402-559-4077
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Credential, e.g., agency login: NICHOLS.JESSICA

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

***Attach Biographical Sketch** 18583_JNicholsBio.pdf Add Attachment | Delete Attachment | View Attachment

Attach Current & Pending Support Add Attachment | Delete Attachment | View Attachment

PROFILE - Senior/Key Person 1

Prefix: Dr. * First Name: Tammy Middle Name: L
* Last Name: Kielian Suffix: PhD
Position/Title: Associate Professor Department: Pathology & Microbiology
Organization Name: University of Nebraska Medical Center Division: College of Medicine
* Street1: 985900 Nebraska Medical Center
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* City: Omaha County: Douglas
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* Country: USA: UNITED STATES * Zip / Postal Code: 68198-5900
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Credential, e.g., agency login: TLKIELIAN

*** Project Role:** Other (Specify) **Other Project Role Category:** Mentor

***Attach Biographical Sketch** 18590_Kielian Bio.pdf Add Attachment | Delete Attachment | View Attachment

Attach Current & Pending Support 18758_Kielian Other Supp.pdf Add Attachment | Delete Attachment | View Attachment

Tracking Number:GRANT10348416 Funding Opportunity Number:PA-09-042 Received Date:2009-06-12T11:22:28-04:00

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2

Prefix: Dr. * First Name: Paul Middle Name: D
* Last Name: Fey Suffix: PhD
Position/Title: Associate Professor Department: Pathology & Microbiology
Organization Name: University of Nebraska Medical Center Division: College of Medicine
* Street1: 985900 Nebraska Medical Center
Street2:
* City: Omaha County: Douglas
* State: NE: Nebraska Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 68198-5900
* Phone Number: 402-559-2122 Fax Number: 402-559-4077
* E-Mail: pfey@unmc.edu

Credential, e.g., agency login: FEY.PAUL

*** Project Role:** Other (Specify) **Other Project Role Category:** Advisory Panel

***Attach Biographical Sketch** 18618_feyBiosketch.pdf Add Attachment Delete Attachment View Attachment

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PROFILE - Senior/Key Person 3

Prefix: Dr. * First Name: Mark Middle Name:
* Last Name: Rupp Suffix: MD
Position/Title: Professor Department: Internal Medicine
Organization Name: University of Nebraska Medical Center Division:
* Street1: 984031 Nebraska Medical Center
Street2:
* City: Omaha County: Douglas
* State: NE: Nebraska Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 68198-4031
* Phone Number: 402-559-5276 Fax Number:
* E-Mail: merupp@unmc.edu

Credential, e.g., agency login: RUPP.MARK

*** Project Role:** Other (Specify) **Other Project Role Category:** Advisory Panel

***Attach Biographical Sketch** 18620_Bio Rupp.pdf Add Attachment Delete Attachment View Attachment

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Tracking Number:GRANT10348416 Funding Opportunity Number:PA-09-042 Received Date:2009-06-12T11:22:28-04:00

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 4

Prefix: Dr. * First Name: Mark Middle Name:
* Last Name: Smeltzer Suffix: PhD
Position/Title: Professor Department: Microbiology and Immunology
Organization Name: University of Arkansas for Medical Sciences Division:
* Street1: 4301 West Markham Street Slot 511
Street2:
* City: Little Rock County:
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* Country: USA: UNITED STATES * Zip / Postal Code: 72205
* Phone Number: 501-686-7958 Fax Number: 501-686-5359
* E-Mail: smeltzermarks@uams.edu

Credential, e.g., agency login:

*** Project Role:** Other (Specify) **Other Project Role Category:** Advisory Panel

***Attach Biographical Sketch** 18622_Smeltzer Bio.pdf [Add Attachment] [Delete Attachment] [View Attachment]

Attach Current & Pending Support [Add Attachment] [Delete Attachment] [View Attachment]

PROFILE - Senior/Key Person 5

Prefix: Dr. * First Name: Joyce Middle Name:
* Last Name: Solheim Suffix: PhD
Position/Title: Associate Professor Department: Eppley Institute
Organization Name: University of Nebraska Medical Center Division:
* Street1: 986805 Nebraska Medical Center
Street2:
* City: Omaha County: Douglas
* State: NE: Nebraska Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 68198-6805
* Phone Number: (402)559-4539 Fax Number:
* E-Mail: jsolheim@unmc.edu

Credential, e.g., agency login: SOLHEIM.JOYCE

*** Project Role:** Other (Specify) **Other Project Role Category:** Advisory Panel

***Attach Biographical Sketch** 18625_solheimbio.pdf [Add Attachment] [Delete Attachment] [View Attachment]

Attach Current & Pending Support [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 6

Prefix: Dr. * First Name: Paul Middle Name:

* Last Name: Dunman Suffix: PhD

Position/Title: Assistant Professor Department: Pathology & Microbiology

Organization Name: University of Nebraska Medical Center Division: College of Medicine

* Street1: 985900 Nebraska Medical Center

Street2:

* City: Omaha County: Douglas

* State: NE: Nebraska Province:

* Country: USA: UNITED STATES * Zip / Postal Code: 68198-5900

* Phone Number: 402-559-7745 Fax Number: 402-559-4077

* E-Mail: pdunman@unmc.edu

Credential, e.g., agency login: DUNMAN.PAUL

*** Project Role:** Other (Specify) **Other Project Role Category:** Collaborator

***Attach Biographical Sketch**	18626_Dunman Bio.pdf	Add Attachment	Delete Attachment	View Attachment
Attach Current & Pending Support		Add Attachment	Delete Attachment	View Attachment

ADDITIONAL SENIOR/KEY PERSON PROFILE(S)		Add Attachment	Delete Attachment	View Attachment
Additional Biographical Sketch(es) (Senior/Key Person)		Add Attachment	Delete Attachment	View Attachment
Additional Current and Pending Support(s)		Add Attachment	Delete Attachment	View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

BIOGRAPHICAL SKETCH

Provide the following information for the 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
Nichols, Jessica Renee	Assistant Professor of Pediatrics
eRA COMMONS USER NAME (credential, e.g., agency login) NICHOLS.JESSICA	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Texas A&M University, College Station TX	BS	2000	Medical Science
Texas A&M Health Science Center, College Station TX	MD	2002	Medicine

A. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

Positions

2002-2005 **Pediatric Residency**, East Carolina University/University Health Systems; Greenville NC
2005-2006 **Clinical Instructor, Chief Resident**, East Carolina University/University Health Systems; Greenville NC
2006-2009 **Pediatric Infectious Disease Fellow**, University of Arkansas for Medical Sciences / Arkansas Children's Hospital; Little Rock AR
2009- **Assistant Professor of Pediatrics**, University of Nebraska Medical Center, Omaha, NE

Honors

2009 Best Abstract, Arkansas Children's Hospital (ACH) Fellows Day
2009 Honorable Mention Oral Presentation, ACH Fellows Day
2009 Second Place, UAMS Research Week, Postdoctoral fellow division
2009 SSPR/APA Trainee Travel Award Winner, Presentation at SSPR Annual Meeting
2008 PIDS and IDSA Fellow Travel Award Winner, Presentation at IDSA Annual Meeting
2008 First Place, UAMS Research Week, Postdoctoral fellow division
2007 Best Abstract, Arkansas Children's Hospital (ACH) Fellows Day
2007 Honorable Mention Oral Presentation, ACH Fellows Day
2007 SSPR/APA Trainee Travel Award Winner, Presentation at SSPR Annual Meeting
2006 Outstanding Faculty Teaching Award, East Carolina University
2002 Honor graduate, Texas A&M Health Science Center
1996-2002 Texas A&M Medical Science Scholar, Charter Class

Other Experiences and Professional Memberships

2002- American Academy of Pediatrics
2006- Infectious Disease Society of America
2006-2009 Infection Control Committee, Arkansas Children's Hospital
2006- Pediatric Infectious Disease Society
2009- American Society for Neurochemistry

B. Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation.

Nichols J, Aldrich AL, Mariani MM, Vidlak D, Esen N, Kielian T. Toll-like receptor 2 (TLR2) deficiency leads to increased Th17 infiltrates in experimental brain abscesses. *Journal of Immunology*. 2009 June;

182:7119-7130.

Garg S, **Nichols J**, Esen N, Liu S, Phulwani N, Syed M, Wood W, Zhang Y, Becker K, Aldrich A, Kielian T. MyD88 expression in the CNS is pivotal for eliciting protective immunity in brain abscesses. *ASN Neuro*. 2009 March; 1(2):art:e00007.doi:10.10421AN20090004. *First two authors contributed equally.

Gurley C, **Nichols J**, Liu S, Phulwani N, Esen N, Kielian T. Microglia and Astrocyte Activation by Toll-Like Receptor Ligands: Modulation by PPAR-γ Agonists. *PPAR Research*. 2008 June; 2008:453120.

Nichols JR, Stovall S, Jacobs RF. Human Papillomavirus Infection: the role of vaccination in pediatric patients. *Clinical Pharmacology & Therapeutics*. 2007 April; 81(4):607-10.

C. Research Support. List selected ongoing or completed research projects (federal and non-federal support). Begin with the projects that are most relevant to the research proposed in this application. Briefly indicate the overall goals of the projects and your role (e.g. PI, Co-Investigator, Consultant) in the research project. Do not list award amounts or percent effort in projects.
(Skip this question if you are an NIH intramural employee.)

2008 Children's University Medical Group (CUMG) Award, Development and characterization of a novel murine model of central nervous system catheter infection. PI: **J Nichols.** $6,000.

BIOGRAPHICAL SKETCH

Provide the following information for the 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
Tammy Leigh Kielian	Associate Professor
eRA COMMONS USER NAME TLKIELIAN	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Nebraska-Lincoln, Lincoln NE	B.S.	1991	Biological Sciences
Kansas State University, Manhattan, KS	M.S.	1994	Immunology
Univ. Kansas Medical Center, Kansas City, KS	Ph.D.	1998	Microbiology
Dartmouth Medical School, Lebanon, NH	Postdoc	1998-2001	Neuroimmunology

PROFESSIONAL EXPERIENCE

2008-present Associate Professor, Dept. of Pathology and Microbiology, University of Nebraska Medical Center
2006-2008 Associate Professor, Depts. of Neurobiology and Developmental Sciences and Microbiology and Immunology, University of Arkansas for Medical Sciences
2001-2006 Assistant Professor, Dept. of Neurobiology and Developmental Sciences, University of Arkansas for Medical Sciences
2000- 6/2001 Research Assistant Professor, Department of Pathology, Dartmouth Medical School
1998-2000 Postdoctoral Fellow, Dartmouth Medical School, in the laboratory of Dr. William F. Hickey

AWARDS AND OTHER PROFESSIONAL ACTIVITIES

2010-2014 Chair, American Society for Neurochemistry (ASN) Public Policy and Education Committee
7/2009 NIH/CSR Special Emphasis Panel/Scientific Review Group ZAA1 CC review panel for RFA "The Effects of Alcohol on Glial Cells"
6/2009 Member of ZRG1 BDCN-T (58) RRFA-OD-09-003 Challenge Grant Panel 11; NIH/CSR
1/2009 Ad Hoc reviewer, NIH/CSR Clinical Neuroimmunology and Brain Tumors Study Section (CNBT)
2009-2013 Member of Nominations Committee, American Society for Neurochemistry
2009-2012 Handling Editor and Member of Editorial Board, *Journal of Neurochemistry*
2008-present Member of External Advisory Committee for Meharry Medical College Specialized Neuroscience Research Program funded by NINDS (U54 award mechanism)
2007-present Member of Editorial Board, *Journal of Neuroinflammation*
2008 Chair and Organizer of session at the Winter Conference on Brain Research entitled "*Toll-like receptors in CNS injury and infection*, Snowbird, UT
2007 Chair and Organizer of Colloquium session for 2008 American Society for Neurochemistry (ASN) entitled "*Immune Functions of Astrocytes*", San Antonio, TX
2007-2011 Council Member, American Society for Neurochemistry (elected position)
2007 Ad Hoc member, National Institute of Neurological Disorders and Stroke (NINDS) Special Emphasis Panel ZNS1 SRB-M
2007-2008 President, Arkansas Chapter for the Society for Neuroscience
2007 External reviewer, Multiple Sclerosis Society of Canada
2007 External reviewer, Health Research Board of Ireland
2006-2007 Present-elect, Arkansas Chapter for the Society for Neuroscience
2007-2008 Scientific Program Committee Member, American Society for Neurochemistry (ASN) annual meeting in San Antonio, TX
2006-2010 Member of American Society for Neurochemistry (ASN) Public Policy and Education Committee
2006 Jordi Folch Pi Memorial Award from the American Society for Neurochemistry (ASN) for outstanding contributions to Neuroscience Research
6/2004-2006 Full Member, NIH/CSR, Clinical Neuroimmunology and Brain Tumors Study Section (CNBT)

2006	Chair and organizer of Colloquium session for 2006 American Society for Neurochemistry meeting entitled “Effects of Neuroinflammation on Gap Junction Communication”
2006	Scientific Program Committee Member, American Society for Neurochemistry (ASN) annual meeting in Portland, OR
2005	Ad Hoc reviewer, Canada Council for the Arts, Killam Research Fellowship
2002-2004	Ad Hoc reviewer, NIH/CSR, Clinical Neuroimmunology and Brain Tumors Study Section (CNBT)
2001	Ad Hoc reviewer, National Science Foundation, IBN- Neuronal and Glial Mechanisms
2000	Hitchcock Foundation Award for Biomedical Research, Dartmouth Medical School

PUBLICATIONS (partial list)

1. Andre, A., Wade, T., **Kielian, T.**, Cambier, J.C., and Wade, W.F. Distinct structural compartmentalization of the signal transducing functions of MHC Class II I(s) molecules. J. Exp. Med. 179:763-768, 1994.
2. **Kielian, T.L.** and Blecha, F. CD14 and other recognition molecules for lipopolysaccharide: a review. Immunopharmacology 29:187-205, 1995.
3. **Kielian, T.L.**, Ross, C.R., McVey, D.S., Chapes, S.K., and Blecha, F. Lipopolysaccharide modulation of a CD14-like molecule on porcine alveolar macrophages. J. Leukoc. Biol. 57:581-586, 1995.
4. David, S.A., Silverstein, R., Amura, C.R., **Kielian, T.**, and Morrison, D.C. Lipopolyamines: Novel antiendotoxin compounds that reduce mortality in experimental sepsis caused by gram-negative bacteria. Antimicrob. Agents Chemother. 43:912-919, 1999.
5. Nagai, E., Ogawa, T., **Kielian, T.**, Ikubo, A., and Suzuki, T. Irradiated tumor cells adenovirally engineered to secrete GM-CSF establish anti-tumor immunity and eliminate pre-existing tumors in syngeneic mice. Cancer Immunol. Immunother., 47:72-80, 1998.
6. **Kielian, T.** and Hickey, W.F. Inflammatory thoughts about glioma gene therapy. Nature Med. 5(11):1237-1238, 1999.
7. **Kielian, T.**, Nagai, E., Ikubo, A., Rasmussen, C.A., and Suzuki, T. Granulocyte/macrophage-colony stimulating factor released by adenovirally transduced CT26 cells leads to the local expression of macrophage inflammatory protein 1α and accumulation of dendritic cells at vaccination sites in vivo. Cancer Immunol Immunother., 48:123-131, 1999.
8. **Kielian, T.** and Hickey, W.F. Proinflammatory cytokine, chemokine, and cellular adhesion molecule expression during the acute phase of experimental brain abscess development. Am. J. Pathol., 157:647-658, 2000.
9. **Kielian, T.**, Barry, B., and Hickey, W.F. CXC chemokine receptor-2 ligands are important for neutrophil-mediated host defense in experimental brain abscesses. J. Immunol., 166:4634-4643, 2001.
10. **Kielian, T.**, Cheung, A., and Hickey, W.F. Diminished virulence of an alpha-toxin mutant of *Staphylococcus aureus* in experimental brain abscesses. Infect. Immun., 69:6902-6911, 2001.
11. **Kielian, T.**, Van Rooijen, N., and Hickey, W.F. MCP-1 expression in CNS-1 astrocytoma cells: implications for macrophage infiltration into tumors *in vivo.* J. Neuro-Oncol. 56:1-12, 2002.
12. **Kielian, T.** and Hickey, W.F. Chemokines and neural inflammation in experimental brain abscesses. In: Universes in Delicate Balance: Chemokines and the nervous system. Suzuki, K., Proudfoot, A., Hickey, W.F., Harrison, J., and Ransohoff, R., eds. Chapter 3.7: 217-224, 2002.
13. **Kielian, T.**, Mayes, P., and Kielian, M**.** Characterization of microglial responses to *Staphylococcus aureus*: effects on cytokine, co-stimulatory molecule, and Toll-like receptor expression, J. Neuroimmunol. 130:86-99, 2002.
14. **Kielian, T.** and Drew, P.D. Effects of peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists on CNS inflammation. J. Neurosci. Res. 71:315-325, 2003.
15. **Kielian, T.** Microglia and chemokines in infectious diseases of the nervous system: Views and Reviews”, Frontiers in Bioscience 9:732-750, 2004.
16. Esen, N., Tanga, F.Y., DeLeo, J.A., and **Kielian, T.** Toll-like receptor 2 (TLR2) mediates astrocyte activation in response to the gram-positive bacterium *Staphylococcus aureus*, J. Neurochem., 88:746-758, 2004.
17. **Kielian, T.**, Bearden, E.D., Baldwin, A.C., and Esen, N. IL-1 and TNF-α play a pivotal role in the host immune response in a mouse model of *Staphylococcus aureus*-induced experimental brain abscess, J. Neuropath. Exp. Neurol., 63:381-396, 2004.
18. **Kielian, T.** and Esen, N. Effects of neuroinflammation on glia-glia gap junctional intercellular communication: A perspective, Neurochem. Int., 45:429-436, 2004.
19. Baldwin, A.C., and **Kielian, T.** Persistent immune activation associated with a mouse model of *Staphylococcus aureus*-induced experimental brain abscess, J. Neuroimmunol., 151:24-32, 2004.
20. **Kielian, T.**, McMahon, M., Bearden, E.D., Drew, P.D., and Esen, N. *S. aureus*-dependent microglial activation is selectively attenuated by the cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$- prostaglandin J_2(15d-PGJ_2), J. Neurochem., 90:1163-1172, 2004.
21. **Kielian, T.** Immunopathogenesis of brain abscess, J. Neuroinflamm., 1:1-16, 2004.

22. **Kielian, T.** and Drew, P.D. Cytokines and Brain: Health and Disease. In: Inflammatory Disorders of the Nervous System. Minagar, A. and Alexander, J.S., eds., 2005.
23. **Kielian, T.**, Esen, N., and Bearden, E.D. Toll-like receptor 2 (TLR2) is pivotal for recognition of *S. aureus* peptidoglycan but not intact bacteria by microglia Glia**,** 49:567-576, 2005.
24. Garg, S., Syed, M.Md, and **Kielian, T.** *S. aureus*-derived peptidoglycan induces functional gap junction intercellular communication in microglia, J. Neurochem, 95:475-483, 2005.
25. **Kielian, T.**, Haney, A., Mayes, P.M., and Esen N. Toll-like receptor 2 (TLR2) modulates the proinflammatory milieu in *S. aureus*-induced brain abscess, Infect. Immun., 73:7428-7435, 2005.
26. Esen, N. and **Kielian, T.** Recognition of *S. aureus*-derived peptidoglycan (PGN) but not intact bacteria is mediated by CD14 in microglia, J. Neuroimmunol., 170:93-104, 2005.
27. **Kielian, T.** Toll-like receptors (TLR) in central nervous system glial inflammation and homeostasis, J. Neurosci. Res., 83:711-730, 2006.
28. Esen, N. and **Kielian, T.** Central role for MyD88 in the responses of microglia to pathogen-associated molecular patterns (PAMPs), J. Immunol., 176:6802-6811, 2006.
29. Konat, G.W., **Kielian, T.**, and Marriott, I. The role of Toll-like receptors in CNS response to microbial challenge, J. Neurochem., 99:1-12, 2006.
30. Phulwani, N.K., Feinstein, D.L., Gavrilyuk, V., Akar, C., and **Kielian T.** 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) and ciglitazone modulate astrocyte activation through PPAR-γ-independent pathways, J. Neurochem., 99:1389-1402, 2006.
31. Esen, N., Syed, M. Md., and **Kielian, T.** Modulation of connexin expression and gap junction communication by the gram-positive pathogen *S. aureus*, Glia, 55:104-117, 2007.
32. **Kielian, T.**, Phulwani, N.K., Esen, N., Syed, M.Md., Haney, A.C., McCastlain, K., and Johnson J. MyD88-dependent signals are essential for the host immune response in experimental brain abscess, J. Immunol., 178:4528-4537, 2007.
33. Esen, N. and **Kielian, T.** Effects of low dose GM-CSF on microglial inflammatory profiles to diverse pathogen-associated molecular patterns (PAMPs), J. Neuroinflamm., 4:10-20, 2007.
34. **Kielian, T.**, Esen, N., Haney, A., Phulwani, N.K., Mayes, P.P., Cheung, A.L., and Ruhe, J.J. Minocycline modulates neuroinflammation independent of its anti-microbial activity in *S. aureus*-induced brain abscess, Am. J. Pathol., 171:1199-1214, 2007.
35. Syed, M.Md., Phulwani, N.K., and **Kielian, T.** TNF-α regulates Toll-like receptor 2 (TLR2) expression in microglia, J. Neurochem., 103:1461-1471, 2007.
36. **Kielian, T.,** Syed, M.Md., Liu, S., Phillips, N., Wagoner, G., Drew, P.D., and Esen, N. The synthetic PPAR-γ agonist ciglitazone attenuates neuroinflammation and accelerates encapsulation in bacterial brain abscesses, J. Immunol., 180:5004-5016, 2008. NIHMSID #93615
37. Phulwani, N.K. and **Kielian, T.** Poly (ADP-ribose) polymerases (PARPs) 1-3 regulate astrocyte activation, J. Neurochem., 106:578-590, 2008. NIHMSID #94007
38. **Kielian, T.** Glial Connexins and Gap Junctions in CNS inflammation and disease, J. Neurochem., 106:1000-1016, 2008. NIHMSID #94003
39. Gurley, C., Nichols, J., Liu, S., Phulwani, N.K., Esen, N., and **Kielian, T.** Microglia and astrocyte activation by Toll-like receptor (TLR) ligands: modulation by PPAR-γ agonists, PPAR Research, 2008:453120, 2008. PMC2435222
40. Phulwani, N.K., Syed, M.Md., and **Kielian, T.** Toll-like receptor 2 (TLR2) expression in astrocytes is induced by TNF-α- and NF-κB-dependent pathways, J. Immunol., 181:3841-3849, 2008. NIHMSID #93624
41. Garg, S., Nichols, J.R., Esen, N., Liu, S., Phulwani, N.K., Syed, M.Md., Wood, W.H., Zhang, Y., Becker, K.G., Aldrich, A., and **Kielian, T**. MyD88 expression by CNS-resident cells is pivotal for eliciting protective immunity in brain abscesses, ASN NEURO, doi:10.1042/AN20090004, 2009. NIHMSID #102800
42. Nichols, J.R., Aldrich, A.L., Mariani, M.M., Esen, N., and **Kielian, T.** Toll-like receptor 2 (TLR2) deficiency leads to increased Th17 infiltrates in experimental brain abscesses, J. Immunol., 182:7119-7130, 2009. NIHMSID #108304
43. Esen, N. and **Kielian, T.** Toll-like receptors (TLRs) in brain abscess. Current Topics in Microbiology and Immunology (CTMI), **T. Kielian** volume editor, July 2009.
44. **Kielian, T.** Overview of Toll-like receptors (TLRs) in the CNS. Current Topics in Microbiology and Immunology (CTMI), **T. Kielian** volume editor, July 2009.

RESEARCH PROJECTS ONGOING OR COMPLETED DURING THE LAST 3 YEARS

ACTIVE

1RO1 NS053487-01A1 (PI, Kielian) 08/01/06 – 7/31/11
NIH/NINDS

"Effects of Neuroinflammation on Gap Junction Communication in Glia"
The objective of this proposal is to identify the effects of proinflammatory mediators on gap junction communication in glia and experimental brain abscess.

2 RO1 NS40730 (PI, Kielian) 12/01/03 – 11/30/09
NIH/NINDS
"The Pathogenesis of Brain Abscess" Years 4-9
The goal of this work is to investigate the potential of PPAR-gamma agonists and minocycline to modulate brain abscess development.

9 RO1 NS055385-01A2 (PI, Kielian) 04/16/07 – 04/30/11
Source: NIH/NINDS
"Receptors Involved in Microglial Responses to *S. aureus*"
The objective of this proposal is to identify the role of TLR2 in regulating Th17 development in experimental brain abscesses.

P01 AI083211 (PI, Bayles; PI of Project 4: Kielian) 07/01/09 – 03/31/14
NIH/NIAID
"Staphylococcal Biofilm and Disease"
Title of Project 4: "Innate Immune Response to *S. aureus* Biofilm"
The objective of Project 4 is to determine whether *S. aureus* biofilm growth re-programs the host innate immune response from a classical anti-bacterial response to an anti-inflammatory response that favors bacterial persistence.

PENDING
3 RO1 NS40730 (PI, Kielian) 12/1/09 – 11/30/14
NIH/NINDS
"The Pathogenesis of Brain Abscess" Years 9-13
Priority Score: 141
The goal of this work is to investigate the signals controlling brain abscess encapsulation using MRI modalities.

COMPLETED
1 RO1 MH65297 (PI, Kielian) 06/20/02 – 05/01/07
NIH/NIMH
"Receptors Involved in Microglial Responses to *S. aureus*"
The objective of this proposal was to identify cellular receptors that mediate microglial activation in response to *S. aureus*.

! Principal Investigator/Program Director (Last, first, middle): Fey, P.D

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	Associate Professor
Paul D. Fey	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Kansas State University	B.S.	1990	Microbiology
Creighton University	Ph.D.	1995	Medical Microbiology
Medical College of Virginia	Post-Doctoral Fellowship	1995-1997	Staphylococcal genetics and antibiotic resistance

A. Positions and Honors

Positions and Employment:

1995-1997 Post Doctoral Fellow, Virginia Commonwealth University, Medical College of Virginia, Richmond, VA. Mentor: Gordon L. Archer, M.D.

1997-2003 Assistant Professor, University of Nebraska Medical Center, Departments of Internal Medicine and Pathology and Microbiology, Division of Infectious Disease, Omaha, NE.

1997-2005 Clinical Assistant Professor, Creighton University, Department of Medical Microbiology and Immunology, Omaha, NE.

1999-present Associate Medical Director; Nebraska Public Health Laboratory and Clinical Microbiology Laboratory, University of Nebraska Medical Center, Department of Pathology and Microbiology, Omaha, NE.

2003-2004 Associate Professor, University of Nebraska Medical Center, Departments of Internal Medicine and Pathology and Microbiology, Division of Infectious Disease, Omaha, NE.

2005-2006 Associate Professor, University of Nebraska Medical Center, Department of Pathology and Microbiology, Omaha, NE.

2005-Present Clinical Associate Professor, Creighton University, Department of Medical Microbiology and Immunology, Omaha, NE.

2006 Associate Professor, Tenured, University of Nebraska Medical Center, Department of Pathology and Microbiology, Omaha, NE.

Honors

2002 University of Nebraska Medical Center-Internal Medicine Basic Science Research Award

2004-present Antimicrobial Agents and Chemotherapy-editorial board

2005 NIH NIAID Study Section IDM-M91 Special Emphasis Panel "Bacterial Regulatory Genes".

2006 FDA Center for Women's Health-Scientific expert reviewer.

2006-present NIH NIAID Study Section ZRG IDM-M (11) "Food Safety, non-HIV Infectious Agents Sterilization and Bioremediation"

2007 NIH NIAID Study Section ZRG1 BACP-B (09) (F) - Bacterial Pathogenesis.

2007 The University of Texas at Houston mentor award-nominated by Melissa Diamantis

2007 Department of Health and Human Services Consultant Task force: *A public Health Action Plan to Combat Antimicrobial Resistance*

2008 NSF MCB-Genes and Genome Systems. Ad-Hoc expert grant review.

2008 Biotechnology and Biological Sciences Research Council (United Kingdom). Plant and Microbial Sciences. Ad-Hoc expert grant review.

2008 State of Pennsylvania Department of Health. Antibiotic Resistance Study Section.

2008 Congressional Directed Medical Research Program (CDMRP) Study Section.

B. Peer Reviewed Publications

1. Shlaes, D.M., J.H. Shlaes, S. Vincent, L. Etter, **P.D. Fey**, and R.V. Goering. 1993. Teicoplanin-resistant *Staphylococcus aureus* expresses a novel membrane protein and increases expression of penicillin-binding protein 2 complex. Antimicrob. Agents. Chemother. 37:2432-2437.
2. Goering, R.V., **P.D. Fey**, F.W. Goldstein. 1995. Usefulness of pulsed-field gel electrophoresis in the epidemiological analysis of *Staphylococcus aureus* isolates with decreased susceptibility to teicoplanin. Eur. J. Clin. Microbiol. Infect. Dis. Suppl. 1:3-5.
3. **Fey, P.D.**, M.W. Climo, G.L. Archer. 1998. Determination of the chromosomal relationship between *mecA* and *gyrA* in methicillin-resistant coagulase-negative staphylococci. Antimicrob. Agents. Chemother. 42:306-312.
4. Bergman, K.L., K.M. Olsen, T.E. Peddicord, **P.D. Fey**, and M.E. Rupp. 1999. Antimicrobial activity and postantibiotic effect of physiologic levels of clarithromycin, 14-hydroxy-clarithromycin, and azithromycin against clinical isolates of *Haemophilus influenzae* and *Streptococcus pneumoniae*. Antimicrob. Agents. Chemother. 43:1291-1293.
5. **Fey, P.D.**, J.S. Ulphani, C. Heilmann, F. Götz, and M.E. Rupp. 1999. Characterization of the Relationship between Polysaccharide Intercellular Adhesin and Hemagglutination in *Staphylococcus epidermidis*. J. Infect. Dis. 179:1561-1564.
6. M.E. Rupp, J.S. Ulphani, **P.D. Fey**, and D. Mack. 1999. Characterization of *Staphylococcus epidermidis* polysaccharide intercellular adhesin/hemagglutinin in the pathogenesis of intravascular catheter-associated infection in a rat-model. Infect. Immun. 67:2656-2659.
7. M.E. Rupp, **P.D. Fey**, J.S. Ulphani, K. Bartscht, and D. Mack. 1999. Characterization of the importance of polysaccharide intercelluar adhesin/hemagglutinin of *Staphylococcus epidermidis* in the pathogenesis of biomaterial-based infection in a mouse-foreign body infection model. Infect. Immun. 67:2627-2632.
8. **Fey, P.D.,** T.J. Safranek, M.E. Rupp, E.F. Dunne, E. Ribot, P.C. Iwen, P.A. Bradford, F.J. Angulo, and S.H. Hinrichs. 2000. Pediatric Ceftriaxone-Resistant *Salmonella* infection acquired from infected cattle in the United States. New England J. Med. 342:1242-1249.
9. **Fey, P.D.,** R.A. Wickert, T.J. Safranek, M.E. Rupp, and S.H. Hinrichs. 2000. Prevalence of *Escherichia coli* O157:H7 and other enterohaemorrhagic *Escherichia coli* in the state of Nebraska. Emer. Infect. Dis. 6:530-533.
10. Rebuck, J.A., K.M. Olsen, **P.D. Fey**, K.L. Bergman, and M.E. Rupp. 2000. In vitro activities of parenteral β-lactam antimicrobials against TEM-10, TEM-26, and SHV-5 derived extended-spectrum β-lactamases by time-kill curve methods. J. Antimicrob. Chemother. 46:461-464.
11. Rebuck, J.A., K.M. Olsen, **P.D. Fey**, A.N. Langnas, and M.E. Rupp. 2000. Characterization of an outbreak due to extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in a pediatric intensive care unit transplant population. Clin. Infect. Dis. 31:1368-1372.
12. Rupp, M.E., **P.D. Fey**, C. Heillmann, and F. Gotz. 2000. Characterization of the importance of *Staphylococcus epidermidis* autolysin and polysaccharide intercellular adhesin in the pathogenesis of intravascular catheter-associated infection in a rat model. J. Infect. Dis. 183:1038-1042.
13. Dunne, E.F., **P.D. Fey,** P. Kludt, R. Reporter, F. Mostashari, P. Schillum, J. Wicklund, C. Miller, B. Holland, K. Stamey, T.J. Barrett, J.K. Rasheed, F.C. Tenover, E. Ribot, F.J. Angulo. 2000. Emergence of Domestically acquired Ceftriaxone-resistant *Salmonella* infections in the United States associated with an AmpC β-lactamase. JAMA. 284:3151-3156.
14. Rupp, M.E., **P.D. Fey**, and G.M. Longo. 2001. Effect of LY333328 against vancomycin-resistant *Enterococcus faecium* in a rat central venous catheter-associated infection model. J. Antimicrob. Chemother. 47:705-707.
15. Rupp, M.E., N. Marion, **P.D. Fey**, D.L. Bolam, P.C. Iwen, C.M. Overfelt, L. Chapman. 2001. Outbreak of vancomycin-resistant enterococci in a neonatal intensive care unit. Infect. Control Hosp. Epi. 22:301-303.
16. Fowler, V.G., **P.D. Fey**, L.B. Reller, A.L. Chamis, G.R. Corey, and M.E. Rupp. 2001. The intercellular adhesin locus *ica* is present in clinical isolates of *Staphylococcus aureus* from bacteremic patients with infected and unifected prosthetic joints. Med. Microbiol. Immunol. 189:127-131.
17. Rupp, M.E., **Fey, P.D.** In vivo models to evaluate adhesion and biofilm formation by *Staphylococcus epidermidis*. In Methods in Enzymology. 2001, vol. 336; pp. 206-215. Academic Press, New York, NY.
18. Carattoli, A., Tosini, F., W.P. Giles, Rupp, M.E., Hinrichs, S.H., Angulo, F.J., Barrett, T.J., and **Fey, P.D.** 2002. Characterization of plasmids carrying CMY-2 from expanded-spectrum cephalosporin-resistant *Salmonella* isolated in the United States between 1996 and 1998. Antimicrob. Agents Chemother. 46:1269-1272.
19. **P. D. Fey**, B. Saïd-Salim, M. E. Rupp, S. H. Hinrichs,D. J. Boxrud, C. C. Davis, B. N. Kreiswirth, and P. M. Schlievert. 2003. Comparative Molecular Analysis of Community- or Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 47:196-203.
20. Rupp, M.E., and **P.D. Fey.** 2003. Extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae:* considerations for diagnosis, prevention, and drug treatment options. Drugs. 63:353-365.
21. Rupp, M.E., S.J. Medcalf, **P.D. Fey,** L.D. Handke, and N.D. Marion. 2003. Monsel's solution: a potential vector for nosocomial infection? Infect. Control. Hosp. Epidemiol. 24:142-144.
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23. **Fey, P.D.** and M.E. Rupp. 2003. Molecular epidemiology in the public health and hospital environments. Clin Lab Med 23:885-901.

24. L.D. Handke, K.M. Conlon, S.R. Slater, S. Elbaruni, F. Fitzpatrick, H. Humphreys W.P. Giles, M.E. Rupp, **P.D. Fey** and J.P. O'Gara. 2004. Genetic and Phenotypic analysis of phenotypic variation in multiple *Staphylococcus epidermidis* isolates. Journal of Medical Microbiology 53:367-374.
25. W.P. Giles, A.K. Benson, M.E. Olson, J.M. Whitchard, R.W. Hutkins, P.L. Winokur, and **P.D. Fey**. 2004. DNA sequence analysis of regions surrounding bla_{cmy-2} from multiple *Salmonella* plasmid backbones. Accepted August, Antimicrob. Agents Chemother. 48:2845-2852.

26. M.M. Samrakandi, C. Zhang, M. Zhang, J. Nietfeldt, G. Duhamel, M.E. Olson, **P.D. Fey,** P.C. Iwen, S.H. Hinrichs, J.D. Cirillo, and A.K. Benson. 2004. Genome diversity among *Francisella tularensis* subspecies *tularensis* and *Francisella tularensis* subspecies *holarctica* in North America. FEMS Microbiol Lett. 237:9-17.

27. Z. Yang, J. Kovar, J. Kim, J. Niefteldt, D.R. Smith, R. Moxley, **P.D. Fey,** and A.K. Benson. 2004. Identification of frequent subpopulations of sorbitol-non-fermenting, beta-glucuronidase-negative *Escherichia coli* O157:H7 from bovine production environments and human clinical samples. Applied and Environmental Microbiology, 70:6846-6854.

28. Rupp ME, Fitzgerald T, Marion N, Helget V, Puumala S, Anderson JR, and **Fey P.D.** 2004. Effect of silver-coated urinarycatheters: efficacy, cost-effectiveness, and antimicrobial resistance. Am J Infect Control. 32:445-450

29. S. Kozitskaya, M. E. Olson, **P. D. Fey**, W. Witte, K. Ohlsen, and W. Ziebuhr. 2005. Clonal Analysis of Biofilm-positive and Biofilm-negative Staphylococcus epidermidis Isolates by Multilocus Sequence Typing (MLST). J. Clin. Microbiol. 43:4751-4757.
30. J. M. Whichard, K. Joyce, **P. D. Fey**, J. M. Nelson, F. J. Angulo, and T. J. Barrett. 2005. Extended-spectrum ß-lactam Resistance among Human Clinical Enterobacteriaceae in the United States: Results and Characterization of 2000 NARMS Surveillance. Emer. Infect. Dis. 11:1464-1466.
31. M.E. Olson, K.L. Garvin, **P.D. Fey**, and M.E. Rupp. 2006. Initial Adherence of *Staphylococcus epidermidis* to Orthopedic Biomaterials is Polysaccharide Intercellular Adhesin (PIA) Dependent. Clin. Ortho. and Related Research. 451:21-24.
32. L.D. Handke, S.R. Slater, K.M. Conlon, Sinead T. O'Donnell, M.E. Olson, M.E. Rupp, J.P. O'Gara, and **P.D. Fey**. 2007. $\sigma^B$and SarA independently regulate polysaccharide intercellular adhesin (PIA) production in *Staphylococcus epidermidis*. Can. J. Microbiol. 53:82-91
33. Nelson, J.L., K.C. Rice, S.R. Slater, P.M. Fox, G.L. Archer, K.W. Bayles, **P.D. Fey**, B.N. Krieswirth, and G.A. Somerville. 2007. Vancomycin intermediate *Staphylococcus aureus* have impaired acetate catabolism: Implications for polysaccharide intercellular adhesin synthesis and autolysis. Antimicrobial Agents and Chemotherapy. 51:616-622.
34. M. E. Rupp, L. A Sholtz , D. R. Jourdan, N. D Marion, L. K Tyner, **P. D. Fey**, P. C. Iwen, and J. R. Anderson. 2007. Outbreak of bloodstream infections temporally associated with the use of an intravascular positive-displacement needleless valve. Clinical Infect. Dis. 44:1408-14.
35. M. Dempsey, M. Dobson, C. Zhang, M. Zhang, C. Lion, C. Gutierrez Martin, P. Iwen, **P.D. Fey,** M.E. Olson, D. Niemeyer, S. Francesconi, R. Crawford, M. Stanley, J. Rhoades, D. Wagner, P. Keim, A. Johansson, S.H. Hinrichs, and A.K. Benson. 2007. Identification of a genomic deletion marking an emerging subclone of *F. tularensis* subsp. *holarctica* in the Iberian Peninsula. Appl. Environ. Microbiol. 73:7465-70.
36. Y. Zhu, E. C. Weiss, M. Otto, **P. D. Fey**, M. S. Smeltzer, and G. A. Somerville**.** 2007. *Staphylococcus aureus* metabolism in a biofilm: the influence of arginine on polysaccharide intercellular adhesin synthesis, biofilm formation, and pathogenesis. Infect. Immun. 75:4219-4226.
37. **P.D. Fey**, M.P. Dempsey, M.E. Olson, M.S. Chrustowski, J.L. Engle, J.J. Jay, M.E. Dobson, K.S. Kalasinsky, A.A. Shea, P.C. Iwen, R.W. Wickert, S.C. Francesconi, R.M. Crawford, and S.H. Hinrichs. 2007. Molecular Analysis of *Francisella tularensis* subspecies *tularensis* and *holarctica*. American J. Clin. Pathol. 128:926-35.
38. L.D. Handke, K.L. Rogers, M.E. Olson, G.A. Somerville, T.J. Jerrells, M.E. Rupp, P.M. Dunman, and **P.D. Fey**. 2008. *Staphylococcus epidermidis saeR* is an effector of anaerobic growth and a mediator of acute inflammation. Infect. and Immun. 76:141-52.
39. A. Nusair, D. Jourdan, S. Medcalf, N. Marion, P.C. Iwen, **P.D. Fey**, and M.E. Rupp. 2008. Infection control experience in a transplant cooperative care center. Infect Control Hosp Epidemiol. 29:424-429.
40. M.H. Murthy, M.E. Olson, R.W. Wickert, **P.D. Fey**, and Z. Jalali. 2008. Daptomycin non-susceptible methicillin-resistant *Staphylococcus aureus* USA 300 isolate. J. Med. Microbiol. 57: 1036-1038
41. L. Holland, S. O'Donnell, D. A. Ryjenkov, L. Gomelsky, S. R. Slater, **P. D. Fey**, M. Gomelsky and J. P. O'Gara. 2008. A staphylococcal GGDEF domain protein regulates biofilm formation independently of c-di-GMP. J. Bact. 190:5178-89
42. J. A. Poole, N. E. Alexis, C. Parks, A. K. MacInnes, **P. D. Fey**, L. Larsson, D. Allen-Gipson, S. G. Von Essen, D. J. Romberger. 2008. Repetitive Organic Dust Exposure in vitro Impairs Differentiation and Function of Macrophages. J. Allergy Clin Immunol. 22:375-82
43. K.L. Rogers, M.E. Rupp, and **P.D. Fey**. 2008. The presence of *icaADBC* is detrimental to the colonization of human skin by *Staphylococcus epidermidis*. Appl. Environ. Microbiol. 74(19):6155-6157.
44. M. R. Sadykov, M. E. Olson, S. Halouska, Y. Zhu, **P. D. Fey**, R. Powers, and G. A. Somerville. 2008. Tricarboxylic acid cycle dependent regulation of *Staphylococcus epidermidis* polysaccharide intercellular adhesin synthesis. J. Bact. 190(23):7621-7631.

45. Rogers, K.L., **Fey, P.D.,** Rupp, M.E. 2009. Coagulase negative staphylococcal infections. Infectious Disease Clinics of North America. 23:73-98.

C. **Research Support**

Ongoing Research Support

NIH/NIAID R21 AI081101-01 5/2009-4/2011
"Antibiotic pressure and selection of TCA cycle mutants in *Staphylococcus epidermidis"*
The major goal of this study is to determine if mutations within the TCA cycle abrogate antibiotic efficacy of bactericidal antibiotics
Role: PI

NIH/NIAID P01 AI083211-01 7/2009-4/2014
"Biofilm Biology of *Staphylococcus aureus"*
The major goals of this program project are to study multiple aspects of biofilm maturation. The major goal of project 2 (Fey, PI) is to study the impact of arginine catabolism on biofilm maturation and virulence of *S. epidermidis* and *S. aureus.*
Role: Project leader and PI of project 2 (Bayles, PI of program project)

Department of Defense 5/2009-4/2012
"Fighting drug-resistant infections*"*
The major goals of this study is to create a defined transposon insertion library in *S. aureus* USA300 LAC
Role: Co-PI (Bayles, Fey)

Department of Defense 10/2007 - 9/2010
Rapid identification of biological warfare agents
The major goal of this study is to find new targets for the identification of biological warfare agents
Role: Co-I (Hinrichs PI)

NIH/CDC 1U38 HM000010 10/01/06-9/30/09
"Assessment and Development of an antimicrobial susceptibility program in Nebraska"
The goals of this educational grant are to teach current clinical microbiology antimicrobial susceptibility testing methodologies to clinical microbiology laboratories in the state of Nebraska.
Role: PI

NIH/NIAID 1 R01 AI073780-01 9/1/2008-8/31/2013
"Staphylococcus aureus mRNA turnover"
The goals of this application are to study the regulation of mRNA turnover in *S. aureus* and its relationship to virulence.
Role: Co-I (Dunman PI)

Completed Research Support (last three years)

Cubist Pharmaceuticals 1/01/07-12/31/08
"Efficacy of Daptomycin in *Staphylococcus epidermidis* mediated biofilm infections."
The major goal of this study was to determine the efficacy of daptomycin against *S. epidermidis* biofilms.
Role: PI

NIH/NIGM 1 R21 GM076585 02/01/2006-1/31/2008
"Environmental regulation of *Staphylococcus epidermidis* PIA synthesis"
The major goal of this project was to determine the relationship of the TCA cycle and PIA/biofilm production.
Role: Co-I (Somerville, PI)

NIH/NIAID 5RO1-AI49311 07/01/2002-06/30/2007
ica phenotypic variation in *Staphylococcus epidermidis*
The major goal of this project was to define molecular mechanisms regarding phenotypic variation of biofilm formation in *Staphylococcus epidermidis*.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the 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
Rupp, Mark E.	Professor, Department of Internal Medicine, University of Nebraska Medical Center Director, Department of Healthcare Epidemiology, Nebraska Medical Center
eRA COMMONS USER NAME RUPP.MARK	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Texas at Austin	BS	1981	Chemical Engineering
Baylor College of Medicine, Houston, TX	MD	1985	Medicine

A. Positions and Honors

Positions and Employment

1985-1988 Medical Residency, Internal Medicine, Virginia Commonwealth University
1988-1991 Fellowship, Infectious Diseases, Virginia Commonwealth University
1991-1992 Clinical Instructor, Dept Internal Medicine, Virginia Commonwealth University
1992-1997 Assistant Professor of Internal Medicine, University of Nebraska Medical Center
1996- Medical Director & Hospital Epidemiologist, Dept of Healthcare Epidemiology, Nebraska Medical Center
1997-2004 Associate Professor of Internal Medicine, University of Nebraska Medical Center
2001- Medical Director, Clinical Trials Office, Nebraska Medical Center
2004- Director, Antimicrobial Stewardship Program, Nebraska Medical Center
2004- Professor of Internal Medicine, University of Nebraska Medical Center

Selected Other Experience and Professional Memberships

1990- American Society for Microbiology
2001-04 Division L President-Elect, President, and Past-President
1994- Society for Hospital Epidemiology of America (SHEA)
1997-2002 Annual Meeting Committee; 2001 SHEA Annual Meeting – Scientific Chairman
2002-2004 Academic Councilor/Board of Directors
2004-2006 Treasurer/Board of Directors
2007-2010 Vice-Pres, Pres-Elect, President, Past-President
2002-2007 United States Food and Drug Administration, Anti-Infective Drugs Advisory Committee-Consultant
2003 Centers for Disease Control – Hospital Infection Control Practices Advisory Committee – External Expert Review Panel, "Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings"
2003- International Society on Staphylococci and Staphylococcal Infections
2003 North American Advisory Board, 2004 Abstract Chairman, 11th International Symposium on Staphylococci and Staphylococcal Infections
2004 Centers for Disease Control – Special Emphasis Panel RFA 03120 "Applied Research on Antimicrobial Resistance: Characterization of Strains of Community-Associated Methicillin-Resistant *Staphylococcus aureus*"
2008 Centers for Disease Control – Special Emphasis Panel RFA CI08-001 "Development, Implementation, and Evaluation of Novel Strategies to reduce Inappropriate Antimicrobial use in Community and Healthcare Settings"

Selected Honors

National Merit Scholarship (1977-1981); Chemical Engineering Departmental Merit Scholarship (1977-1981); National Foundation for Infectious Diseases Young Investigator Award (1993); Fellow, American College of Physicians (1994); Fellow, Infectious Diseases Society of America (2001); Alpha Omega Alpha – National Honor Medical Society (2003);

Fellow, Society for Healthcare Epidemiology of America (2004); Best Doctors In America 2003-2008; UNMC Top Teachers in Internal Medicine 2002, 2003, 2005, 2006, 2007, 2008.

B. Selected Publications [Total Bibliography: 102 articles, 1 textbook, 32 chapters, 149 abstracts, 3 US patents, 54 other – letters, reviews, etc]

Textbook:

Biofilms, Infections, and Antimicrobial Therapy. Pace JL, **Rupp ME**, Finch R (eds). CRC Press, New York. 2006.

Articles**:**

Rupp ME, Archer GL. Hemagglutination and Adherence to Plastic by *Staphylococcus epidermidis*. Infect Immun, 60:4322-27, 1992.

Rupp ME, Archer GL. Coagulase-Negative Staphylococci: Pathogens Associated with Medical Progress. Clin Infect Dis. 19:231-45, 1994.

Rupp ME, Han J, Goering RV. Repeated Recovery of *Staphylococcus saprophyticus* from the Urogenital Tracts of Women: Persistence versus Recurrence. Infect Dis Obstet Gynecol, 2:218-22,1995.

Rupp ME, Han J, Gatermann S. Hemagglutination by *Staphylococcus aureus* Strains Responsible for Human Bacteremia or Bovine Mastitis. J Med Microbiol Immunol. 184:33-36,1995.

Rupp ME, Sloot N, Meyer HGW, Han J, Gatermann S. Characterization of the Hemagglutinin of *Staphylococcus epidermidis*. J Infect Dis, 172:1509-18,1995.

Rupp ME, Hamer KE. Effect of ofloxacin, levofloxacin, d-ofloxacin, vancomycin, and cefazolin on the adherence of *Staphylococcus epidermidis* to teflon intravascular catheters. J Antimicrob Chemoth. 41:155-61, 1998.

Rupp ME, Ulphani JS, Fey PD, Mack D. Characterization of the importance of polysaccharide intercellular adhesin/hemagglutinin of *Staphylococcus epidermidis* in the pathogenesis of biomaterial-based infection in a mouse foreign body infection model. Infect Immun. 67:2627-32,1999.

Mack D, Riedewald J, Rohde H, Magnus T, Feucht HH, Elsner HA, Laufs R, **Rupp ME**. Essential functional role of the polysaccharide intercellular adhesin of *Staphylococcus epidermidis* in hemagglutination. Infect Immun. 1999; 67:1004-08.

Ulphani JS, **Rupp ME**. Novel rat model of *Staphylococcus aureus* central venous catheter-associated infection. Lab Animal Sci. 49:283-87,1999.

Fey PD, Ulphani JS, Gotz F, Heilmann C, Mack D, **Rupp ME**. Characterization of the relationship between polysaccharide intercellular adhesin (PIA) and hemagglutination (HA) in *Staphylococcus epidermidis*. J Infect Dis. 179:1561-4, 1999.

Rupp ME, Ulphani JS, Fey PD, Mack D. Characterization of *Staphylococcus epidermidis* polysaccharide intercellular adhesin/hemagglutinin in the pathogenesis of intravascular catheter-associated infection in a rat model. Infect Immun. 67:2656-59,1999.

Rupp ME, Fey PD, Heilmann C, Gotz F. Characterization of the importance of *Staphylococcus epidermidis* polysaccharide intercellular adhesin and autolysin in the pathogenesis of intravascular catheter-associated infection in a rat model. J Infect Dis 183:1038-42, 2001.

Fowler VG, Fey PD, Reller LB, Chamis A, Corey GR, **Rupp ME**. The intercellular adhesin locus *ica* is present in clinical isolates of *Staphylococcus aureus* from bacteremic patients with infected and uninfected prosthetic joints. J Microbiol Immun, 189:127-131, 2001.

Rupp ME, Fey PD. In-vivo models to evaluate adhesion and biofilm formation by *Staphylococcus epidermidis*. Methods in Enzymology. 336:206-215, 2001.

Fey PD, Salim B, **Rupp ME**, Hinrichs SH, Boxrud DJ, Davis CC, Kreiswirth BN, Schlievert PM. Comparative molecular analysis of community-acquired methicillin-resistant *Staphylococcus aureus* and hospital-acquired methicillin-resistant *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy. 47:196-203, 2003.

Fey PD, **Rupp ME**. Molecular Epidemiology in the Public Health and Hospital Environments. Clinics in Laboratory Medicine. 23:885-891, 2003.

Handke L, Conlon KM, Slater SR, Elbaruni S, Fitzpatrick F, Humphreys H, Giles WP, **Rupp ME**, Fey PD, O'Gara JP. Genetic and Phenotypic analysis of phenotypic variation in multiple *Staphylococcus epidermidis* isolates. J Medical Microbiology. 53:367-374, 2004.

Rupp ME, Lisco S, Lipsett P, Perl T, Keating K, Civetta J, Mermel L, Lee D, Dellinger EP, Donahoe M, Giles D, Pfaller M, Maki DG, Sherertz R. Effect of a second-generation chlorhexidine/silver sulfadiazine-impregnated

central venous catheter on catheter-associated infections in intensive care unit patients. A randomized, double-blinded, controlled trial. Annals of Internal Medicine, 143:570-580, 2005.

Olson ME, Garvin KL, Fey PD, **Rupp ME**. Initial Adherence of *Staphylococcus epidermidis* to orthopedic biomaterials is polysaccharide intercellular adhesin dependent. Clinical Orthopaedics and Related Research. 451:21-24, 2006.

Handke LD, Slater SR, Conlon KM, **Rupp ME**, O'Gara JP, Fey PD. Sigma-B and SarA independently regulate polysaccharide intercellular adhesin (PIA) production in *Staphylococcus epidermidis*. Canadian Journal of Microbiology. 53:82-91, 2007.

Fowler VG, Boucher HW, Corey R, Abrutyn E, Karchmer AW, **Rupp ME**. Levine DM, Chambers HF, Talley FP, Vigliani G, Cabell CH, Link AS, DeMeyer I, Filler S, Zervos M, Cook P, Parsonnet J, Berstein JM, Price CS, Forrest G, Fatkenheur G, Gareca M, Rehm SJ, Brodt HR, Tice A, Cosgrove SE & *S. aureus* Endocarditis and Bacteremia Study Group. Daptomycin versus Standard Therapy for *Staphylococcus aureus* Bacteremia and Endocarditis. N Eng J Med. 355:653-65, 2006.

Rupp ME, Holley HP, Jr, Lutz P, Dicpinigaitis PV, Woods C, Levine DP, Veney N, Fowler VG, Jr. A phase II, randomized, multicenter, double-blind, placebo-controlled, trial of a polyclonal anti-capsular immunoglobin in the treatment of *Staphylococcus aureus* bacteremia. Antimicrobial Agents and Chemotherapy. 51:4249-54, 2007.

Rupp ME, Lee SA, Jourdan DR, Marion ND, Tyner LK, Fey PD, Iwen PC, Anderson JR. Outbreak of bloodstream infections temporally associated with the use of an intravascular positive-displacement needleless valve Clinical Infectious Diseases. 44:1408-14, 2007.

Handke, L D, Rogers KL, Olson ME, Jerrells TJ, **Rupp ME**, Dunman PM, Fey PD. *Staphylococcus epidermidis sae*R is an effector of anaerobic growth and a mediator of acute inflammation. Infection and Immunity. 76:141-152, 2008.

Rogers KL, **Rupp ME**, Fey PD. The Presence of *ica*ADBC is Detrimental to the Colonization of *Staphylococcus epidermidis* on Human Skin. Applied and Environmental Microbiology. 74:6155-7, 2008.

Rogers K, Fey PD, **Rupp ME**. Coagulase-Negative Staphylococcal Infections. Infectious Diseases Clinics of North America. 23:73-98, 2009.

Chapters

Rupp ME. Infections Associated with Intravascular Devices. In: Baddour L, Gorbach S (eds) Therapy of Infectious Diseases. Harcourt Health Sciences. New York, 141-151, 2003

Rupp ME. Nosocomial Bloodstream Infections. In: C Glen Mayhall (ed). Hospital Epidemiology and Infection Control, 3rd edition. Lippincott Williams and Wilkins, Philadelphia, 253-265, 2004.

Rupp ME. Control of Gram-Positive Multidrug Resistant Pathogens. In: Lautenbach E, Woeltje K (eds). A Practical Handbook for Hospital Epidemiologists, 2nd Edition. Slack, Inc. Thorofare, NJ. 179-187, 2004.

Rogers KL, Fey PD, **Rupp ME**. Epidemiology of Infections due to Coagulase-Negative Staphylococci. In: Crossley K, Archer GL, Fowler V (eds), The Staphylococci in Human Disease, 2nd Edition. Churchill Livingstone Inc., New York. 2009.

Patents

Rupp ME. United States Patent: Inhibition of Adherence of *Staphylococcus epidermidis* to Biomaterial Surfaces by Treatment with Carbohydrates, #5718694, 1998.

C. Research Support

Ongoing Research Support

NIAID (HHSN266200400066C) AI-40066 Loeb (McMaster University) PI (Rupp, Co-I) 2005-2009
NIH/NIAID $206,799 1.2 calendar
"Population Genetics Analysis on West Nile Virus"
This project seeks to characterize host determinants that predispose to neuorinvasive disease

Microbiotix, Inc. NIH (1 R43AI074161-01) Opperman (Microbiotix) UNMC Subcontract (Rupp) 2008-2009
NIH $107,040 1.4 calendar
"Novel Inhibitors of Staphylococcal Biofilm Formation

This project seeks to characterize novel putative inhibitors of staphylococcal biofilm formation in a mouse foreign body infection model.

Molnlycke Health Care US Rupp ME, PI 2009-2010
Molnlycke $120,000 0.6 calendar
"Effectiveness of chlorhexidine gluconate (CHG) general skin cleansing in reducing the occurrence of catheter-associated bloodstream infections and the transmission and/or infection rate due to multi-drug resistant organisms in hospitalized patients."

Cardinal Health Foundation Rupp ME, PI 2009-2010
Cardinal Health Foundation $35,000 0.3 Calendar
"Prevention of Healthcare-Associated Infections Through Optimized Patient Care Environment Cleaning and Disinfection"

Recent Completed Research Support

Cubist Pharmacueticals Fey PI (Rupp Co-I) 2007-2008
Cubist Pharmaceuticals $78,416 0.6 calendar
"The efficacy of daptomycin, using in-vitro and in-vivo methodologies, to eradicate PIA (biofilm)-producing *Staphylococcus epidermidis"*

3M Health Care Rupp ME, PI 2007
3M Health Care $84,415.34 0.6 calendar
"Clinical Performance of the 3M Tegaderm Chlorhexidine Bordered Dressing" Health Care"

5RO1 AI49311 Fey PI (Rupp Co-I) 2002-2006
NIH/NIAID $150,000 1.8 calendar
"ica phase variation in *Staphylococcus epidermidis*"

BIOGRAPHICAL SKETCH

Provide the following information for the 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
Mark S. Smeltzer	Professor Department of Microbiology and Immunology Department of Orthopaedic Surgery
eRA COMMONS USER NAME	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Washburn Univ. of Topeka, Topeka, KS	BS	1982	Biology/Chemistry
Kansas State Univ., Manhattan, KS	MS	1987	Microbiology
Kansas State Univ., Manhattan, KS	PhD	1990	Microbiology
Kansas State Univ., Manhattan, KS	Post-doct.	1993	Bacteriology

A. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

EMPLOYMENT HISTORY:

1982-1984: Medical Technician, Department of Pathology.
1984-1987: Microbiologist I (M.S. student), Department of Pathology.
1987-1990: Graduate Research Assistant (Ph.D. student), Department of Pathology.
1990-1993: Post-doctoral Research Associate, Department of Pathobiology.
1993-1998: Assistant Professor, Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR.
1993-1998: Assistant Professor, Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR.
1995-present: Section Head, Musculoskeletal Infection Group, Department of Orthopaedic Surgery, University of Arkansas for Medical Sciences, Little Rock, AR.
1998-present: Assistant Professor, Department of Orthopaedic Surgery, University of Arkansas for Medical Sciences, Little Rock, AR.
1998-present: Associate Professor, Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR.
1998-present: Associate Professor, Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR.
1999-present Coordinator of Graduate Studies, Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR.
1999-present Director, Molecular Microbiology Core Facility, Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR
2001-present: Professor, Department of Orthopaedic Surgery, University of Arkansas for Medical Sciences, Little Rock, AR.
2001-present: Professor, Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR.
2001-present: Professor, Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR.

HONORS AND AWARDS:

Kansas Health Foundation Postdoctoral Scholar, Kansas State University (1991-1993)
Phi Zeta Award for Excellence in Basic Research, Kansas State University (1992)
New Investigator Award, Orthopaedic Research Society (1995)
Randall Award, Outstanding Young Faculty Member, South Central ASM (1996)

PROFESSIONAL ACTIVITIES:

Memberships: Orthopaedic Reseach Society, American Society for Microbiology, Musculoskeletal Infection Society.

Grant reviews: Department of Veterans Affairs, Wellcome Trust, NIH Special Review Panel for Targeted Research on Oral Microbial Biofilms, NIH NRSA Fellowship Review Panel, American Heart Association Microbiology and Immunology Study Section

Journal reviews: Clinical Orthopaedics and Related Research, Journal of Orthopaedic Research, Journal of Bone and Mineral Research, Trends in Microbiology, Microbiology, FEMS Microbiology Letters, Journal of Bacteriology, Infection and Immunity, Journal of Infectious Disease, Molecular Microbiology

B. Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation.

PEER-REVIEWED PUBLICATIONS

1. **Weiss, B.D., Weiss, E.C., Haggard, W.O., McLaren, S.G. and *Smeltzer, M.S.*** 2009. Optimized elution of daptomycin from polymethymethacrylate (PMMA) beads. *Antimicrobial Agents and Chemotherapy,* **53**:264-266.
2. **Tsang, L.H., Cassat, J.E., Shaw, L.N., Beenken, K.E. and *Smeltzer, M.S.*** 2008. Factors contributing to the biofilm-deficient phenotype of *Staphylococcus aureus sarA* mutants. PLoS ONE, **3(10)**:e3361.
3. **Cassat, J.E., Lee, C.Y., and *Smeltzer, M.S.*** 2007. Investigation of biofilm formation in clinical isolates of *Staphylococcus aureus*. Methods Mol. Biol., **391**:127-144.
4. **Rice, K.C., Mann, E.E., Endres, J.L., Weiss, E.C., Cassat, J.E., *Smeltzer, M.S.*, and Bayles, K.W.** 2007**.** The *cidA* murein hydrolase regulator contributes to DNA release and biofilm development in *Staphylococcus aureus*. Proceedings of the National Academy of Sciences (USA), **104**:8113-8118.
5. **Zhu, Y., Weiss, E.C., Otto, M., Fey, P.D., *Smeltzer, M.S.*, and Somerville, G.A.** 2007. *Staphylococcus aureus* biofilm metabolism and the influence of arginine on polysaccharide intercellular adhesin synthesis, biofilm formation, and pathogenesis. Infection and Immunity, 75:4219-4226.
6. **Tsang, L.H., Daily, S., Weiss, E.C., and *Smeltzer, M.S.* 2007.** Mutation of *traP* in clinical isolates of *Staphylococcus aureus* has not impact on expression of *agr* or on biofilm formation. Infection and Immunity, 75:4528-4533.
7. **Cassat, J.E., Weiss, E.C., Beenken, K.E., Yang, S-J., Bayles, K.W. and *Smeltzer, M.S.*** Disruption of acetolactate synthase (*alsS*) and acetolactate decarboxylase (*alsD*) in *Staphylococcus aureus* limits biofilm formation *in vitro* and *in vivo*. Microbiology, *submitted.*
8. **Sambanthamoorthy, K., *Smeltzer, M.S.* and Elasri, M.O.,** Identification and characterization of *asa* (SA1233), a gene that activates expression of *sarA* and regulates several virulence factors in *Staphylococcus aureus. Microbiology,* **152**:2559-2572.
9. **Cassat, J.E., Dunman, P.M., Murphy, E.J., Projan, S.J., Beenken, K.E., Palm, K.J., Yang, S-J., Rice, K.C., Bayles, K.W. and *Smeltzer, M.S.*,** Transcriptional profiling of the *Staphylococcus aureus* clinical isolate UAMS-1 and its isogenic *sarA* and *agr* mutants, *Microbiology,* **152**:3075-3090.
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11. **Zharov, V.P., Mercer, K.E., Galitovskaya, E.N. and *Smeltzer, M.S.*,** Photothermal nanotherapeutics and nanodiagnostics for selective killing of bacteria targeted with gold nanoparticles. 2006. *Biophysical Journal*, **90**:619-627.
12. **Jones-Jackson, L., Walker, R., Purnell, G., McLaren, S.G., Skinner, R.A., Thomas, J.R., Suva, L., Anaissie, E., Miceli, M., Nelson, C.L., Ferris, E. and *Smeltzer, M.S.*,** Early detection of bone infection and differentiation from post-surgical inflammation using 2-deoxy-2-[18F]-fluoro-D-glucose positron emission tomography (FDG-PET) in an animal model. 2005. *Journal of Orthopaedic Research*, **23**:1484-1489.

13. **Nelson, C.L., McLaren, A.C., McLaren, S.G., Johnson, J.W. and *Smeltzer, M.S.*,** Is aseptic loosening really aseptic? 2005. *Clinical Orthopaedics and Related Research*, **437**:25-30.
14. **Cassat J.E., Dunman, P.M., McAleese, F., Murphy, E., Projan, S.J. and *Smeltzer, M.S.*,** Comparative genomics of *Staphylococcus aureus* musculoskeletal isolates. 2005. *Journal of Bacteriology,* **187**:576-592.
15. **Koenig, R.L., Ray, J.L., Maleki, S.J., Smeltzer, M.S. and Hurlburt, B.K.,** *Staphylococcus aureus* AgrA binding to the RNAIII-agr regulatory region. 2004*. Journal of Bacteriology,* **186**:7549-7555.
16. **Beenken, K.E. Dunman, P.M., McAleese, F., Macapagal, D., Murphy, E., Projan, S.J., Blevins, J.S. *Smeltzer, M.S.*,** 2004. Global gene expression in *Staphylococcus aureus* biofilms, *Journal of Bacteriology*, 186:4665-4684.
17. **O'Leary, J.O., Langevin, M.J., Price, C.T.D., Blevins, J.S., Smeltzer, M.S. and Gustafson, J.E.** 2004. Effects of *sarA* inactivation on the intrinsic multidrug resistance mechanism of **Staphylococcus aureus**. FEMS Microbiology Letters, 237:297-302
18. **Beenken, K.E., Blevins, J.S. and *Smeltzer, M.S.*,** 2003. Mutation of *sarA* in *Staphylococcus aureus* limits biofilm formation. *Infection and Immunity*, **71**:4206-4211.
19. **Sterba, K.M., Mackintosh, S.G., Blevins, J.S., Hurlburt, B.K. and *Smeltzer, M.S*.**, 2003. Characterization of *Staphylococcus aureus* SarA binding sites. *Journal of Bacteriology*. **185**:4410-4417.
20. **Blevins, J.S., Elasri, M.O., Allmendinger, S.D., Beenken, K.E., Skinner, R.A., Thomas, J.R. and *Smeltzer, M.S.,*** 2003. Role of *sarA* in the pathogenesis of *Staphylococcus aureus* musculoskeletal infection. *Infection and Immunity*, **71**:516-523.
21. **Blevins, J.S., Beenken, K.E., Elasri, M.O., Hurlburt, B.K. and *Smeltzer, M.S.*** 2002. Strain-dependent differences in the regulatory roles of *sarA* and *agr* in *Staphylococcus aureus. Infection and Immunity,* 70:470-480*.*
22. **Nelson, C.L., McLaren, S.G., Skinner, R.A., *Smeltzer, M.S.,* Thomas, J.R. and Olsen, K.M., 2002.** The treatment of experimental osteomyelitis by surgical debridement and the implantation of calcium sulfate tobramycin pellets. *Journal of Orthopaedic Research* **20**:643-647.
23. **Elasri,, M.O., Thomas, J.R., Skinner, R.A., Blevins, J.S., Beenken, K.E., Nelson, C.L. and *Smeltzer, M.S.*** 2002. Role of the *Staphylococcus aureus* collagen adhesin in bone and joint infection. *Bone,* 30:275-280*.*
24. **Ellington, J.K., Reilly, S.S., Warner, K.R., Ramp, W.K., *Smeltzer, M.S.*, Kellam, J.F. and Hudson, M.C.** Mechanisms of *Staphylococcus aureus* invasion of cultured osteoblasts. 1999*. Microbial Pathogenesis*. **26**:317-323.
25. **Blevins, J.S., Gillaspy, A.F., Rechtin, T.M., Hurlburt, B.K. and *Smeltzer, M.S.*** 1999. The staphylococcal accessory regulator (*sar*) controls expression of the *Staphylococcus aureus* collagen adhesin gene (*cna*) in an *agr*-independent manner. *Molecular Microbiology*. **33**:317-326.
26. **Rechtin, T.M., Gillaspy, A.F., Schumacher, M., Brennan, R.G., *Smeltzer, M.S.* and Hurlburt, B.K.** 1999. Characterization of SarA virulence gene regulator of *Staphylococcus aureus*. *Molecular Microbiology*. **33**:307-316.
27. **Snodgrass, J.L., Mohammed, N., Ross, J.M., Sau, S., Lee, C.Y. and *Smeltzer, M.S.*** 1998. Functional characterization of the *Staphylococcus aureus* collagen adhesin B domain. *Infection and Immunity*, 67:3952-3959.
28. **Gillaspy, A.F., Lee. C.Y., Sau, S., Cheung, A.L. and *Smeltzer, M.S.*** 1998. Factors affecting the collagen binding capacity of *Staphylococcus aureus. Infection and Immunity*. **66**:3170-3178.
29. ***Smeltzer, M.S.*, Gillaspy, A.F., Pratt, F.L., Jr. and Thames, M.D.** 1997. Comparative evaluation of *cna, fnbA, fnbB* and *hlb* genomic fingerprinting for the epidemiological typing of *Staphylococcus aureus*. *Journal of Clinical Microbiology*. **35**:2444-2449.
30. ***Smeltzer, M.S.*, Gillaspy, A.F., Pratt, F.L. and Iandolo, J.J.** 1997. Prevalence and chromosomal map location of *Staphylococcus aureus* adhesin genes. *Gene.* **196**:249-259.
31. **Gillaspy, A.F., Patti, J.M., Pratt, F.L., Jr., and *Smeltzer, M.S.*** 1997. The *Staphylococcus aureus* collagen adhesin-encoding gene (*cna*) is within a discrete genetic element. *Gene.* **196**:239-248*.*
32. ***Smeltzer, M.S.*, Thomas, J.R., Hickmon, S.G., Skinner, R.A., Nelson, C.L., Griffith, D., Parr, T.R., Jr. and Evans, R.P.** 1997. Characterization of a rabbit model of staphylococcal osteomyelitis. *Journal of Orthopaedic Research,* **15**:414-421.

33. **Gillaspy, A.F., Patti, J.M., Pratt, F.L., Jr., and *Smeltzer, M.S.* 1997.** Transcriptional regulation of the *Staphylococcus aureus* collagen adhesin gene (*cna*). *Infection and Immunity,* 65:1536-1540.
34. ***Smeltzer, M.S.*, Pratt, F.L., Jr., Gillaspy, A.F. and Young, L.A. 1996.** Genomic fingerprinting for the epidemiological differentiation of *Staphylococcus aureus* clinical isolates. *Journal of Clinical Microbiology*, 34:1364-1372.
35. **Gillaspy, A.F., Hickmon, S.G., Skinner, R.A., Thomas, J.R., Nelson, C.L**. and ***Smeltzer, M.S*.** 1995. Role of the accessory gene regulator (*agr*) in the pathogenesis of staphylococcal osteomyelitis. *Infection and Immunity,* **63**:3373-3380.

C. Research Support. List selected ongoing or completed (during the last three years) research projects (federal and non-federal support). Begin with the projects that are most relevant to the research proposed in this application. Briefly indicate the overall goals of the projects and your role (e.g. PI, Co-Investigator, Consultant) in the research project. Do not list award amounts or percent effort in projects.

RESEARCH PROJECTS ONGOING OR COMPLETED IN THE LAST 3 YEARS:

Title: *sar*-mediated regulation in *Staphylococcus aureus*. Funding agency: National Institute of Allergy and Infectious Disease (NIAID). Type: R01 (R01-AI43356). Period: December 15, 2003-December 14, 2008. Role on project: PI. The objective of this proposal is to define the virulence factors that define *S. aureus* as the preeminent musculoskeletal pathogen. The specific aims are 1) to identify genes that are conserved among S. aureus strains that cause musculoskeletal infection, 2) to correlate transcriptional changes associated with mutation of *sarA* with the adaptive response required for persistence within a biofilm and 3) to identify genes that contribute to biofilm formation and musculoskeletal disease. Currently in a no-cost extension status.

Title: Detection, diagnosis and treatment of staphylococcal musculoskeletal infection. Funding agency: National Institute of Allergy and Infectious Disease (NIAID). Type: R01 (R01-AI069087). Period: Feb 1, 2007-Jan 31, 2012. Role on project: PI. The objectives of this proposal are to optimize methods for the detection, diagnosis and treatment of staphylococcal musculoskeletal infection. The specific aims are 1) to optimize FDG-PET for the early detection of musculoskeletal infection, 2) to develop a rapid diagnostic method that will facilitate determinative antimicrobial therapy of staphylococcal musculoskeletal infection, and 3) to optimize antibiotic therapy for biofilm-associated musculoskeletal infection.

Title: Regulation of *Staphylococcus aureus* biofilm formation by *rbf*. Funding agency: National Institute of Allergy and Infectious Disease (NIAID). Type: R01 (R01-AI067857). Period: July 5, 2007-July 4, 2012.
Role on project: Co-I. The major goals of this project are to study the molecular architecture of the *rbf* locus involved in biofilm regulation in *S. aureus*, to study the regulatory mechanism of Rbf, and to investigate the role of Rbf in pathogenesis.

Title: Regulation of biofilm formation in clinical isolates of *Staphylococcus aureus*. Funding agency: National Institute of Allergy and Infectious Disease (NIAID). Type: R01 (R01-AI074935). Period: July 1, 2007-June 30, 2012. Role on project: PI. The specific aims of this proposal are 1) to define the regulatory loci that contribute to biofilm formation, and their impact on virulence, in clinical isolates of *S. aureus*, 2) to assess the impact of biofilm-associated loci on the efficacy of antimicrobial therapy, and 3) to define the interaction between SarA and its DNA targets.

Title: Capsule regulatory network in *S. aureus* pathogenesis. Funding agency: National Institute of Allergy and Infectious Disease. Type: R01 (R01- AI37027). Period: December 1, 2008-November 30, 2013. Role on project: Co-investigator The major goals of this project are to characterize the regulatory network affecting capsule, to elucidate the mechanisms of regulation, and to correlate regulation network to pathogenesis.

BIOGRAPHICAL SKETCH

Provide the following information for the 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
Joyce Solheim	Associate Professor
eRA COMMONS USER NAME SOLHEIM.JOYCE	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Northwest Missouri State University	B.S.	1978-1982	Medical Technology
Southern Illinois University, Carbondale, IL	Ph.D.	1987-1992	Microbiol. & Immunology
Washington University, St. Louis, MO	Post-doc	1992-1997	Immunology

A. Positions and Honors

Positions

1997-1999 Assistant Professor, Department of Microbiology and Immunology, University of South Dakota School of Medicine, Vermillion, SD

1999-2003 Assistant Professor, Eppley Institute (with adjunct appointments in the Departments of Pathology & Microbiology and Biochemistry & Molecular Biology), University of Nebraska Medical Center, Omaha, NE

2003-present Associate Professor, Eppley Institute (with adjunct appointments in the Departments of Pathology & Microbiology and Biochemistry & Molecular Biology), University of Nebraska Medical Center, Omaha, NE

Other Experience and Professional Memberships

1998 & 2001 Minisymposium co-chair, national AAI Annual Meeting/Experimental Biology Meeting
1999-2003 Member, NIH Study Section to review National Research Service Award applications
2000 Associate Editor for the *Journal of Immunology*
2000-present UNMC Eppley Institute Director's Advisory Committee
2002-2003 Grant application reviewer for the United Kingdom Wellcome Trust
2004-present UNMC Eppley Cancer Center Internal Advisory Board
2004-present UNMC Eppley Cancer Center Senior Leadership Council
2005-present Section Editor for the *Journal of Immunology*
2005-present Director, UNMC Cancer Research Graduate Program
2005 Member, DOD Breast Cancer Research Program Immunological Sciences Study Section
2005 Member, NIH ZRG1 IMM-C 02M Special Emphasis Panel
2006 Ad hoc member, NIH Cellular and Molecular Immunology - B Study Section
2007 Member, NIH ZRG1 IMM-E 02M Special Emphasis Panel
2007 Ad hoc member, NIH Hypersensitivity, Autoimmune, and Immune-mediated Diseases Study Section ZRG HAI-G 09F
2007 Ad hoc member, NIH Cellular and Molecular Immunology - A Study Section (two cycles)
2007 Minisymposium chair, AAI Annual Meeting
2008 Ad hoc member, NIH Cellular and Molecular Immunology - A Study Section
2009 Ad hoc member, NIH Cellular and Molecular Immunology - A Study Section
2009 AAI Annual Meeting Abstract Programming Chair (Antigen Processing and Presentation)

Honors

2006 UNMC Distinguished Scientist Award
2008 Recipient of UNMC Outstanding Faculty Mentor of Graduate Students Award
2008 Nominated for the Alvin M. Earle Outstanding Health Sciences Educator Award

B. Selected peer-reviewed publications, in chronological order

Solheim, J. C. Class I MHC molecules: assembly and antigen presentation. *Immunological Rev.* 172:11-19, 1999.

Hansen, T., G. Balendiran, J. Solheim, A. Young, D. Ostrov, and S. Nathenson. Structural differences in mouse class I molecules define features that allow each to play a specialized role in antigen presentation. *Immunol. Today* 21:83-88, 2000.

Turnquist, H. R., H. J. Thomas, K. R. Prilliman, C. T. Lutz, W. H. Hildebrand, and J. C. Solheim. HLA-B polymorphism affects interactions with multiple endoplasmic reticulum proteins. *Eur. J. Immunol.* 30:3021-3028, 2000.

Turnquist, H. R., S. E. Vargas, M. M. McIlhaney, S. D. Sanderson, S. Li, P. Wang, B. Gubler, P. van Endert, and J. C. Solheim. A region of tapasin that affects L^d binding and assembly. *J. Immunol.* 167:4443-4449, 2001.

Harris, M. R., L. Lybarger, N. B. Myers, C. Hilbert, J. C. Solheim, T. H. Hansen, and Y. Y. L. Yu. Interactions of HLA-B27 with the peptide loading complex as revealed by heavy chain mutations. *Int. Immunol.* 13:1275-1282, 2001.

Turnquist, H. R., S. E. Vargas, and J. C. Solheim. Loss of a glycine in the α2 domain affects MHC peptide binding but not chaperone binding. *Biochem. Biophys. Res. Commun.* 289:825-831, 2001.

Reber, A. J., H. R. Turnquist, H. J. Thomas, C. T. Lutz, and J. C. Solheim. Expression of invariant chain can cause an allele-dependent increase in the surface expression of MHC class I molecules. *Immunogenetics* 54:74-81, 2002.

Hildebrand, W. H., H. R. Turnquist, K. R. Prilliman, H. D. Hickman, E. L. Schenk, M. M. McIlhaney, and J. C. Solheim. HLA class I polymorphism has a dual impact on ligand binding and chaperone interaction. *Human Immunol.* 63:248-255, 2002.

Turnquist, H. R., S. E. Vargas, E. L. Schenk, M. M. McIlhaney, A. J. Reber, and J. C. Solheim. The interface between tapasin and MHC class I: identification of amino acid residues in both proteins that influence their interaction. *Immunologic Res.* 25:261-269, 2002.

Turnquist, H. R., S. E. Vargas, M. M. McIlhaney, S. Li, P. Wang, and J. C. Solheim. Calreticulin binds to the α1 domain of MHC class I independently of tapasin. *Tissue Antigens* 59:18-24, 2002.

Turnquist, H. R., E. L. Schenk, M. M. McIlhaney, H. D. Hickman, W. H. Hildebrand, and J. C. Solheim. Disparate binding of chaperone proteins by HLA-A subtypes. *Immunogenetics* 53:830-834, 2002.

Petersen, J. L., C. R. Morris, and J. C. Solheim. Virus evasion of MHC class I. *J. Immunol.* 171:4473-4478, 2003.

Morris, C. R., J. L. Petersen, S. E. Vargas, H. R. Turnquist, M. M. McIlhaney, S. D. Sanderson, J. T. Bruder, Y. Y. L. Yu, H.-G. Burgert, and J. C. Solheim. The amyloid precursor-like protein 2 and the adenoviral E3/19K protein both bind to a conformational site on $H\text{-}2K^d$ and regulate $H\text{-}2K^d$ expression. *J. Biol. Chem.* 278:12618-12623, 2003.

Reber, A. J., A. E. Ashour, S. N. Robinson, J. E. Talmadge, and J. C. Solheim. Flt3-ligand bioactivity and pharmacology in neoplasia. *Curr. Drug Targets – Immune, Endocr. & Metab. Disorders* 4:149-156, 2004.

Turnquist, H. R., J. L. Petersen, S. E. Vargas, M. M. McIlhaney, E. Bedows, W. E. Mayer, A. G. Grandea III, L. Van Kaer, and J. C. Solheim. The Ig-like domain of tapasin influences intermolecular interactions. *J. Immunol.* 2976-2984, 2004.

Ma, W., H. Yu, Q. Wang, H. Jin, J. Solheim, and V. Labhasetwar. A novel approach for cancer immunotherapy: tumor cells with anchored superantigen SEA generate effective antitumor immunity. *J. Clin. Immunol.* 24:294-301, 2004.

Turnquist, H. R., K. G. Kohlgraf, M. M. McIlhaney, R. L. Mosley, M. A. Hollingsworth, and J. C. Solheim. Tapasin decreases immune responsiveness to a model tumor antigen. *J. Clin. Immunol.* 24:462-470, 2004.

Morris, C. R., A. J. Reber, J. L. Petersen, and J. C. Solheim. Association of intracellular proteins with folded major histocompatibility complex class I molecules. *Immunologic Res.* 30:171-179, 2004.

Petersen, J. L., H. D. Hickman-Miller, M. M. McIlhaney, S. E. Vargas, A. W. Purcell, W. H. Hildebrand, and J. C. Solheim. A charged amino acid residue in the transmembrane/cytoplasmic region of tapasin influences MHC class I assembly and maturation. *J. Immunol.* 174:962-969, 2005.

Sang, H., V. M. Pisarev, J. Chavez, S. Robinson, Y. Guo, L. Hatcher, C. Munger, J. C. Solheim, R. K. Singh, and J. E. Talmadge. Murine mammary adenocarcinoma cells transduced with p53 and/or Flt3L induce anti-tumor immune responses. *Cancer Gene Ther.*, 12:427-437, 2005.

Ambagala, A. P., J. C. Solheim, and S. Srikumaran. Viral interference with MHC class I antigen presentation pathway: the battle continues. *Vet. Immunol. Immunopathol.* 107:1-15, 2005.

Chen, Z., M. L. Varney, M. W. Backora, K. Cowan, J. C. Solheim, J. E. Talmadge, and R. K. Singh. Down-regulation of vascular endothelial cell growth factor-C expression using small interfering RNA vectors in mammary tumors inhibits tumor lymphangiogenesis and spontaneous metastasis and enhances survival. *Cancer Res.* 65:9004-9011, 2005.

Ashour, A., J. L. Petersen, M. M. McIlhaney, J. M. Vose, and J. C. Solheim. Effect of linkage of tranduction domain sequences to a lymphoma idiotype DNA vaccine on vaccine effectiveness. *Hybridoma* 25:306-308, 2006.

Ashour, A. E., H. R. Turnquist, R. Singh, J. Talmadge, and J. C. Solheim. CCL21-induced immune cell infiltration. *Int. Immunopharm*., 7:272-276, 2007.

Turnquist, H. R., X, Lin, A. E. Ashour, M. A. Hollingsworth, R. K. Singh, J. E. Talmadge, and J. C. Solheim. CCL21 induces extensive intratumoral immune cell infiltration and specific anti-tumor cellular immunity. *Int. J. Oncol.* 30:631-639, 2007.

Solheim, J. C., A. J. Reber, A. E. Ashour, S. Robinson, M. Futakuchi, S. G. Kurz, K. Hood, R. R. Fields, L. R. Shafer, D. Cornell, S. Sutjipto, S. Zurawski, D. M. LaFace, R. K. Singh, and J. E. Talmadge. Dendritic cell expansion by intravenously injected adenovirus-Flt3L is suppressed in mammary tumors. *Cancer Gene Ther.* 14:364-371, 2007.

Ashour, A. E., H. R. Turnquist, N. Burns, X. Wang, X. Lin, J. Tremayne, M. A. Hollingsworth, J. M. Blonder, G. J. Rosenthal, J. E. Talmadge, and J. C. Solheim. Flt3 ligand delivered in a pluronic formulation prolongs the survival of mice with orthotopic pancreatic adenocarcinoma. *Cancer Biother. Radiopharm.* 22:235-238, 2007.

Ashour, A. E., X. Lin, X. Wang, H. R. Turnquist, N. M. Burns, A. Tuli, A. Sadanandam, K. Suleiman, R. K. Singh, J. E. Talmadge, and J. C. Solheim. CCL21 is an effective surgical neoadjuvant for treatment of mammary tumors. *Cancer Biol. Ther.* 6:1206-1210, 2007.

Tuli, A., M. Sharma, X. Wang, N. Naslavsky, S. Caplan, and J. C. Solheim. Specificity of amyloid precursor-like protein 2 interactions with MHC class I molecules. *Immunogenetics* 60:303-313, 2008 [NIHMS66130].

Tuli, A., M. Sharma, M. M. McIlhaney, J. E. Talmadge, N. Naslavsky, S. Caplan, and J. C. Solheim. Amyloid precursor-like protein 2 increases the endocytosis, instability, and turnover of the H2-K^d MHC class I molecule. *J. Immunol.* 181:1978-1987, 2008 [NIHMS66137].

Simone, L. C., X. Wang, A. Tuli, M. M. McIlhaney, and J. C. Solheim. Influence of the tapasin C-terminus on the assembly of MHC class I allotypes. *Immunogenetics* 61:43-54, 2009; epub 2008. [NIHMS101042]

Lin, X., X. Wang, H. Capek, L. Simone, A. Tuli, C. R. Morris, A. J. Reber, and J. C. Solheim. Effect of invariant chain on major histocompatibility complex class I molecule expression and stability on human breast tumor cell lines. *Cancer Immunol. Immunother.*, epub 2008 [NIHMS70756].

Tuli, A., M. Sharma, X. Wang, L. C. Simone, H. L. Capek, S. Cate, W. H. Hildebrand, N. Naslavsky, S. Caplan, and J. C. Solheim. Amyloid precursor-like protein 2 association with HLA class I molecules. *Cancer Immunol. Immunother.,* epub 2009 [NIHMS101247].

Simone, L. C., X. Wang and J. C. Solheim. A transmembrane tail: interaction of tapasin with TAP and the MHC class I molecule. *Mol. Immunol.*, 46:2147-2150, 2009 [Publisher will submit to PMC].

Wang, X., L. C. Simone, A. Tuli, and J. C. Solheim. Comparative analysis of the impact of a free cysteine in tapasin on the maturation and surface expression of murine MHC class I allotypes. *International Journal of Immunogenetics*. Epub ahead of print 2009 [Publisher will submit to PMC].

Donkor, M. K., E. Lahue, T. A. Hoke, L. R. Shafer, U. Coskun, J. C. Solheim, D. Gulen, J. Bishay, and J. E. Talmadge. Mammary tumor heterogeneity in the expansion of myeloid-derived suppressor cells. *Int. Immunopharmacol.* Epub ahead of print 2009.

Abe, F., A. J. Dafferner, M. Donkor, S. N. Westphal, E. M. Scholar, J. C. Solheim, R. K. Singh, T. A. Hoke, and J. E. Talmadge. Myeloid-derived suppressor cells in mammary tumor progression in FVB Neu transgenic mice. *Cancer Immunol. Immunother*. Epub ahead of print 2009.

C. Research Support

Ongoing Research Support

R01 GM57428 Solheim (PI) 2005-2010
NIH/NIGMS
ER Protein Effect on Class I MHC Assembly
The goal of this project is to determine the factors regulating the presentation of antigenic peptides by MHC class I molecules to T lymphocytes.
Role: Principal Investigator

Eppley Cancer Center Pediatric Cancer Research Grant 2008-2009
Role of Amyloid Precursor-Like Protein 2 in Ewing's Sarcoma Evasion of Immunity
The goal of this project is to ascertain whether the amyloid-precursor like protein 2 functions in the resistance of Ewing's sarcoma to elimination by the immune system.
Role: Principal Investigator

Cancer & Smoking Disease Research Grant 2008-2009
Nebraska Dept. of Health and Human Services
Role of APLP2 in the Regulation of Antigen Presentation
The goal of this project is to determine the impact of APLP2 on the cell surface expression of MHC class I molecules and the recognition of MUC1 antigenic epitopes.
Role: Principal Investigator

Breast Cancer Concept Award (DOD BCRP BC075621) Singh (PI) 2008-2009
Department of Defense
Molecular Mechanism of Lymph Node Metastasis in Breast Cancer
The major goal of this project is to determine the causal relationship between HA-LYVE-1 and SLC-mediated regulation of lymph node metastasis in breast cancer. The knowledge gained from these studies will provide a foundation to develop diagnostic markers and novel therapeutics to inhibit breast cancer metastasis.
Role: Co-investigator

Completed Research Support

Eppley Cancer Center Grant Solheim (PI) 2007-2008
CCL21 Neoadjuvant Therapy for Breast Cancer
The goal of this project is to evaluate the chemokine CCL21 as a neoadjuvant breast tumor treatment.
Role: Principal Investigator

Nebraska Research Initiative Grant Talmadge (PI) 2006-2008
Translational Biotechnology Research
The goal of this project is to apply new biotechnologies, such as recombinant cytokines, in preclinical and clinical cancer therapy studies.
Role: Co-Investigator

BIOGRAPHICAL SKETCH

Provide the following information for the 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
Dunman, Paul Michael	Assistant Professor
eRA COMMONS USER NAME DUNMAN,PAUL	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Delaware Valley College, Doylestown, PA	BS	1988-1992	Biology
University of Medicine and Dentistry of New Jersey, Newark, NJ	PhD	1992-1999	Microbiology and Molecular Genetics
Wyeth Infectious Disease Dept., Pearl River, NY		1999-2001	Postdoctoral Training

A. Positions and Honors.

Positions and Employment

2001-2003 Research Scientist, Wyeth Research Infectious Disease Dept., Pearl River, NY
2003-2004 Sr. Research Scientist, Wyeth Research Bacterial Vaccines Dept., Pearl River, NY
2004-Present Assistant Professor, University of Nebraska Medical Center, Omaha, NE

Other Experience and Professional Memberships

2001-Present Member American Society of Microbiology
2004 International Symposium on Staphylococci and Staphylococcal Infections (ISSSI) Advisory Board
2004-Present Antimicrobial Agents and Chemotherapy (AAC) Editorial Board
2004 American Pediatric Society, Neonatal Sepsis Guest Panel, San Francisco CA
2005-2008 Consultant Merck Pharmaceuticals, Bacterial Vaccines, West Point, PA
2005-Present American Heart Association, Microbiology and Immunology Review Panel
2008 American Heart Association, Microbiology and Immunology Co-Chair
2008 Consultant Wyeth Pharmaceuticals, Bacterial Vaccines, Pearl River, NY

Honors

2003 Interrscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) Young Investigator Award
2009 University of Nebraska Gilmore Outstanding Investigator

B. Selected peer-reviewed publications (in chronological order).

1. Palejwala VA, Pandya GA, Bhanot OS, Solomon JJ, Murphy HS, Dunman PM, and Humayun MZ. 1994. UVM, an ultraviolet-inducible RecA-independent mutagenic phenomenon in Escherichia coli. J Biol Chem 269**:**27433-27440.
2. Wang G, Palejwala VA, Dunman PM, Aviv DH, Murphy HS, Rahman MS, and Humayun MZ. 1995. Alkylating agents induce UVM, a recA-independent inducible mutagenic phenomenon in Escherichia coli. Genetics 141**:**813-823. PMCID: **PMC1206846**.
3. Murphy HS, Palejwala VA, Rahman MS, Dunman PM, Wang G, and Humayun MZ. 1996. Role of mismatch repair in the Escherichia coli UVM response. J Bacteriol 178**:**6651-6657. PMCID: **PMC178557**.
4. Rahman MS, Dunman PM, Wang G, Murphy HS, and Humayun MZ. 1996. Effect of UVM induction on mutation fixation at non-pairing and mispairing DNA lesions. Mol Microbiol 22**:**747-755.

5. Wang G, Dunman PM, and Humayun MZ. 1997. Replication of M13 single-stranded DNA bearing a site-specific ethenocytosine lesion by Escherichia coil cell extracts. Cell Res 7**:**1-12.
6. Dunman PM, Ren L, Rahman MS, Palejwala VA, Murphy HS, Volkert MR, and Humayun MZ. 2000. Escherichia coli cells defective for the recN gene display constitutive elevation of mutagenesis at 3,N(4)-ethenocytosine via an SOS-induced mechanism. Mol Microbiol 37**:**680-686.
7. Dunman PM, Murphy E, Haney S, Palacios D, Tucker-Kellogg G, Wu S, Brown EL, Zagursky RJ, Shlaes D, and Projan SJ. 2001. Transcription profiling-based identification of *Staphylococcus aureus* genes regulated by the agr and/or sarA loci. J Bacteriol 183**:**7341-7353. PMCID: **PMC95583**.
8. Livermore DM, Warner M, Hall LM, Enne VI, Projan SJ, Dunman PM, Wooster SL, and Harrison G. 2001. Antibiotic resistance in bacteria from magpies (Pica pica) and rabbits (Oryctolagus cuniculus) from west Wales. Environ Microbiol 3**:**658-661.
9. Said-Salim B, Dunman PM, McAleese FM, Macapagal D, Murphy E, McNamara PJ, Arvidson S, Foster TJ, Projan SJ, and Kreiswirth BN. 2003. Global regulation of *Staphylococcus aureus* genes by Rot. J Bacteriol 185**:**610-619. PMCID: **PMC145333**.
10. Utaida S, Dunman PM, Macapagal D, Murphy E, Projan SJ, Singh VK, Jayaswal RK, and Wilkinson BJ. 2003. Genome-wide transcriptional profiling of the response of *Staphylococcus aureus* to cell-wall-active antibiotics reveals a cell-wall-stress stimulon. Microbiology 149**:**2719-2732.
11. Beenken KE, Dunman PM, McAleese F, Macapagal D, Murphy E, Projan SJ, Blevins JS, and Smeltzer MS. 2004. Global gene expression in *Staphylococcus aureus* biofilms. J Bacteriol 186**:**4665-4684. PMCID: **PMC438561**
12. Bischoff M, Dunman P, Kormanec J, Macapagal D, Murphy E, Mounts W, Berger-Bachi B, and Projan S. 2004. Microarray-based analysis of the *Staphylococcus aureus* sigmaB regulon. J Bacteriol 186**:**4085-4099. PMCID: **PMC421609**.
13. Dunman PM, Mounts W, McAleese F, Immermann F, Macapagal D, Marsilio E, McDougal L, Tenover FC, Bradford PA, Petersen PJ, Projan SJ, and Murphy E. 2004. Uses of *Staphylococcus aureus* GeneChips in genotyping and genetic composition analysis. J Clin Microbiol 42**:**4275-4283. PMCID: **PMC516287**.
14. Weinrick B, Dunman PM, McAleese F, Murphy E, Projan SJ, Fang Y, and Novick RP. 2004. Effect of mild acid on gene expression in *Staphylococcus aureus*. J Bacteriol 186**:**8407-8423. PMCID: **PMC532443**.
15. Cassat JE, Dunman PM, McAleese F, Murphy E, Projan SJ, and Smeltzer MS. 2005. Comparative genomics of *Staphylococcus aureus* musculoskeletal isolates. J Bacteriol 187**:**576-592. PMCID: **PMC543526**.
16. Entenza JM, Moreillon P, Senn MM, Kormanec J, Dunman PM, Berger-Bachi B, Projan S, and Bischoff M. 2005. Role of sigmaB in the expression of *Staphylococcus aureus* cell wall adhesins ClfA and FnbA and contribution to infectivity in a rat model of experimental endocarditis. Infect Immun 73**:**990-998. PMCID: **PMC547034**.
17. McAleese F, Murphy E, Babinchak T, Singh G, Said-Salim B, Kreiswirth B, Dunman P, O'Connell J, Projan SJ, and Bradford PA. 2005. Use of ribotyping to retrospectively identify methicillin-resistant *Staphylococcus aureus* isolates from phase 3 clinical trials for tigecycline that are genotypically related to community-associated isolates. Antimicrob Agents Chemother 49**:**4521-4529. PMCID: **PMC1280157**.
18. McAleese F, Petersen P, Ruzin A, Dunman PM, Murphy E, Projan SJ, and Bradford PA. 2005. A novel MATE family efflux pump contributes to the reduced susceptibility of laboratory-derived *Staphylococcus aureus* mutants to tigecycline. Antimicrob Agents Chemother 49**:**1865-1871. PMCID: **PMC1087644**.
19. Truong-Bolduc QC, Dunman PM, Strahilevitz J, Projan SJ, and Hooper DC. 2005. MgrA is a multiple regulator of two new efflux pumps in *Staphylococcus aureus*. J Bacteriol 187**:**2395-2405. PMCID: **PMC1065235**.
20. Anderson KL, Roberts C, Disz T, Vonstein V, Hwang K, Overbeek R, Olson PD, Projan SJ, and Dunman PM. 2006. Characterization of the *Staphylococcus aureus* heat shock, cold shock, stringent, and SOS responses and their effects on log-phase mRNA turnover. J Bacteriol 188**:**6739-6756. PMCID: **PMC1595530**.

21. Cassat J, Dunman PM, Murphy E, Projan SJ, Beenken KE, Palm KJ, Yang SJ, Rice KC, Bayles KW, and Smeltzer MS. 2006. Transcriptional profiling of a *Staphylococcus aureus* clinical isolate and its isogenic agr and sarA mutants reveals global differences in comparison to the laboratory strain RN6390. Microbiology 152**:**3075-3090.
22. Luong TT, Dunman PM, Murphy E, Projan SJ, and Lee CY. 2006. Transcription Profiling of the mgrA Regulon in *Staphylococcus aureus*. J Bacteriol 188**:**1899-1910. PMCID: **PMC1426550**.
23. McAleese F, Wu SW, Sieradzki K, Dunman P, Murphy E, Projan S, and Tomasz A. 2006. Overexpression of genes of the cell wall stimulon in clinical isolates of *Staphylococcus aureus* exhibiting vancomycin-intermediate- S. aureus-type resistance to vancomycin. J Bacteriol 188**:**1120-1133. PMCID: **PMC1347359**.
24. Richardson AR, Dunman PM, and Fang FC. 2006. The nitrosative stress response of *Staphylococcus aureus* is required for resistance to innate immunity. Mol Microbiol 61**:**927-939.
25. Roberts C, Anderson KL, Murphy E, Projan SJ, Mounts W, Hurlburt B, Smeltzer M, Overbeek R, Disz T, and Dunman PM. 2006. Characterizing the effect of the *Staphylococcus aureus* virulence factor regulator, SarA, on log-phase mRNA half-lives. J Bacteriol 188**:**2593-2603. PMCID: **PMC1428411**.
26. Tenover FC, McDougal LK, Goering RV, Killgore G, Projan SJ, Patel JB, and Dunman PM. 2006. Characterization of a strain of community-associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. J Clin Microbiol 44**:**108-118. PMCID: **PMC1351972**.
27. Yang SJ, Dunman PM, Projan SJ, and Bayles KW. 2006. Characterization of the *Staphylococcus aureus* CidR regulon: elucidation of a novel role for acetoin metabolism in cell death and lysis. Mol Microbiol 60**:**458-468.
28. Torres VJ, Stauff DL, Pishchany G, Bezbradica JS, Gordy LE, Iturregui J, Anderson KL, Dunman PM, Joyce S, and Skaar EP. 2007. A *Staphylococcus aureus* regulatory system that responds to host heme and modulates virulence. Cell Host Microbe 1**:**109-119. PMCID: **PMC2083280**.
29. Corbin BD, Seeley EH, Raab A, Feldmann J, Miller MR, Torres VJ, Anderson KL, Dattilo BM, Dunman PM, Gerads R, Caprioli RM, Nacken W, Chazin WJ, and Skaar EP. 2008. Metal chelation and inhibition of bacterial growth in tissue abscesses. Science 319**:**962-965.
30. Handke LD, Rogers KL, Olson ME, Somerville GA, Jerrells TJ, Rupp ME, Dunman PM, and Fey PD. 2008. *Staphylococcus epidermidis* saeR is an effector of anaerobic growth and a mediator of acute inflammation. Infect Immun 76**:**141-152. PMC2223648.
31. Stauff DL, Bagaley D, Torres VJ, Joyce R, Anderson KL, Kuechenmeister L, Dunman PM, and Skaar EP. 2008. *Staphylococcus aureus* HrtA is an ATPase required for protection against heme toxicity and prevention of a transcriptional heme stress response. J Bacteriol 190**:**3588-3596. PMCID: **PMC2395006**.
32. Chen PR, Nishida S, Poor CB, Cheng A, Bae T, Kuechenmeister L, Dunman PM, Missiakas D, and He C. 2009. A new oxidative sensing and regulation pathway mediated by the MgrA homologue SarZ in *Staphylococcus aureus*. Mol Microbiol 71**:**198-211.
33. Kuechenmeister LJ, Anderson KL, Morrison JM, and Dunman PM. 2009. The use of molecular beacons to directly measure bacterial mRNA abundances and transcript degradation. J Microbiol Methods 76**:**146-151. PMCID: **PMC2654336**.
34. Yang SJ, Xiong YQ, Dunman PM, Schrenzel J, Francois P, Peschel A, and Bayer AS. 2009. Regulation of mprF in daptomycin-nonsusceptible *Staphylococcus aureus*. Antimicrob Agents Chemother. In press.
35. Goering RV, Larsen AR, Skov R, Tenover FC, Anderson KL, and Dunman PM. 2009. Comparative genomic analysis of European community-associated methicillin-resistant *Staphylococcus aureus* (CC80-MRSA-IV) isolates by high-density microarray. Clin Micro Infect. In press.

C. Research Support.

Ongoing Research Support

NIH/NIAID
1R01AI073780-01 (Dunman - PI) 01/01/2009-12/31/2013
Title: *Staphylcoccus aureus* RNA turnover properties

Characterize *S. aureus* native and stress responsive mRNA turnover machinery.
Role: PI

American Heart Association National Scientist Development Award
0535037N (Dunman - PI) 07/01/2005-06/30/2009
Title: Role of the *Staphylococcus aureus* accessory regulator SarA in post-transcriptional virulence factor regulation
Define the mechanism of sarA-mediated target transcript stabilization
Role: PI

Nebraska Research Initiative Award
No Number (Dunman - PI) 06/01/2006-06/01/2009 (reviewed annually)
Title: *Staphylococcus aureus* antimicrobial agents
Identify novel antibacterial compounds that target the *S. aureus* RNase III ribonuclease
Role: PI

Completed Research Support (past 3 years only)

Merck Research Award
No Number (Dunman - PI) 06/01/2005-06/01/2007
Title: Development of microarray tools and technologies
Role: PI

For New and Competing Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel

PHS 398/2590 OTHER SUPPORT

KIELIAN, T.L.

ACTIVE

2 RO1 NS40730 (P.I. Kielian) 12/1/03 – 11/30/09 25%
NIH/NINDS $213,750
The Pathogenesis of Brain Abscess
The goal of this work is to investigate the potential of PPAR-gamma agonists and minocycline to modulate brain abscess development.

1RO1 NS053487-01A1 (P.I. Kielian) 8/01/06 – 7/31/11 25%
NIH/NINDS $202,500
Effects of Neuroinflammation on Gap Junction Communication in Glia
The objective of this proposal is to identify the effects of proinflammatory mediators on gap junction communication in glia and experimental brain abscess.

9 RO1 NS055385 (P.I. Kielian) 05/01/07 – 04/30/11 25%
NIH/NINDS $196,875
Receptors Involved in Microglial Responses to *S. aureus*
The objective of this proposal is to identify the role of TLR2 in regulating Th17 development in experimental brain abscesses.

P01 AI083211 (P.I. Project 4, Kielian) 07/1/09 – 06/30/14 20%
NIH/NIAID $284,507
Staphylococcal Biofilm and Disease
Title of Project 4: Innate Immune Response to *S. aureus* Biofilm
The objective of Project 4 is to determine whether *S. aureus* biofilm growth re-programs the host innate immune response from a classical anti-bacterial response to an anti-inflammatory response that favors bacterial persistence.

PENDING

3 RO1 NS40730 (P.I. Kielian) 12/1/09 – 11/30/14 25%
NIH/NINDS $225,000
The Pathogenesis of Brain Abscess
The goal of this work is to investigate the signals controlling brain abscess encapsulation using MRI modalities.

Budget Justification

Personnel

Jessica Nichols MD – (9 person months) Dr. Nichols will be the recipient of the comprehensive training program detailed in this grant application. She will perform the studies outlined in the specific aims, as well as attend the conferences, classes and teaching sessions described in the application to enhance her professional development. During the K08 training period, she will acquire a variety of microbiologic and immunologic skills and techniques. The nature of the studies proposed and the need to acquire a substantial number of new skills justifies the necessity for a full five years of funding. Dr. Nichols is committed to a career as a physician scientist.

Tammy Kielian PhD – Mentor and member of Scientific Advisory Panel (no salary); Associate Professor of Pathology/Microbiology. Dr. Kielian will oversee all aspects of Dr. Nichols training, including the performance of studies proposed, acquisition of new skills, manuscript preparation and career development. Dr. Kielian is an independent investigator with a long-standing history of multiple NIH funding in the study of immune responses during *S. aureus* brain abscess development.

Paul Fey PhD – Collaborator and member of Scientific Advisory Panel (no salary); Associate Professor of Pathology/Microbiology. Dr. Fey will participate in the quarterly Scientific Advisory Committee meetings and will assist Dr. Nichols in experiments examining bacterial growth kinetics in the CNS catheter infection model. Dr. Fey is currently NIH funded and a respected expert in the field of staphylococcal biology.

Mark Rupp MD – Member of Scientific Advisory Panel (no salary); Professor of Internal Medicine and Medical Director of the Department of Healthcare Epidemiology and the Clinical Trials Office. Dr. Rupp will participate in the quarterly Scientific Advisory Committee meetings and will guide Dr. Nichols in overall career development. Dr. Rupp is a respected expert in the field of staphylococcal biology and hospital-acquired infections.

Mark Smeltzer PhD - Collaborator and member of Scientific Advisory Panel (no salary); Professor of Microbiology/Immunology and Orthopedics at the University of Arkansas for Medical Sciences. Dr. Smeltzer will participate in the quarterly Scientific Advisory Committee meetings via video teleconference and will assist Dr. Nichols in experiments utilizing IVIS (*in vitro imaging system*) to define bacterial growth kinetics in the CNS catheter infection model. Dr. Smeltzer has a long-standing track record of NIH funding and is a respected expert in the field of staphylococcal biology.

Joyce Solheim PhD – Member of Scientific Advisory Panel (no salary); Associate Professor of the Eppley Institute at the University of Nebraska Medical Center. Dr. Solheim will participate in the quarterly Scientific Advisory Committee meetings and will guide Dr. Nichols in overall career development. Dr. Solheim is a respected expert in the field of immunology and a successful scientist who will provide valuable advice as Dr. Nichols pursues the work outlined in this proposal.

Paul Dunman PhD – Collaborator (no salary); Assistant Professor of Pathology/Microbiology. Dr. Dunman will assist Dr. Nichols with experiments examining bacterial growth kinetics and staphylococcal gene expression in the CNS catheter infection model using *S. aureus* Affymetrix GeneChips. Dr. Dunman is currently NIH funded and a respected expert in the field of staphylococcal biology and has collaborated with numerous laboratories throughout the country by performing *S. aureus* Affymetrix GeneChip arrays.

Supplies ($20,000)

I am requesting $20,000 to fund the purchase of supplies needed to conduct the experiments outlined in my research plan, including bacterial culture media and supplies, ELISA reagents, multi-analyte microbead arrays, and Affymetrix GeneChip microarrays, in addition to consumable laboratory reagents. Additional supply support will come from the Department of Pediatrics at the University of Nebraska Medical Center and Dr. Kielian.

Animal Expenses ($7,000)

A total of $7,000 is requested to cover the costs of purchasing mice and per diem charges during the course of the experiments outlined in this proposal. Currently, C57BL/6 mice are $19.50 each at Harlan Laboratories and I have estimated that a total of 1,500 mice will be required to successfully execute my studies. In addition, the per diem rate at UNMC for a standard mouse cage (capacity of 5 adult mice/cage) is $0.87 per cage per day. These costs, averaged over the 5 year period, will require an estimated $7,000 per year.

Travel/Publications ($3,000)

I am requesting $3,000 to assist with travel expenses to national meetings to present my work and for the costs associated with publication, including manuscript submission and color figure fees as well as page charges.

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

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

Prefix: Dr.
* First Name: Jessica
Middle Name: R
* Last Name: Nichols
Suffix: MD

* New Investigator? ☐ No ☒ Yes

Degrees: MD

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: Ms.
* First Name: Deborah
Middle Name: K
* Last Name: Vetter
Suffix:

* Phone Number: 402-559-7456
Fax Number: 402-559-2957
Email: spadmin@unmc.edu

* Title: Director

* Street1: 987835 NEBRASKA MEDICAL CENTER
Street2: N/A
* City: OMAHA
County: Douglas
* State: NE: Nebraska
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 68198-7835

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/registry/index.asp. 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.

PHS 398 Checklist

OMB Number: 0925-0001
Expiration Date: 9/30/2007

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 ☐

OMB Number. 0925-0001
Expiration Date: 9/30/2007

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. Assurances/Certifications (see instructions)

In agreeing to the assurances/certification section 18 on the SF424 (R&R) form, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the agency's application guide, when applicable. Descriptions of individual assurances/certifications are provided at: http://grants.nih.gov/grants/funding/424

If unable to certify compliance , where applicable, provide an explanation and attach below.

Explanation: Add Attachment | Delete Attachment | View Attachment

PHS 398 Career Development Award Supplemental Form

OMB Number: 0925-0001

1. Application Type:

From SF424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated here for your reference, as you attach the sections that are appropriate for this Career Development Award.

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Career Development Award Attachments:

Please attach applicable sections, below.

Introduction (if applicable)				
1. Introduction to Application *(for RESUBMISSION applications only)*		Add Attachment	Delete Attachment	View Attachment
Candidate Information				
2. Candidate's Background	18645_CandidateBack.pdf	Add Attachment	Delete Attachment	View Attachment
3. Career Goals and Objectives	18646_CareerGoalObj.pdf	Add Attachment	Delete Attachment	View Attachment
4. Career Development/Training Activities During Award Period	18666_CareerdevelAct.pdf	Add Attachment	Delete Attachment	View Attachment
5. Training in the Responsible Conduct of Research	18647_ResponsibleConduct.pdf	Add Attachment	Delete Attachment	View Attachment
6. Mentoring Plan *(when applicable)*		Add Attachment	Delete Attachment	View Attachment
Statements of Support				
7. Statements by Mentor, Co-Mentors, Consultants, Contributors *(as appropriate)*	18743_mentoring.pdf	Add Attachment	Delete Attachment	View Attachment
Environment and Institutional Commitment to Candidate				
8. Description of Institutional Environment	18629_Descrip of Institutional En	Add Attachment	Delete Attachment	View Attachment
9. Insitutional Commitment to Candidate's Research Career Development	18635_JSparksLetterSupport.pdf	Add Attachment	Delete Attachment	View Attachment
Research Plan				
10. Specific Aims	18642_Specific Aims.pdf	Add Attachment	Delete Attachment	View Attachment
11. Background and Significance	18644_BackandSig.pdf	Add Attachment	Delete Attachment	View Attachment
12. Preliminary Studies/Progress Report	18643_Preliminary Data.pdf	Add Attachment	Delete Attachment	View Attachment
13. Research Design and Methods	18701_Experimental.pdf	Add Attachment	Delete Attachment	View Attachment
14. Inclusion Enrollment Report *(for RENEWAL applications only)*		Add Attachment	Delete Attachment	View Attachment
15. Progress Report Publication List *(for RENEWAL applications only)*		Add Attachment	Delete Attachment	View Attachment
Human Subject Sections				
16. Protection of Human Subjects		Add Attachment	Delete Attachment	View Attachment
17. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
18. Targeted/Planned Enrollment		Add Attachment	Delete Attachment	View Attachment
19. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

Tracking Number:GRANT10348416 Funding Opportunity Number:PA-09-042 Received Date:2009-06-12T11:22:28-04:00

PHS 398 Career Development Award Supplemental Form

2. Career Development Award Attachments (continued):

Other Research Plan Sections

20. Vertebrate Animals	18710_Vertebrate Animals.pdf	Add Attachment	Delete Attachment	View Attachment
21. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
22. Consortium/Contractual Arrangements		Add Attachment	Delete Attachment	View Attachment
23. Resource Sharing Plan(s)	18709_RESOURCESHARING.pdf	Add Attachment	Delete Attachment	View Attachment

Appendix (if applicable)

24. Appendix Add Attachments | Delete Attachments | View Attachments

*3. Citizenship:

☒ U.S. Citizen or noncitizen national

☐ Permanent Resident of U.S.
(If a permanent resident of the U.S., a notarized statement must be provided by the time of award)

☐ Non-U.S. Citizen with temporary U.S. visa

Candidate's Background

I am pediatrician, specializing in infectious diseases, who completed my fellowship at the University of Arkansas for Medical Sciences (UAMS) in June 2009. In terms of training, I completed the Medical Science Scholars program at Texas A&M University, in which I was chosen as a high school senior to complete an accelerated program of undergraduate and medical school training. I was accepted to medical school at the Texas A&M Health Science Center as a second year college student and went on to receive my M.D. in 2002, graduating with honors. As a pediatric resident at East Carolina University, I participated in clinical trials and developed a project examining the health practices of residents and the impact of the work hours of residency on these practices during my chief residency.

At the beginning of my fellowship at UAMS, I was very new to basic science research. I was extremely interested in the pathology and host response to central nervous system (CNS) infections, but had limited exposure to physician scientists in this area of research in my prior education. A large factor in my choice of the University of Arkansas for my fellowship was the opportunity to work with Dr. Toni Darville, a respected Chlamydia immunologist and a very successful physician scientist. While the prospect of bench research was intimidating, I was motivated and inspired by Dr. Darville's ability to relate the work we did in the laboratory to the work we performed in the hospital as consultants. My experiences in the laboratory and in the clinical realm have continued to reinforce my initial interest in becoming a physician scientist.

During the first year of my pediatric infectious disease fellowship, I trained in the laboratory of Dr. Darville. The research I performed there focused on the immune response to genital tract infection with *Chlamydia trachomatis* in a mouse model. I was trained in animal research (specifically, animal handling, infections and sample collection), multiple immunologic assays, as well as *in vitro* cell culture and infection techniques. I also completed graduate course work, including an introduction to biostatistics and participation in the graduate student immunology journal club. This work culminated in a presentation of my findings at the international Chlamydia Basic Research Society meeting in 2007. In addition, a manuscript summarizing these studies, "MyD88 deficiency delays resolution of chlamydial genital tract infection due to abrogation of NK cell IFN-gamma production and subsequent blunting of the adaptive Th1 response", is currently being prepared for publication. I was also the recipient of travel awards to present this research at the Southern Society for Pediatric Research annual meeting and received the Best Abstract and Best Presentation awards at the Arkansas Children's Hospital Fellows Day. At the end of my first year of training, Dr. Darville was offered the opportunity to become the Chief of Pediatric Infectious Disease at the University of Pittsburgh Medical Center. With her guidance, I chose to remain at the University of Arkansas to continue my fellowship and my research with a new mentor rather than accept the fellowship position I was offered at the University of Pittsburgh.

In June 2007, I transitioned to the laboratory of Tammy Kielian, Ph.D., to begin work studying the immune response to bacterial infection within the CNS. This work applied the immunologic tools I had learned under Dr. Darville to a new model and was uniquely suited to my interest in CNS infections. During this time, I learned to generate brain abscesses in mice, both wild type and genetically manipulated (Toll-like receptor deficient) animals. I also became proficient in the use of flow cytometry and immunohistochemical staining to evaluate the influx of immune cells into the CNS. I successfully generated bone marrow chimeras to dissect the relative contribution of CNS resident cells (astrocytes, microglia) versus infiltrating peripheral immune cells during brain infection. This work culminated in a presentation of my findings at the American Society of Neurochemistry meeting in 2008, as well at UAMS research week, where I received an award for Best Postdoctoral presentation. In addition, I am the first author on two manuscripts that have recently been published as a result of this work ("Toll-like receptor 2 deficiency leads to increased Th17 infiltrates in experimental brain abscesses" in the Journal of Immunology; and "MyD88 expression in the CNS is pivotal for eliciting protective immunity in brain abscesses" in ASN NEURO, co-first author).

In December 2007, I spent two weeks working on the infectious disease consult service and managed several children with recurrent staphylococcal CNS catheter infections. I was saddened by the suffering these children endured, caused initially by the symptoms of their infection and exacerbated by the prolonged hospitalization and multiple procedures required to treat the infections. I returned to the laboratory intrigued by the interactions between the bacterial biofilms that form on CNS catheters and the innate immune system in the CNS, as these interactions may provide information about adjuvant therapies for CNS catheter infections. Upon reviewing the literature and discussing the issue with my research mentor, Dr. Kielian, I discovered that very little was known about these interactions. To explore these issues, I have developed a novel model of CNS catheter infection in the mouse, which serves as the impetus of the current K08 application. This technique results in a reproducible

catheter-associated infection and ventriculitis in the mouse, similar to the findings seen in humans with ventricular shunt infections. I have been primarily responsible for the development of this model with input and advice from Dr. Kielian, performing the *in vitro* preparations and testing, as well as all animal surgeries, sample collection, culturing and immunologic assays. I have also performed the tissue sectioning and staining (H&E, Luxol blue, FluoroJade) on the brain tissue samples. The development of this novel model is an important advancement as it now allows us to investigate the pathology and host response to these infections. Central nervous system catheter infections present a challenge that is costly in both patient morbidity and healthcare expense. A better understanding of the host response in this setting can help develop targeted therapies that will greatly improve management of this difficult clinical problem.

In spring 2008, Dr. Kielian was offered an opportunity to join the staphylococcal research group in the Department of Pathology and Microbiology at the University of Nebraska Medical Center. Due to the lack of a pediatric infectious disease fellowship at UNMC at that time, I was not able to move with Dr. Kielian to continue my work in her laboratory. I remained at the University of Arkansas to continue my research on the newly developed CNS catheter infection model with the continued guidance of Dr. Kielian, through weekly telephone calls and almost daily e-mail contact, and the on-site support of Dr. Mark Smeltzer, a well-respected expert in *S. aureus* biofilm infection in the Department of Microbiology at UAMS. Dr. Smeltzer's career guidance and scientific input in the biofilm culture techniques used in the development of this model have been invaluable. In addition to the current proposal, he and I will collaborate on future projects investigating the molecular pathogenesis of *S. aureus* within the CNS. For the last year, I have worked independently in a laboratory within the Department of Neurobiology to develop this novel animal model and perform the preliminary studies described in the current K08 application. This has been a valuable educational experience as I have navigated the many issues involved with laboratory management as a relatively independent investigator. My ability to apply the research skills and knowledge obtained during my diverse research experiences while continuing to develop this new and exciting avenue of independent research, despite the changes in faculty mentoring, speaks highly of my focus and perseverance and my likelihood of success as an independent physician scientist.

After completing my pediatric infectious disease fellowship, I will embark upon my independent physician scientist career as an Assistant Professor in Pediatrics at the University of Nebraska Medical Center (UNMC) effective July 1, 2009. I chose to join the faculty at UNMC to continue my very productive mentoring relationship with Dr. Kielian. In addition, the strong staphylococcal research group at UNMC will be a valuable resource as I move forward with this avenue of research (www.unmc.edu/dept/pathology/lsr/). I have negotiated well protected time for research as well as adequate office and laboratory space, administrative support and start-up funds. These funds will provide equipment necessary for the experiments outlined in this proposal as well as a research technologist to assist in this work. In addition, I will have wet lab space adjacent to Dr. Kielian's laboratory, as well as the staphylococcal research group. I will also have access to core laboratories for flow cytometry, multiplex analysis, IVIS imaging, confocal microscopy, and other techniques utilized in this K08 proposal. My clinical service will be limited to 4 weeks per year on the inpatient infectious disease consult service and a half-day infectious disease outpatient clinic weekly, representing less than 20% effort for clinical duties. My teaching and administrative responsibilities will also be minimal, providing very little interference with my daily research activities.

Career Goals

Physician scientists play a valuable role in recognizing the need to develop new basic science research projects to address relevant issues in the clinic and in applying research from the basic science arena to clinical care. An excellent case in point was my observation that very little basic science research had been applied to the topic of CNS catheter infections that are pertinent to the pediatric population, leading me to establish a novel mouse model to study this condition. Physician scientists understand the diagnostic and management challenges encountered by clinicians and patients and are able to apply their diverse research skills and knowledge to solve these issues. In addition, physician scientists understand the applications of scientific discovery in the laboratory in ways that allow for optimal translation of these findings to patient care. This unique insight into both the scientific and practical aspects of these complex clinical problems allows them to pursue innovative approaches in the clinic and in the laboratory.

I have worked for the last 2 years as the sole clinician in a neurobiology research group and have been impressed by the differences between those of us with a clinical background and those with a basic science background. Both perspectives are equally valuable and I am grateful for the opportunity to contribute and to bridge the gap between the bench and the bedside. I plan to continue work as a physician scientist throughout my career, integrating clinical practice and the research outlined in this K08 proposal to maximize my contributions to both areas of discipline. I have been fortunate to have several highly effective mentors throughout my research career and hope that I can assist other clinicians in the development of their own research careers in the future.

This model holds vast potential for future studies as very little has been published regarding the role of the innate immune system in the CNS in response to catheter-associated infections. Understanding the interactions between the neuroimmune system and the biofilms that form on infected catheters will allow us to explore novel management strategies for these infections. These possibilities include immune adjuvants that could be used in conjunction with systemic antibiotics, potentially preserving CNS catheters in some patients. One of the collaborators on this project, Dr. Paul Dunman, has a novel compound library that can be screened for potential therapeutic agents in future studies once we obtain a better understanding of the relevant immune components. In addition, a better appreciation of these immune interactions may allow us to screen patients at higher risk of infection and offer targeted prophylactic therapy for these patients, decreasing the risk of infection for these children.

Career Development Award Objectives

This K08 grant proposal will be serving three primary objectives. First, the intensive research training period and protected time for research activities outlined in this application will allow me to continue my studies characterizing the immune response to CNS catheter-associated infections. These studies complement my prior work and will allow me to pursue my interests in innate immune host defense responses. By developing this unique model of CNS catheter infection, I now have an important tool that can be used to investigate the interactions between the immune system and infected CNS catheters, an area that remains largely unexplored. My clinical perspective, combined with the research skills and knowledge I have obtained over the last few years, provides me with an advantage in both the design and translational impact of these studies. My goal is that the knowledge gained can be translated into prevention and therapeutic strategies targeting CSF shunt infections. Secondly, I plan to further develop my knowledge base in the areas of staphylococcal biology, immunology and neuroscience through the multiple educational venues available at UNMC. This knowledge will be invaluable as I design future studies to continue pursuing my independent research objectives. Finally, through these proposed studies, I will gain additional laboratory skills that will advance my development towards an independent investigator. The research techniques I will master include static and dynamic biofilm culture techniques, transcriptional profiling using Affymetrix microarrays, immunohistochemical staining, confocal microscopy and IVIS *in vivo* imaging. Under the guidance of Dr. Kielian, I will also master the skills required for independent laboratory management, including scientific writing for funding proposals and peer-reviewed publications, laboratory personnel supervision and budget management.

Future Research Avenues

The mouse CNS catheter infection model offers several avenues of investigation that I plan to explore in the future. In addition to the studies outlined in the current proposal, I plan to explore the role of Toll-like receptor 2 (TLR2) in response to CNS biofilm infections, as it is known to play a very important role in parenchymal brain

infections. This will build upon my prior research experience in this field (1,2). I will also investigate the impact of systemic antibiotic therapy with various agents on CNS biofilm formation. The changes in immune markers, bacterial kinetics and pathology in response to antibiotic therapy have not yet been examined but will be explored using this model. In addition, I plan to adapt this CNS biofilm model to generate infection with coagulase-negative staphylococci, which currently account for approximately half of all CNS catheter infections (3). The differences in pathology and immune responses between infections with coagulase-negative staphylococci and the more aggressive *S. aureus* will provide valuable clues to assist in the management of both of these issues from a clinical perspective. My continued collaboration with Dr. Kielian will be invaluable as I move forward in this avenue of investigation based on her long-standing interest in *S. aureus* pathogenesis in the CNS and expertise in neuroimmunology.

Career Development/Training Activities
In order to achieve the goals outlined above, I have designed an educational plan and organized an advisory panel to oversee my development as an independent investigator. This educational plan includes didactic coursework, participation in laboratory meetings and hands-on training in new laboratory techniques. My plans will follow the anticipated timeline as outlined below.

Activity	Year 1	Year 2	Year 3	Year 4	Year 5
Specific Aim 1	X	X	X		
Specific Aim 2			X	X	X
Scientific courses	X	X			
Career development courses	X	X			
Responsible conduct of research	X	X	X	X	X
Conferences / Meetings	X	X	X	X	X
Peer Reviewed publications		X	X	X	X
Develop & submit RO1				X	X

Course Curriculum. In order to supplement my knowledge base, I plan to participate in the following courses offered by the University of Nebraska Medical Center. I anticipate this coursework to occupy <5% of my time during years 1 and 2.

- **Current topics in Staphylococcal Biology.** This special topics course is led by Drs. Paul Dunman and Paul Fey in the Department of Pathology and Microbiology and the Laboratory for Staphylococcal Research, and addresses emerging issues and current topics in Staphylococcal biology with an emphasis on *S. aureus* and *S. epidermidis*.
- **Advanced Immunobiology.** This course is a conceptual study of cellular and biomolecular immunology. Topics include mechanisms of immune recognition, regulatory and effector functions, interleukins and clinical immunology, with discussion of current literature.
- **Neuroimmunology.** This objective of this course is to provide essential knowledge towards a better understanding of the principals of neuroimmunology as they apply to the pathogenesis and immunotherapeutics of neurologic disorders. This course is designed for in depth study of neurobiology, neuroimmunology, neuropharmacology, immunotherapy, and neurodegenerative disorders.

Statistical analysis is an essential component of any sound scientific study. During my fellowship, I completed coursework in introductory biostatistics through the University of Arkansas for Medical Sciences School of Public Health. To supplement this educational background, I will participate in the Biostatistics II course offered through the UNMC College of Public Health (BIOS 808), which is designed to help graduate students understand and apply advanced biostatistical methods needed in the design and analysis of biomedical investigations. These include multiple linear regression, analysis of covariance, logistic regression, survival analysis, and repeated measures analysis.

Courses in Professional Career Development. The University of Nebraska Medical Center offers numerous courses aimed at faculty development. These include periodic courses in administrative skill development, educational techniques and a monthly seminar series focused on skills needed for early career success. In addition, the Department of Pediatrics has developed a monthly discussion group targeting career development of young faculty. The Office of Postdoctoral Education at UNMC also presents an annual grant writing seminar. I plan on participating in these courses when available and relevant to my educational goals.

Special Courses. I will attend the Annual Workshop on the Pathology of Mouse Models for Human Disease at Jackson Laboratories (Bar Harbor, ME) during my first two-year training period. This workshop provides a week of intensive training sessions in pathology and histopathology as well as didactic sessions in which particular disease areas and models will be discussed. Topics and models to be covered include: nomenclature, basic mouse genetics, concepts of mouse model generation, approaches to working with

mutant mice and the pathology of multiple organ systems. This information will be valuable as I progress in my studies with this novel CNS catheter infection model.

Conferences. I will participate in the following local educational forums during my 5 year research period.

- **Kielian laboratory meetings.** These meetings occur weekly in the Durham Research Center conference room. The meetings are designed to discuss work in progress and technical problems to devise possible solutions if necessary, review and critique new findings and assist in manuscript and grant preparations. The forum consists of one 30 minute PowerPoint presentation by one laboratory member followed by research updates from other members of the research group. These meetings are also used to review new data published by other laboratories in the field. I will participate in these meetings in addition to weekly mentor meetings scheduled with Dr. Kielian to assess progress with my own work.
- **Immunology Interest Group.** This is a monthly meeting of individuals interested in the field of immunology, attended by faculty, post-doctoral students and research fellows from the University of Nebraska Medical Center and surrounding schools. Participants present a 45 minute overview of current research progress with a significant amount of time allotted for feedback and interaction with the audience. In addition, I will participate in the Immunology journal club, which reviews emerging data and new articles published in this field.
- **Mid America Consortium of Gram Positive Pathogens, Work in Progress (WIP) Meetings.** This is a multi-institutional group of staphylococcal researchers that meets via monthly teleconference. This group includes staphylococcal researchers from the University of Nebraska Medical Center, the University of South Dakota and the University of Arkansas for Medical Sciences. The forum consists of one 45 minute presentation of ongoing research projects from a given investigator with extensive dialog and feedback from attendees.
- **Department of Pathology and Microbiology Seminars.** The Department of Pathology and Microbiology offers several educational opportunities throughout the year. These include Grand Rounds, graduate student research seminars, basic science seminars and a translational research seminar series.
- **Departments of Neurology and Pharmacology and Experimental Neuroscience.** The Department of Neurology offers regular Grand Rounds presentations and recurrent scientific seminars are sponsored by the department of Pharmacology and Experimental Neuroscience.
- **Numerous other educational opportunities are offered on the UNMC campus.** These include Grand Rounds for the Department of Pediatrics, InBRE seminars, Center for Clinical and Translational Research seminars and numerous other research-oriented conferences. These will be taken advantage of as topics are presented relevant to my career objectives and time available dictates.

National and International Meetings / Conferences. I will submit abstracts and present data for at least one of the following national meetings annually: American Society of Neurochemistry (ASN), Infectious Disease Society of America (IDSA), American Society of Microbiologists (ASM) or possibly others depending on the thematic program of the meeting. In addition, I will participate in several smaller venue meetings during my training period. This will include the Gordon Research Conference on Staphylococcal Diseases and the FASEB Summer Research Conference on Neuro-Immune Interactions. These are both small meetings that draw prominent national and international scientists, allowing a unique opportunity to interact with other researchers and discuss innovative data and techniques. I have previously presented my research in both poster (ASN, Pediatric Academic Societies, IDSA) and oral presentation (Chlamydia Basic Research Society, IDSA, Southern Society for Pediatric Research) formats, and look forward to building on these positive experiences.

Training in laboratory techniques. I have designed this K08 research training program so that I will have the opportunity to master several new research techniques. In the Kielian laboratory, I will expand my current knowledge of immunologic staining methodologies by becoming familiar with immunofluorescence staining and confocal microscopy. In generating my preliminary data for the current K08 application, I have gained some familiarity with basic tissue preparation techniques, but I am not independent in this skill set and therefore, require further training and assistance. I will also work with Dr. Kielian to learn confocal microscopy and 3D image analysis, as well as advanced small animal imaging techniques (IVIS, MRI) applicable to my animal

model. I will learn to independently perform assays that I have been exposed to in previous work in Dr. Kielian's laboratory, including RT-PCR, Western blotting, lymphocyte proliferation assays and gentamicin protection assays to demonstrate intracellular survival of bacteria in various CNS cell types. I will work with Drs. Paul Dunman and Paul Fey to master biofilm culture techniques and with Dr. Dunman on RNA isolation from bacteria and subsequent transcriptional profiling of *S. aureus* gene expression using Affymetrix gene arrays. Dr. Fey will also help me learn to use pulse-field gel electrophoresis to determine similarities in staphylococcal clinical isolates, which will be essential as I evaluate the propensity of various staphylococcal clinical strains to cause disease in the mouse CNS catheter infection model.

R01. I plan to write and submit an application for NIH-R01 funding in years 4 and 5 based on the data acquired during the experiments outlined in this mentored scientist development award. I have institutional support from Dr. John Sparks (Chair of Department of Pediatrics) to progress towards this goal, as described in Dr. Sparks' letter of support that is included with this K08 application.

Total Effort Allocation. I have been guaranteed at least 75% protected time to devote to this research project. My clinical service will be limited to 4 weeks per year on the inpatient infectious disease consult service and a half-day infectious disease outpatient clinic weekly, representing less than 20% effort for clinical duties. My teaching and administrative responsibilities will also be minimal, providing very little interference with my daily research activities.

Mentorship and Scientific Advisory Committee. I have organized a rigorous Scientific Advisory Committee (SAC) composed of individuals who are outstanding investigators in their respective fields to oversee my progress in the proposed K08 project and my ongoing profession development. These scientists have strong clinical and research backgrounds in immunology and staphylococcal biology. There will be quarterly meetings of my SAC at the University of Nebraska Medical Center in Omaha, Nebraska, with off-site advisors participating via teleconference. At these meetings, I will present progress on my research project, as well as my educational track. The feedback and suggestions provided by these distinguished researchers will serve as a valuable tool for monitoring and evaluating my progress. These meetings will be documented to supplement the annual report completed by my primary research mentor, Dr. Kielian. It is anticipated that such meetings with the SAC will stimulate further research ideas and add to the work I will be completing under the guidance of Dr. Kielian. The details of the members of my advisory committee are listed below.

> **Tammy Kielian, PhD**, Associate Professor in the Department of Pathology and Microbiology at the University of Nebraska Medical Center, is an excellent role model of a successful and innovative researcher, who will continue her role as my mentor for the duration of my next phase of training. She is a very well-respected, independent investigator with a long history of NIH-funding in the immune response to staphylococcal infection, particularly within the CNS. She will provide training in research techniques, equipment and use of reagents as needed. Her laboratory and office are immediately adjacent to my own in Durham Research Center II at the University of Nebraska Medical Center. In addition to serving as a member of the SAC, Dr. Kielian will meet with me weekly to discuss my progress with the experiments outlined in this application and in my development as an independent investigator. We will have almost daily contact, given the proximity of our laboratory spaces and offices. She is also a participant in several of the conferences outlined above, including the Immunology Interest Group and the Mid America Consortium of Gram Positive Pathogens Work in Progress Meetings.
>
> **Paul Fey, PhD**, an Associate Professor in the Department of Pathology and Microbiology at the University of Nebraska Medical Center, will serve as a collaborator and member of the SAC. Dr. Fey is currently NIH funded and a respected expert in the field of staphylococcal biology and serves as the Associate Medical Director of the Nebraska Medical Center Clinical Microbiology Laboratory and the Nebraska Public Health laboratory. In addition to providing scientific and career guidance as part of the advisory committee, Dr. Fey will assist in experiments examining bacterial growth kinetics in the CNS catheter infection model. Dr. Fey is also a participant in the Mid America Consortium of Gram Positive Pathogens Work in Progress Meetings.

Mark Rupp MD, Professor in the Department of Internal Medicine and Medical Director of the Department of Healthcare Epidemiology and the Clinical Trials Office, will serve as a member of the SAC. Dr. Rupp will participate in the quarterly SAC meetings and will offer guidance in overall career development. Dr. Rupp is a respected expert in the field of staphylococcal biology and hospital-acquired infections.

Mark Smeltzer, PhD, Professor in the Departments of Microbiology/Immunology and Orthopedics at the University of Arkansas for Medical Sciences, will serve as a collaborator and SAC member. Dr. Smeltzer will participate in the quarterly SAC meetings via video teleconference and will assist in experiments utilizing IVIS (*in vitro imaging system*) to define bacterial growth kinetics in the CNS catheter infection model. In addition, we actively correspond by e-mail and phone on a regular basis. Dr. Smeltzer has a long-standing track record of NIH funding and is a respected expert in the field of staphylococcal biology. He is also a participant in the Mid America Consortium of Gram Positive Pathogens Work in Progress Meetings.

Joyce Solheim PhD, Associate Professor of the Eppley Institute at the University of Nebraska Medical Center, will serve as a member of the SAC. Dr. Solheim will participate in the quarterly SAC meetings and offer guidance in overall career development. Dr. Solheim is a respected expert in the field of immunology and a successful scientist who will provide valuable advice as I pursue the work outlined in this proposal.

Responsible Conduct of Research. I will participate in the Responsible Conduct of Research ethics course yearly during my training. This course is offered by the UNMC Office of Post-Doctoral Education at least once each year. This course is a UNMC-developed, web-based training program followed by a faculty-led discussion of a series of cases on scientific integrity and responsible conduct in research. The mandatory, web-based components provide an extensive literature-based review of the NIH-required topics and conclude each section with a brief content-based quiz. The web models comprise a review of data management, policies and definitions for scientific misconduct, conflict of interest, responsible use of human subjects and animal research, mentoring and authorship. All trainees are also provided an extensive bibliography covering the areas of mentoring and responsible conduct of research for further reading and reference.

UNIVERSITY OF Nebraska Medical Center

PATHOLOGY AND MICROBIOLOGY

NEBRASKA'S HEALTH SCIENCE CENTER

June 8, 2009

NIH K08 Review Panel

Dear Committee members,

This letter is written in enthusiastic support of Dr. Jessica Nichols' Mentored Clinical Scientist Development Award (K08) application entitled "Characterization of a novel murine model of central nervous system catheter infection". I have known Jessica since May 2007 when she performed research in my laboratory during the second year of her Pediatric Infectious Disease fellowship at the University of Arkansas for Medical Sciences. I continued to serve as a scientific mentor for Jessica from afar through weekly phone conversations when I relocated my laboratory to the University of Nebraska Medical Center in July 2008; however, this mentoring relationship will resume face-to-face interactions once Jessica begins her independent research career as an Assistant Professor in the Department of Pediatrics at the University of Nebraska Medical Center effective July 1, 2009. During the time that I have known her, words cannot convey my enthusiasm for Jessica as a blossoming young scientist and person. The breadths of her accomplishments to date are among the best, in my opinion, compared to other peers at her level.

I would like to begin by providing a short background on how I came to know Dr. Nichols' and how she has excelled as a young clinician scientist in just a few years. My laboratory investigates host immune responses to *S. aureus* in the central nervous system (CNS) utilizing an experimental brain abscess model developed by our research group. During her year-long tenure in my laboratory, Jessica worked on a NIH-funded project to investigate the dynamic relationship between Toll-like receptor 2, a key receptor for *S. aureus* recognition, and Th17 cells, a newly identified T cell subset that plays pivotal roles in anti-bacterial responses as well as autoimmunity. Although Jessica brought with her a solid immunology background when entering the laboratory from the training she received during the first year of her fellowship working with a mouse Chlamydia model with Dr. Toni Darville, she was new to the discipline of neuroimmunology. Within a short time frame, Jessica had immersed herself in the field and displayed an excellent working knowledge of the subject that continues to expand. Her exceptional productivity in my laboratory during this one year research period was reflected by two first-author manuscripts, one in the Journal of Immunology and another as co-first author in the recently launched journal ASN NEURO, the official journal of the American Society for Neurochemistry (ASN). In addition, Jessica was the first-author on an abstract that was presented at the 2008 ASN national meeting. This degree of productivity was outstanding in such a relatively short time frame and speaks highly to the dedication and talent that Dr. Nichols exhibits in the research arena.

Around December 2007, following a clinical service block, Jessica indicated that she treated several children with complications stemming from CSF catheter infections and was intrigued since not much is known about bacterial biofilms and the CNS innate immune response to infection. Since that time, Jessica has worked diligently and in a largely independent manner to successfully establish a novel mouse model for CNS catheter biofilm infection with *S. aureus*. She recently presented data with this innovative model at the 2009 American Society for Neurochemistry meeting, where it was very well received. I would like to emphasize that establishing this original model of CNS catheter infection was spearheaded solely by Dr. Nichols since I had already relocated to Nebraska and was not able to provide any hands-on assistance to facilitate her progress. This placed Jessica in an accelerated position of independence. The fact that she has flourished under these circumstances when others would have surely failed, speaks to her tenacity and

drive as a research scientist. This level of scientific achievement is unusual in such a short time frame and with regard to her performance thus far, I would rank Jessica in the top 1% of senior level postdoctoral fellows/junior faculty that I have either trained or closely interacted with. To add to this level of certainty is the fact that Jessica's writing style is outstanding and she has the ability to logically link clinical findings to basic science applications, a talent that is not automatic to every scientist.

I would also like to speak to the significant impact that Jessica's proposed work would have on the field of neuroimmunology and CNS infectious diseases in general. To date, there is no information available regarding how the immune response interfaces with bacterial biofilms within the CNS compartment. In fact, there is a relative paucity of knowledge dealing with immune responses to *S. aureus* biofilm infections in the periphery, a point that I can confirm since we recently received a grant to investigate this very question in the framework of a PO1 proposal. Therefore, Jessica's K08 research proposal is truly innovative and seeks to describe the unique interactions between the CNS innate immune response and *S. aureus* biofilms. To heighten the impact of her proposal, one needs to look no further than the fact that a significant portion of the pediatric population that requires CSF shunts for the management of hydrocephalus, suffer from catheter-related infections. The significance of this problem is highlighted by the fact that these biofilm infections are recalcitrant to conventional antibiotic therapy and require removal of the shunt and replacement after the residual infection has been eliminated. This represents a serious complication and Jessica's novel mouse model presents an opportunity to decipher the pathogenesis of CNS biofilm infection in a comprehensive manner that is simply not possible in pediatric patients. It is highly likely that the basic science information that Jessica obtains over the years using this mouse model of CNS infection will uncover pathways that may be targeted to improve the therapeutic management of infected CSF shunts. Jessica's vision and talent to wed basic and clinical science questions will no doubt serve her well during the successful execution of her work.

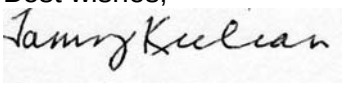
I have been, and remain fully committed, to the sustained support of Jessica's career development and research objectives. This mentoring relationship will continue to prosper based on the one-on-one interactions that we will experience upon Jessica's relocation to Omaha. We have made arrangements to hold weekly formal meetings to discuss Jessica's research progress and talk about upcoming meetings to prepare for abstract submissions as well as pending grant deadlines. In addition, the proximity of our offices (i.e. next door to each other) will no doubt lead to frequent daily informal conversations, which are absolutely essential, in my opinion, for mentoring success and propelling research projects forward. Jessica has been assigned approximately 500 sq. ft. of independent laboratory space immediately adjacent to my research laboratory. Therefore, Jessica will have full access to all of my research equipment that will be pivotal for the successful execution of her proposal, including our confocal microscope and IVIS system. In addition, based on the close proximity of our research laboratories, Jessica will benefit from the expertise of my research associates that have extensive laboratory experience and can assist her with routine lab-related issues that one encounters on a daily basis. Jessica has negotiated a start-up package that includes funds to support an experienced technician for a 3-year period; therefore, I fully expect Jessica to hit the ground running once she arrives at UNMC and will no doubt be productive in this nurturing environment in a timely manner. Through the close collaboration between our laboratories, my research support staff can help provide the necessary training for Jessica's new technician to further accelerate her research productivity. In addition to providing these examples of support, I am also fully committed to assist Jessica with any budgetary needs that may arise during the course of her studies outlined in the current K08 application that would extend beyond the start-up funds that she has negotiated for performing her experiments. I will also continue to provide guidance in terms of manuscript and grant preparation, which are key concepts that must be mastered to be a successful scientist. Although I am relatively young in my research tenure, I have demonstrated consistent productivity as evident by my 3 current NIH RO1 awards in addition to a project on a PO1 that has just received funding. My laboratory averages nearly 4 peer-reviewed publications per year, a trajectory that we plan to either meet or exceed in the coming years. I have instilled this concept of productivity in Jessica and it is evident from her accomplishments to date, that she is up to the task and, in my mind, will no doubt be successful.

In addition to the mentoring track record and plans described above for Jessica, I have also successfully mentored both graduate students and postdoctoral fellows during my research career. My first Ph.D. student graduated in 2007 and achieved 3 first-author peer-reviewed publications in high-tier journals such as the Journal of Immunology, in addition to being a co-author on 5 other papers. Another Ph.D. student will graduate in May 2010 and will also have a total of 3 first-author publications, one of which is currently in revision in the Journal of Immunology. Many of my current and former mentees, including graduate and undergraduate students and postdocs, have received various honors including competitive travel awards to attend national meetings as well as awards for poster and oral presentations at local research forums. I have also trained numerous post-doctoral fellows, who over the years have generated a total of 20 peer-reviewed publications. In addition to this mentoring, I have been successful at maintaining multiple NIH RO1 awards throughout my 8 year tenure as an independent scientist. Based on what I perceive to be a successful mentoring situation to date in terms of Jessica's recent research and professional achievements, I am confident that I can provide Jessica with a nurturing environment to continue to foster her independent research career in conjunction with the truly outstanding Scientific Advisory Committee that she has assembled to oversee her K08 award, should it receive funding. Regardless, this committee is committed to Jessica's growth and success and will be supportive in any way possible in this endeavor. I was fortunate to have several positive role models and outstanding mentors throughout my research training, which was key to my current success as a research scientist. Based on these experiences, my mentoring philosophy is to provide sufficient support along with realizing that each young scientist needs to blossom and embrace their independence, such that a balance between these two directives is achieved. My mentoring outcomes to date strongly support that I will be fully capable of promoting Jessica's professional development to that of independence.

In closing, I would like to reiterate my highest enthusiasm for Dr. Jessica Nichols' future as a productive young clinician scientist. Overall, her research is clinically significant, rationally designed, and most importantly, entirely conceived by herself based on her clinical experiences as a pediatric infectious disease physician. Jessica possesses all of the requisite tools needed to be a successful independent researcher and by funding her career development award application you will be making a sound investment towards the future of an industrious young clinical researcher. I wholeheartedly endorse her application.

Please do not hesitate to contact me if you need any additional information.

Best wishes,



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University of Nebraska Medical Center

NEBRASKA'S HEALTH SCIENCE CENTER

PATHOLOGY AND MICROBIOLOGY

NIH K08 Review Panel

May 24, 2009

Re: Characterization of a Novel Murine Model of CNS Catheter Infection

To Whom It May Concern:

It is with pleasure that I support Dr. Jessica Nichols' K08 Career Development Award Proposal by providing her my expertise in staphylococcal biology as a collaborator and a member of her Research Advisory Committee.

I am an Associate Professor in the Department of Pathology and Microbiology. My research focuses on the genetics of *Staphylococcus epidermidis* and the factors involved in virulence and biofilm formation. The knowledge gained from these studies can be used to develop a better understanding of staphylococcal pathology and better therapies for treatment of these infections. This experience in staphylococcal research will prove valuable as I advise Dr. Nichols in her development as a clinician scientist.

Dr. Nichols has proposed to study the neuroimmune response to a catheter-associated central nervous system infection. This work utilizes a novel mouse model to investigate the host response to CSF shunt infections, a frequent complication of the treatment of hydrocephalus. She has designed a proposal that seeks to define the bacteriologic and immunologic responses in this infectious model, using a clinical *Staphylococcus aureus* specimen as well as a genetically modified isolate, impaired in biofilm formation. As a collaborator and advisor for Dr. Nichols, I will work with her to master biofilm culture techniques and genetic evaluation of staphylococci, as well as the use of genetically modified bacteria in an animal model. Understanding the interactions between the immune system and the biofilms that form on infected catheters can lead to the development of novel management strategies for these infections.

I am happy to serve as one of Dr. Nichols' advisors for this research proposal and to support her throughout her career. As part of her Research Advisory Committee, I will ensure that she continues to direct her attention to scientific questions relevant to her current proposal. I will be able to provide both scientific and career guidance as she pursues the course of study outlined in this project.

I have no doubt that Dr. Nichols will be highly successful in her work. I look forward to continued collaboration with her. I give this application my strongest support.

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Sincerely,

Paul D. Fey, Ph.D.
Associate Professor
University of Nebraska Medical Center

University of Nebraska Medical Center

NEBRASKA'S HEALTH SCIENCE CENTER

June 1, 2009

NIH K08 Review Panel

Re: Jessica R Nichols, M.D. "Characterization of a Novel Murine Model of CNS Catheter Infection"

Dear NIH Study Section,

It is with pleasure that I support Dr. Jessica Nichols' K08 Career Development Award Proposal by providing her my expertise in staphylococcal biology as a member of her Research Advisory Committee.

I am a Professor in the Department of Internal Medicine and the Director of the Nebraska Medical Center Department of Healthcare Epidemiology. My research interests include the epidemiology of hospital acquired infections and staphylococcal biology. This experience, integrating clinical issues with bench tools in staphylococcal research, will prove valuable as I advise Dr. Nichols in her development as a clinician scientist.

Dr. Nichols has proposed to study the neuroimmune response to a catheter-associated central nervous system infection. This work utilizes a novel mouse model to investigate the host response to CSF shunt infections, a frequent complication of the treatment of hydrocephalus. Understanding the interactions between the immune system and the biofilms that form on infected CSF shunt catheters can lead to the development of novel management strategies for these infections. She has designed a proposal that seeks to define the bacteriologic and immunologic responses in this infectious model, using a clinical *Staphylococcus aureus* specimen as well as a genetically modified isolate, impaired in biofilm formation. This approach will provide valuable insights into the relationship between the host and biofilm infections, an arena in which very little is currently known.

I am happy to serve as one of Dr. Nichols' advisors for this research proposal and to support her throughout her career. As part of her Research Advisory Committee, I will ensure that she continues to direct her attention to scientific questions relevant to her current proposal. I will be able to provide both scientific and career guidance as she pursues the course of study outlined in this project.

I have no doubt that Dr. Nichols will be highly successful in her work. I look forward to continued collaboration with her. I give this application my strongest support.

Sincerely,

Mark E. Rupp, M.D.

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DEPARTMENT OF
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June 1, 2009

NIH K08 Review Panel

Re: Jessica R Nichols, M.D. "Characterization of a Novel Murine Model of CNS Catheter Infection"

Dear NIH Study Section,

It is with pleasure that I support Dr. Jessica Nichols' K08 Career Development Award Proposal by providing her my expertise in staphylococcal biology as a member of her Scientific Advisory Committee and as a collaborator in the experiments outlined in this proposal. I am a Professor in the Departments of Microbiology/Immunology and Orthopedics at the University of Arkansas for Medical Sciences. My research focuses on three major facets of *S. aureus* infection. The first is directed toward understanding how *S. aureus* forms a biofilm and the nature of the adaptive response that allows *S. aureus* to persist within a biofilm. The second is directed toward defining the regulatory circuits that control expression of *S. aureus* virulence factors. The third research focus is a collaborative effort with faculty in the Department of Orthopaedic Surgery and the Department of Radiology and Nuclear Medicine aimed at the detection, diagnosis and treatment of *S. aureus* musculoskeletal infection. Taken together, we believe these three research areas represent a comprehensive approach that will ultimately lead to the development of new therapeutic agents and better methods of using these agents to treat staphylococcal musculoskeletal infection. This experience, integrating clinical issues with bench tools in staphylococcal research, will prove valuable as I advise Dr. Nichols in her development as a clinician scientist.

Dr. Nichols has proposed to study the neuroimmune response to a catheter-associated central nervous system infection. This work utilizes a novel mouse model to investigate the host response to CSF shunt infections, a frequent complication of the treatment of hydrocephalus. Understanding the interactions between the immune system and the biofilms that form on infected CSF shunt catheters can lead to the development of novel management strategies for these infections. She has designed a proposal that seeks to define the bacteriologic and immunologic responses in this infectious model, using a clinical *Staphylococcus aureus* specimen as well as a genetically modified isolate, impaired in biofilm formation. I will assist in experiments utilizing IVIS (*in vitro imaging system*) to define bacterial growth kinetics in the CNS catheter infection model and will design the modified bacterial strains utilized in this proposal (*lux*-expressing *S. aureus* and *sarA* deficient *S. aureus* strains).

I am happy to serve as one of Dr. Nichols' advisors for this research proposal and to support her throughout her career. As part of her Scientific Advisory Committee, I will ensure that she continues to direct her attention to scientific questions relevant to her current proposal. I will participate in the quarterly Scientific Advisory Committee meetings via video teleconference. In addition, Dr. Nichols and I actively correspond by e-mail and telephone on a regular basis. I am also a participant in several conferences outlined in Dr. Nichols' education plan, including the

upcoming Gordon Conference on Staphylococcal disease and the Mid America Consortium of Gram Positive Pathogens Work in Progress Meetings. I am confident that Dr. Nichols and I will be able to continue the collaboration and mentoring relationship that we established during her fellowship at the University of Arkansas for Medical Sciences.

I have no doubt that Dr. Nichols will be highly successful in her work. I look forward to continued collaboration with her. I give this application my strongest support.

Sincerely,

Mark Smeltzer Ph.D.
Direct phone: 501-686-7958
e-mail: smeltzermarks@uams.edu

University of Nebraska Medical Center

Eppley Institute for Research in Cancer & Allied Diseases
UNMC Eppley Cancer Center

May 21, 2009

K08 Review Panel Members
c/o Scientific Review Officer
National Institutes of Health
Bethesda, Maryland

Re: Characterization of a Novel Murine Model of CNS Catheter Infection

Dear Members of the Study Section,

It is my pleasure to support Dr. Jessica Nichols' K08 Career Development Award Proposal by providing her my expertise in immunology as a member of her Research Advisory Committee.

I am an Associate Professor at the Eppley Cancer Institute with courtesy appointments in the Departments of Pathology and Microbiology, and Biochemistry and Molecular Biology. My research principally focuses on the assembly and trafficking of class I MHC molecules, with the goal of identifying ways in which those processes influence the presentation of pathogen and tumor antigens to T-lymphocytes. The knowledge gained from these studies on antigen presentation can be used to develop better treatments for infectious diseases and cancers. In addition, we have been engaged in many translational studies involving development of cytokine immunotherapies for breast cancer and pancreatic cancer. This experience integrating bench research with clinical issues will prove valuable as I advise Dr. Nichols in her development as a clinician scientist.

Dr. Nichols has proposed to study the neuroimmune response to a catheter-associated central nervous system infection. This work utilizes a novel mouse model to investigate the host response to CSF shunt infections, a frequent complication of the treatment of hydrocephalus. She has designed a proposal that seeks to define the bacteriologic and immunologic responses in this infectious model, using a clinical *Staphylococcus aureus* specimen as well as a genetically modified isolate, impaired in biofilm formation. Understanding the interactions between the immune system and the biofilms that form on infected catheters can lead to the development of novel management strategies for these infections.

I am happy to serve as one of Dr. Nichols' advisors for this research proposal and to support her throughout her career. As part of her Research Advisory Committee, I will ensure that she continues to direct her attention to scientific questions relevant to her current proposal. I will be able to provide both scientific and career guidance as she pursues the course of study outlined in this project.

I have no doubt that Dr. Nichols will be highly successful in her work. I look forward to continued collaboration with her. I give this application my strongest support.

Sincerely,

Joyce Solheim, Ph.D.
Associate Professor and Director,
Cancer Research Graduate Program

University of Nebraska Medical Center

NEBRASKA'S HEALTH SCIENCE CENTER
A Partner with Nebraska Health System

PATHOLOGY AND MICROBIOLOGY

June 1, 2009

NIH K08 Review Panel

Re: Jessica R Nichols, M.D. "Characterization of a Novel Murine Model of CNS Catheter Infection"

Dear NIH Study Section,

It is with pleasure that I support Dr. Jessica Nichols' K08 Career Development Award Proposal by providing her my expertise in staphylococcal biology as a collaborator on this project.

I am an Assistant Professor in the Department of Pathology and Microbiology at the University of Nebraska Medical Center. The focus of my laboratory is to both characterize the *S. aureus* factors that influence the organism's ability to cause disease, as well as define the regulatory mechanism(s) that modulate the expression of these genes. This experience will prove valuable as I work with Dr. Nichols in the coming years.

Dr. Nichols has proposed to study the neuroimmune response to a catheter-associated central nervous system infection. This work utilizes a novel mouse model to investigate the host response to CSF shunt infections, a frequent complication of the treatment of hydrocephalus. As part of this work, she will be evaluating the changes in bacterial gene expression over time as the biofilm matures in the presence of central nervous system immune cells. I will work with Dr. Nichols to learn the microarray technology needed for these experiments, as I have used these extensively in my own research.

I am happy to serve as one of Dr. Nichols' collaborators for this research proposal and to support her throughout her career. I will be able to provide scientific guidance as she pursues the course of study outlined in this project.

I have no doubt that Dr. Nichols will be highly successful in her work. I look forward to continued collaboration with her. I give this application my strongest support.

Sincerely,

Paul M. Dunman, Ph.D.
Assistant Professor
Department of Pathology and Microbiology
University of Nebraska Medical Center

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DESCRIPTION OF THE INSTITUTIONAL ENVIRONMENT

1. The University of Nebraska Medical Center is a rapidly growing research enterprise, with research funding from external sources now exceeding $82 million annually and the opening of the new Durham Research Center II (DRC II), in May 2009. This state of the art center provides the space and tools needed for UNMC scientists to develop cutting-edge research and interdisciplinary collaborations that are anticipated to stimulate significant advances in medical care. Importantly, for Dr. Nichols' career development, the 7th floor of DRC II houses the Laboratory for Staphylococcal Research (www.unmc.edu/dept/pathology/lsr/), a well-respected and well-funded group of scientists interested in basic and applied research involving *Staphylococcus aureus* and *Staphylococcus epidermidis.* This group includes Dr. Nichols' mentor, Dr. Tammy Kielian, collaborators Drs. Paul Fey and Paul Dunman, as well as Dr. Kenneth Bayles and Dr. Steve Hinrichs. In addition to working with Dr. Kielian and her collaborators, frequent interactions with the Laboratory for Staphylococcal Research and members of Dr. Nichols' Scientific Advisory Committee will play an important role in enriching Dr. Nichols' experience. Interactions with other investigators including students, postdoctoral fellows and principal investigators, will take place through seminars and meetings, as well as through informal interactions enhanced by the "open" architectural design of the laboratory facilities. Dr. Nichols' primary laboratory space is located on the 7th floor of DRC II, immediately adjacent to her mentor, Dr. Kielian. The laboratories are all connected, without dividing walls, and organized around multiple central equipment cores. The facilities in DRC II also include a state-of-the-art vivarium where Dr. Nichols will share a BSL2 animal housing suite and adjoining procedure room with Dr. Kielian for performing animal surgery. While this equipment and research space will certainly facilitate the candidate's project, the intellectual discourse with numerous other investigators within UNMC will contribute significantly to Dr. Nichols' scientific development. This interaction is encouraged by the design of the laboratories and shared equipment cores, as well as the numerous formal and informal seminars, journal clubs and other sessions outlined below and in the candidate's Career Development Activities. The environment at UNMC is ideally suited for Dr. Nichols to carry out the experiments proposed in this application and advance her professional development towards an independent clinician scientist.

2. Key Faculty for Dr. Nichols' Career Development

The Mentor, Dr. Kielian, is multiply funded by the NIH to study immune responses to staphylococcal infection, particularly within the CNS. These grants explore multiple avenues, including the peripheral immune response to *S. aureus* biofilm infection, the role of Toll-like receptors (TLRs) in facilitating *S. aureus* recognition in the brain, the signals that regulate fibrotic encapsulation of brain abscesses and the effects of pro-inflammatory cytokines on gap junction communication. These projects will provide an infrastructure of technical support and general lab supplies for immunologic studies and expertise with an *in vivo* model of CNS infection that will foster Dr. Nichols' independent research project. Specifically, this will allow Dr. Nichols access to shared equipment, as outlined in the Resources section, as well assistance from Dr. Kielian's technicians, which collectively have over 40 years of laboratory experience, as Dr. Nichols' and her technician are trained in new research techniques. Scientific Advisory Committee member and collaborator, Dr. Smeltzer, is also multiply funded by the NIH to study the molecular pathogenesis of staphylococcal disease. This funding provides the infrastructure needed to develop the bacterial constructs that will be utilized in this proposal. Drs. Paul Fey and Paul Dunman are also funded by the NIH to study various aspects of staphylococcal disease, providing the equipment and support that will allow them to assist Dr. Nichols in the studies outlined in this proposal, as well as training Dr. Nichols' in advanced staphylococcal research techniques.

3. Seminars

Numerous seminar programs exist on the UNMC campus for Dr. Nichols to take advantage of and present her work. These seminars are described in detail in the candidate's Career Development Activities and include monthly Immunology Interest Group and journal club meetings, Department of Pathology and Microbiology seminars, Departments of Neurology and Pharmacology and Experimental Neuroscience seminars and Department of Pediatrics Grand Rounds. These seminars consist of both internal and external speakers. In addition, Dr. Nichols will participate in the monthly Work in Progress (WIP) teleconference of the Mid America Consortium of Gram Positive Pathogens. Dr. Nichols will also participate in Dr. Kielian's weekly laboratory meeting, which is designed to discuss work in progress and troubleshooting technical problems in the laboratory, as well as review new data published by other laboratories in the field.

4. Computer

The Department of Pediatrics provides a desktop computer for each faculty member. Hardware upgrades are made on approximately a 3-year rotational basis. In the lab and office, Dr. Nichols has access to the internet and the local area network. All data saved to the server is backed up nightly at an off-site secure location. Dedicated technical support is available for all hardware and software problems or concerns.

5. Library
The UNMC McGoogan Library houses over 80,000 volumes and subscribes to 2,200 journals, either in print form or electronically. This is a fully automated medical library featuring computer terminals that provide access to its collections from any of the several hundred networked desktop computers throughout the Medical Center.

6. Education Center
UNMC has recently constructed a new Education Center, encompassing 20,000 square feet within the Medical College and Graduate School of Meidcal Sciences. All formal courses are taught in the Education Center. In close proximity to the McGoogan Library and the Biomedical Information Center, the new complex integrates the classroom, study, laboratory and computer resources.

University of Nebraska Medical Center

NEBRASKA'S HEALTH SCIENCE CENTER

DEPARTMENT OF PEDIATRICS
Office of the Chair

June 8, 2009

RE: Dr. Jessica Nichols

Dear Colleagues:

I am writing in strong support of Dr. Jessica Nichols, who is applying for a K08 award, entitled "Characterization of a Novel Model of Murine Central Nervous Infection". Dr. Nichols is just completing her fellowship in Pediatric Infectious Diseases at the University of Arkansas, and will be joining our faculty in the Department of Pediatrics in July. She will be appointed full-time as an Assistant Professor of Pediatrics, with a non-salaried secondary appointment in Pathology and Microbiology.

Dr. Nichols began her fellowship research in Arkansas with her research mentor, Dr. Tammy Kielian, working on the development of a unique model for CNS shunt infection. Dr. Kielian is a well-respected and well-funded investigator in infectious diseases, and was recruited last summer to the University of Nebraska Medical Center in the Department of Pathology and Microbiology. Dr. Nichols has continued her work at Arkansas on central nervous system infections, and has continued to collaborate closely with Dr. Kielian over the last year.

I have had the pleasure of getting to know Dr. Nichols since late fall, when she inquired about a position on our faculty. She impressed everyone with her intelligence, knowledge, creativity, and personal commitment to research. I am very excited that Dr. Nichols will be joining our group. Dr. Nichols interests overlap the Department of Pediatrics and the Department of Pathology and Microbiology. Both departments were enthusiastically engaged in her recruitment, including the development of resources and support for her position.

Dr. Nichols is clearly dedicated to a career in research, and has set as a personal goal the development of skills and experience necessary to become an independent investigator. Towards that end, she is has set a goal to obtain independent NIH funding, such as an R01. We support her in achieving these goals, and have put in place the protected time, mentorship, laboratory environment and resources to allow her to succeed. It is important to emphasize that Dr. Nichols will receive these resources, whether or not she receives the K08.

Dr. Tammy Kielian will mentor Dr. Nichols in her research at UNMC, with progress independently monitored by quarterly meetings of a distinguished Scientific Advisory Committee as detailed in the application. Dr. Kielian's CV is attached to the grant application, and demonstrates a remarkable level of productivity. Dr. Kielian is also well funded in her own studies. Dr. Kielian is a full time faculty member of the Department of Pathology and Microbiology. Dr. Steve Hinrichs, Chair of the Department of Pathology and Microbiology, is

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extremely enthusiastic about Dr. Nichols' research, and has been instrumental both in supporting Dr. Kielian's time to mentor Dr. Nichols, and in assisting identification of laboratory space for Dr. Nichols adjacent to Dr. Kielian's labs. Dr. Nichols will have her own lab in the Durham Research Center, a newly opened research facility at UNMC. Dr. Nichols will also receive $50,000 in startup equipment, and $90,000 per year for technician support, supplies, animals, and other expenses for a minimum of three years. She will also have full access to the research resources of the Department of Pediatrics, and the Department of Pathology and Microbiology.

From a time perspective, Dr. Nichols' position will allow her to focus her creative energies on her research interests, while maintaining enough patient contact to maintain her clinical skills. Dr. Nichols will enjoy 80% protected time to pursue her research interests. She will attend on the Pediatric Infectious Diseases clinical service, but her time on the service will initially be limited to 4 weeks of clinical service per year to allow her to develop her research. She will also participate in our educational activities, such as grand rounds and major departmental and divisional conferences. To protect her time, she will not be asked initially to participate in administrative activities, such as departmental or medical school committees.

I have reviewed the proposal from Dr. Nichols, and discussed it with her on multiple occasions. Her proposal is very creative and addresses an issue that is clinically important, but has been remarkably neglected from a basic research perspective. As a neonatologist myself, we care for a significant number of kids who require shunts. Infection is a major complication of CNS shunts and correlates with significant morbidity, yet little is known of the biology of CNS shunt infection.

I came to the University of Nebraska as Chair of the Department of Pediatrics in early 2008, after 16 years at the University of Texas School at Houston, including 10 years as Department Chair. I have had personal experience with a large number of young faculty with early research awards, including a number of K08's. I will meet regularly with Dr. Nichols regarding her career development, and help her to address issues such as resource needs, scientific questions, clinical challenges, institutional process or other career needs.

I am very impressed that Dr. Nichols has the personal characteristics to succeed in her K08 and develop into an independent investigator. She is bright, creative, motivated, and above all personally driven to develop new knowledge. She has the personal characteristics to become a leader in pediatric infectious diseases. This project joins a highly motivated trainee, an energetic mentor with a proven track record, strong institutional support, and a scientific question that is both important and clinically relevant. I believe this is a perfect combination for a K08 award. I am personally committed to her development into a productive, independent investigator, and as Department Chair, commit our institution to her support.

Please contact me if you need further information or have any questions.

Best Regards,

John W. Sparks MD

John W. Sparks, M.D.
Chairman and Stokes-Shackleford Professor of Pediatrics
Department of Pediatrics

INSTITUTIONAL COMMITMENT TO THE CANDIDATE'S RESEARCH CAREER DEVELOPMENT

Dr. Nichols currently has 80% protected time for research. The Department of Pediatrics and the Infectious Disease section are committed to continue to protect Dr. Nichols' time so she can devote her time and effort toward the outlined program of research training and development directed toward an independent career as a physician scientist. Dr. Nichols will have minimal clinical responsibilities that include one half day clinic weekly and 4 weeks of inpatient consult service each year. Administrative and teaching responsibilities will be limited as described in the candidate's Career Development Plan, as well in the institutional letter of commitment from Dr. John Sparks (Chairman, Department of Pediatrics).

Specific Aims

Cerebrospinal fluid (CSF) shunt infections are a frequent and serious complication in the treatment of hydrocephalus in the pediatric population, with a reported incidence of 5-15% (3). The most common organisms responsible for these central nervous system (CNS) catheter infections, *Staphylococcus epidermidis* and *Staphylococcus aureus,* are both known to form biofilms (4,5). These biofilms are organized communities of bacterial cells that aggregate on the catheter surface, enclosed in a self-produced matrix that protects the organisms. The biofilm's ability to evade the host immune response and antimicrobial agents makes it difficult to manage CNS catheter infections non-surgically, such that catheter removal is currently required to effectively treat these infections. **While the growth characteristics and other adaptations of the bacteria required for biofilm formation are being extensively investigated by microbiologists, very little is known about the host interaction with the biofilm, particularly with regard to the immune response to catheter biofilm infections.**

Prior to the initiation of my work, no rodent model had been developed to investigate interactions between the CNS immune response and bacteria during biofilm growth. Therefore, to explore the neuroimmune response to CNS catheter infections, I have developed a novel model of CNS catheter infection in the mouse. This technique results in a consistent catheter-associated infection with *S. aureus* and ventriculitis, mimicking what is seen in humans with ventricular shunt infections. Establishment of this model provides a powerful tool to identify important factors in the host immune response to CNS biofilms through the use of genetically engineered knockout or transgenic mouse strains. My objective in this study is to utilize this model of CNS catheter infection to investigate the kinetics of bacterial growth and the host innate immune response during biofilm growth. Understanding the interactions between the neuroimmune system and the biofilms that form on infected catheters will allow us to explore novel management strategies for these CNS infections that are classically recalcitrant to conventional antibiotic therapy.

The overall ***hypothesis*** of this proposal is that the host innate immune response in the brain is actively attenuated in response to biofilm colonization of a CNS catheter**.** The following specific aims will be performed to address this hypothesis.

1) **To characterize the bacterial growth kinetics and innate immune response in a murine model of CNS catheter infection**. In these studies, I will investigate the kinetics of bacterial growth and gene expression associated with the catheter as well as the surrounding brain parenchyma and other organs to assess the potential for systemic bacterial colonization. In addition, I will define the influx of innate immune cells (microglia, macrophages, dendritic cells and neutrophils) into the tissues surrounding infected CNS catheters and evaluate their activation status.
2) **To define the role of bacterial regulatory factors in the development of CNS catheter infection.** In these studies, I will describe the impact of mutation of the staphylococcal accessory regulatory gene *sarA* on the bacterial growth kinetics and immune response to a CNS catheter infection using a *S. aureus sarA* mutant strain.

Background

Clinical impact of CNS catheter infections. Cerebrospinal fluid (CSF) shunt placement for the treatment of hydrocephalus is one of the most common procedures performed by pediatric neurosurgeons in the United States, with tens of thousands of shunts implanted annually (3). Unfortunately, 30-40% of all CSF shunts placed in pediatric patients fail within the first year, resulting in a shunt revision to primary placement ratio of 3:1 in many healthcare centers (3,5). One of the most common causes of shunt failure is infection, reported in 5-30% of cases (5). In addition to shunt failure, these catheter infections are associated with a higher risk of seizures, decreased intellectual performance and a two-fold increase in long-term mortality (5). Therefore, studies designed to advance our understanding as to how bacteria colonize and evade anti-microbial killing in the CNS compartment could have a dramatic impact on the development of treatment modalities for these serious infections.

Role of biofilms in CNS catheter infections. The most common organisms responsible for CNS catheter infections, *Staphylococcus epidermidis* and *Staphylococcus aureus,* are both known to form biofilms (4,5). These biofilms are organized communities of bacterial cells that aggregate on the catheter surface, enclosed in a self-produced matrix that protects the organisms. Within the biofilms, bacteria can survive in a protected environment, shielded from the host immune response and antimicrobial agents by a bacterially-derived extracellular matrix and other immunomodulatory factors (6). Most of the bacteria within the biofilm are sessile but occasionally individual planktonic bacteria are released, allowing dissemination of the organism to other tissues (6). The biofilm's ability to evade the host immune response and antimicrobial agents makes it difficult to manage CNS catheter infections non-surgically, such that catheter removal is currently required for effective treatment. While the growth characteristics and other adaptations of the bacteria required for biofilm formation are being extensively investigated by microbiologists, very little is known about the host interaction with the biofilm, particularly with regard to the immune response to catheter biofilm infections. It is vital to obtain a better understanding of the interactions between the immune system and the bacteria present in the biofilm in order to make progress towards the management of CNS catheter infections.

Innate immune response to biofilms. Preliminary investigations in *in vitro* biofilm models suggest that there are multiple factors that protect the biofilm from the host immune system. The extracellular matrix is thought to reduce the penetration of immune cells into the biofilm and is known to decrease the ability of phagocytes to actively kill bacteria through mechanisms that have yet to be defined. Poly-N-acetyl glucosamine (PNAG) is one of the exopolysaccharides that form the biofilm matrix in *S. epidermidis* and *S. aureus* (7). The host produces opsonic antibody in response to PNAG that can penetrate the biofilm, but the antibody is quickly saturated and functionally inactivated by excessive PNAG secretion within the biofilm (7). In addition, studies using flow cells have shown that biofilms in static or low flow settings, as would be expected in a CNS shunt, have a thicker matrix than seen in biofilms in high shear settings such as endovascular infection (8). In the presence of the thicker extracellular matrix, leukocytes can adhere to irregularities in the biofilm surface, but do not effectively penetrate the biofilm (8). In high shear settings, leukocytes can more effectively penetrate the biofilm through nutrient channels but are unable to effectively kill bacteria, suggesting biofilms have other mechanisms of protection from the host immune system in addition to the barrier protection offered by the matrix (7,8). These *in vitro* models revealed that *S. aureus* biofilms were primarily associated with a Th1 response typified by elevated IL-1β, IL-6, IL-12 and IFN-γ levels, similar to the responses seen in planktonic *S. aureus* infections (7,8). In *in vitro* models of *S. epidermidis* infections, elevated IL-1β and IL-6 levels are also observed, but IL-12 and IFN–γ levels are lower than those observed with *S. aureus* infections (8). **However, it is important to note that relatively little information is currently available regarding the innate immune response to *S. aureus* biofilms *in vivo* and to our knowledge, no studies have examined this question with regard to CNS shunt infections, the topic of the experiments described in the current K08 application.** The inflammatory mediator profiles observed following infection can provide important information about the types and activation status of immune cells in the area surrounding the catheter and will be evaluated using this *in vivo* model of CSF catheter infection.

Animal models of CNS catheter infection. To the best of our knowledge, no murine model of CNS catheter infection has been reported in the literature. An intracranial infection model, using New Zealand White rabbits, has been used to study rifampin-impregnated CSF shunt catheters, but no other animal models for CNS catheter infection have been reported to date (9). Therefore, the mouse model of CNS catheter infection that I

have developed represents a powerful tool to identify important factors in the host immune response to CNS biofilms through the use of genetically engineered knockout or transgenic mouse strains.

Immune responses within the CNS. Establishing a catheter model within the CNS was necessary because the immune response in this compartment often differs from that seen in the periphery due to the inherent anti-inflammatory environment of the normal CNS (9). However, in response to injury or infection, the CNS is capable of mounting a robust immune response, albeit deviant in some respects, from similar insults in the periphery (9,10,11). Resident glial cells in the CNS parenchyma are activated in addition to the peripheral immune cells, such as neutrophils and macrophages, which invade the CNS in response to infection (9,10). In particular, microglia and astrocytes play an important role in the defense against invading microbes in the brain and must be investigated in this unique setting (13). This is one of the objectives in the current K08 application.

SIGNFICANCE AND CLINICAL RELEVANCE OF THE PROPOSED STUDIES. Deciphering the interactions between the neuroimmune system and the biofilms that form on infected catheters will allow us to explore novel management strategies for these infections in future studies. A better understanding of the immune responses involved in CNS catheter infections would present several potential therapeutic advantages. First, identification of crucial immune response components could help identify patients at higher risk for catheter infection, towards whom targeted prevention can be directed. There are now multiple genetic polymorphisms in the innate immune system that have been identified and are known to predispose patients to specific diseases. For example, certain Toll-like receptor (TLR) 2 polymorphisms predispose individuals to bacterial infections (14,15). Identification of the crucial immune components involved in biofilm infections within the CNS could be used to screen patients and provide better preventative management. In addition, if our studies better define the immune response to biofilm-associated infections, this information could be exploited to allow for selective manipulation of immune responses to favor disease resolution. This could provide potential adjunctive therapy to current antibiotics and prevent catheter removal in some patients.

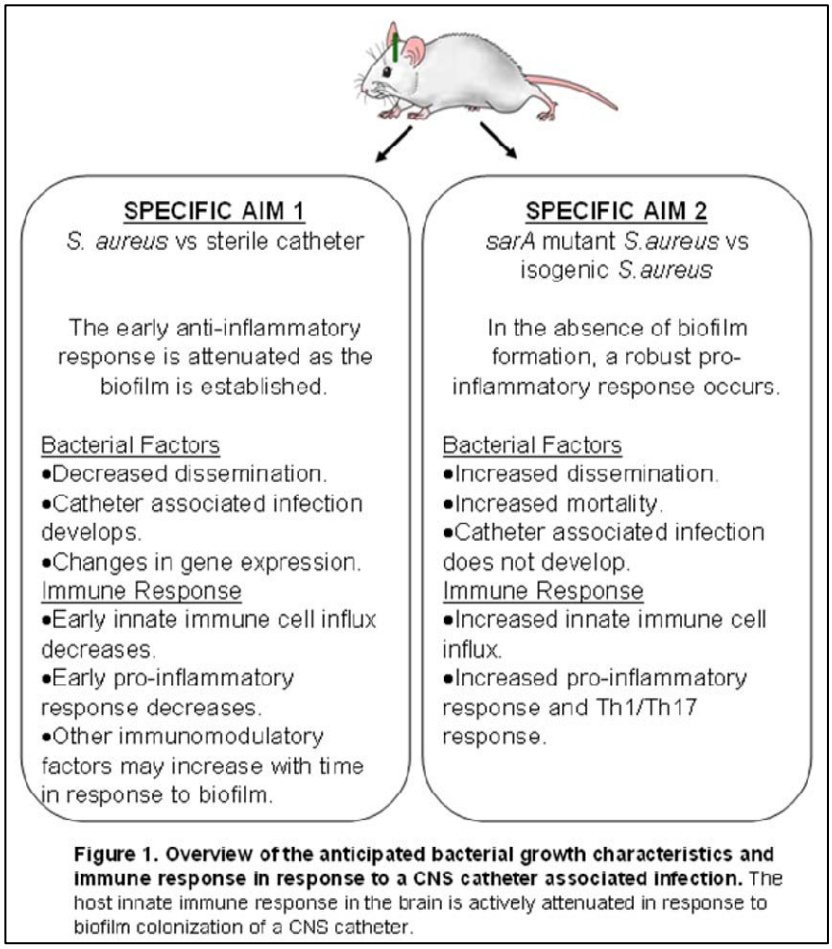

Figure 1. Overview of the anticipated bacterial growth characteristics and immune response in response to a CNS catheter associated infection. The host innate immune response in the brain is actively attenuated in response to biofilm colonization of a CNS catheter.

In the current proposal, we will utilize this model of CNS catheter infection to characterize the host immune response to a CNS biofilm infection with *S. aureus* by investigating the kinetics of bacterial growth and the host innate immune response in this setting. The experimental objectives of this application are summarized in Figure 1. The interactions between host immune cells and the biofilm represent a relatively unexplored niche, particularly in the CNS, and the novel mouse model we have developed will be a valuable tool for investigating these interactions. **Another important advantage of our approach is the fact that all of our studies will be performed *in vivo*, which will take into account all of the complex interactions occurring between the biofilm and the host response in the CNS, relationships that cannot be accurately replicated *in vitro*.** This strategy has the highest likelihood of identifying mechanisms that could be directly applicable to CNS shunt infections in the pediatric population.

Preliminary Data

I have developed a novel model of CNS catheter infection in the mouse using *S. aureus* to begin exploring the immune response to catheter infections within the brain. This technique results in a consistent catheter-associated infection and ventriculitis, mimicking the course of disease seen in humans with ventricular shunt infections.

The *S. aureus* strain used in this mouse CNS catheter infection model is a clinically-derived isolate of methicillin-susceptible *S. aureus* from a patient presenting with a CSF shunt infection. This isolate was selected because it is capable of forming a catheter-associated biofilm within the CNS, increasing the clinical relevance of this model. Silicone catheter fragments coated with *S. aureus* or serum alone (as a control) were stereotactically inserted into the lateral ventricle of C57BL/6 mice and evaluated at days 1, 3, 5, and 7 post-insertion. This procedure is described in detail in the General Methods section. Animals tolerated the procedure well, with no clinical signs of illness or bacterial growth seen in the control group. In contrast, animals implanted with *S. aureus*-coated catheters were clinically ill, exhibiting ruffled fur, weight loss and decreased activity on days 1-3, with both weight and behavioral changes returning to baseline after 4-5 days (data not shown). Excised catheters from infected mice also demonstrated a slime coat, consistent with biofilm formation, whereas those collected from uninfected control mice remained sterile.

1. Bacterial growth associated with CNS catheters is significantly elevated compared to the surrounding brain parenchyma. A major objective of the mouse CNS catheter infection model was to create a catheter-associated biofilm; thus it was important to confirm that this infection model generated a catheter-associated infection as opposed to a planktonic infection in the parenchyma surrounding the catheter. Indeed, catheter-associated bacterial titers were found to increase progressively over time, whereas relatively few bacteria were detected in the brain parenchyma, consistent with biofilm formation and minimal planktonic spread of infection (Figure 2). Both catheters and the surrounding brain parenchyma of mice implanted with sterile catheters were negative for bacterial growth and therefore, are not depicted in this graph.

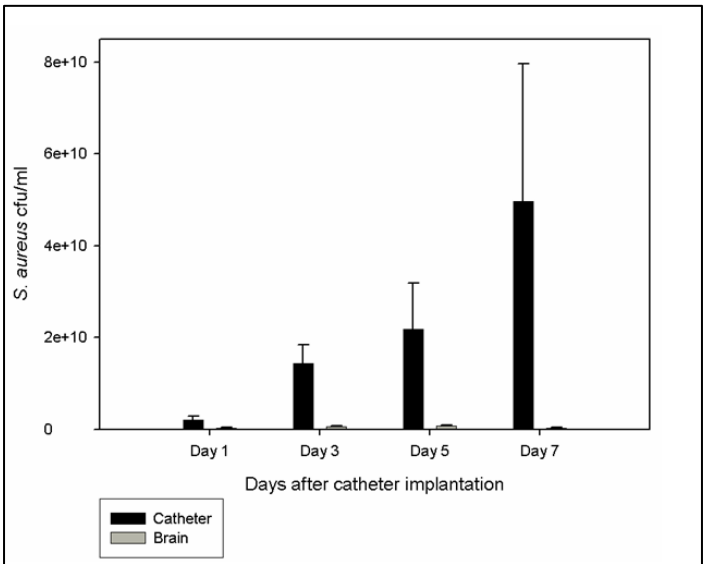

Figure 2: Bacterial growth is elevated along the catheter in comparison to the bacterial growth in the brain parenchyma.

2. Inflammatory infiltrates are associated with infected CNS catheters. Preliminary histologic examination of brain tissues was performed to confirm catheter placement within the lateral ventricle, the site that coincides with human ventricular shunt placements. Catheters were removed from the brain prior to rapid freezing for tissue sectioning. Hematoxylin and eosin (H & E) staining revealed bilateral ventriculitis as well as inflammatory cells associated with the insertion track of *S. aureus* infected catheters (Figure 3, arrows). A greater amount of leukocyte influx was detected in the parenchyma surrounding infected catheters on day 3,

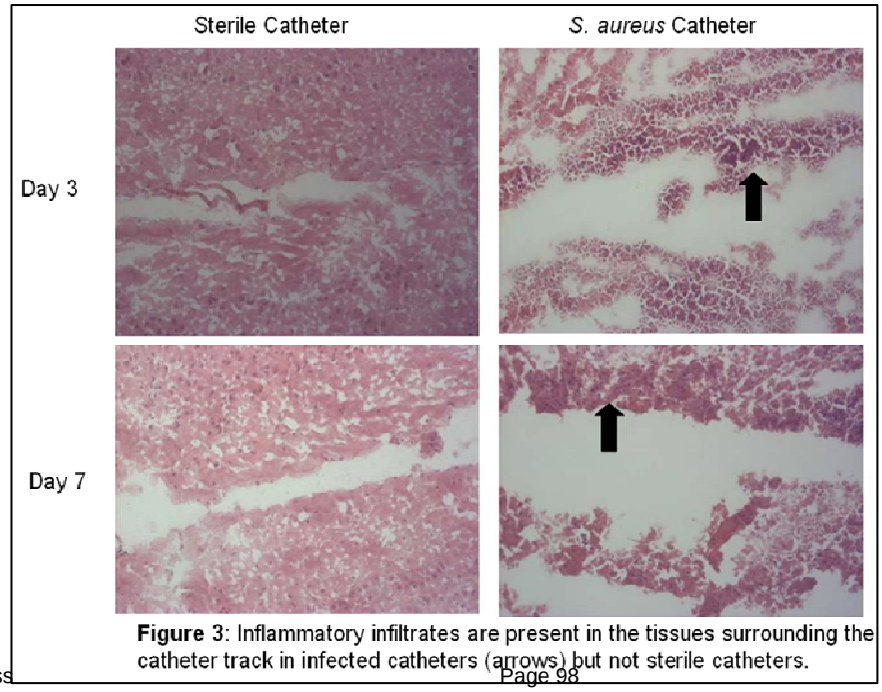

Figure 3: Inflammatory infiltrates are present in the tissues surrounding the catheter track in infected catheters (arrows) but not sterile catheters.

as compared with day 7, suggesting that the inflammatory response may subside over time despite the continued presence of biofilm-associated bacteria. Importantly, rampant inflammatory cell infiltrates were not observed in response to sterile catheters (Figure 3). Collectively, these findings demonstrate the reliability of the mouse CNS catheter infection model to mimic the course of CSF shunt infections in humans and as such, will provide the opportunity to investigate the role of host immune mechanisms in regulating biofilm growth and persistence.

3. Chemokine and pro-inflammatory cytokine expression is elevated in tissues surrounding infected CNS catheters. Preliminary evaluation of inflammatory mediator expression in the parenchyma surrounding sterile and infected CNS catheters indicated that the levels of several chemokines and pro-inflammatory cytokines are elevated in response to *S. aureus* biofilm infection. Specifically, MCP-1, KC, IL-17 and IL-1β levels were markedly elevated, as shown in Figure 4. Importantly, chemokine (KC and MCP-1) expression began to decline at later time points post-infection, which correlated with the reduction in inflammatory cell infiltrates detected by H & E staining (Figure 3). These preliminary findings indicate that the host immune response may be down-regulated as the biofilm matures. This possibility will be further examined in the studies outlined in Specific Aim 1.

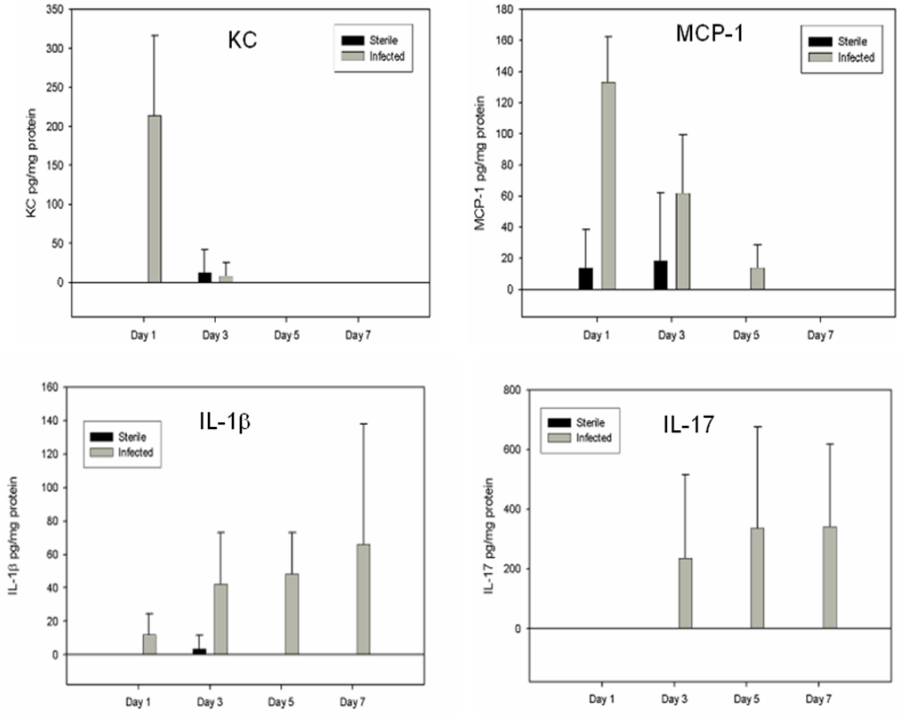

Figure 4: Chemokines and anti-microbial cytokines are elevated in the tissues surrounding infected catheters.

4. Biofilm infection is associated with neuronal cell death in regions immediately surrounding infected catheters. Known complications of CNS catheter infections in children include increased seizure risk and loss of intellectual capacity, suggesting damage to neurons and neuronal signaling pathways. Catheter-associated tissues were recovered from infected and sterile treated mice and stained with FluoroJade C© to visualize areas of neuronal apoptosis. FluoroJade C staining was apparent in tissues immediately surrounding infected catheters at days 5 and 7 following *S. aureus* exposure (Figure 5 and data not shown), whereas no staining was detected in mice implanted with sterile catheters (data not shown). These findings suggest that biofilm infection leads to localized neuronal cell death, which could be reversed if we could attain a better understanding of host-biofilm interactions, an objective of the current K08 proposal.

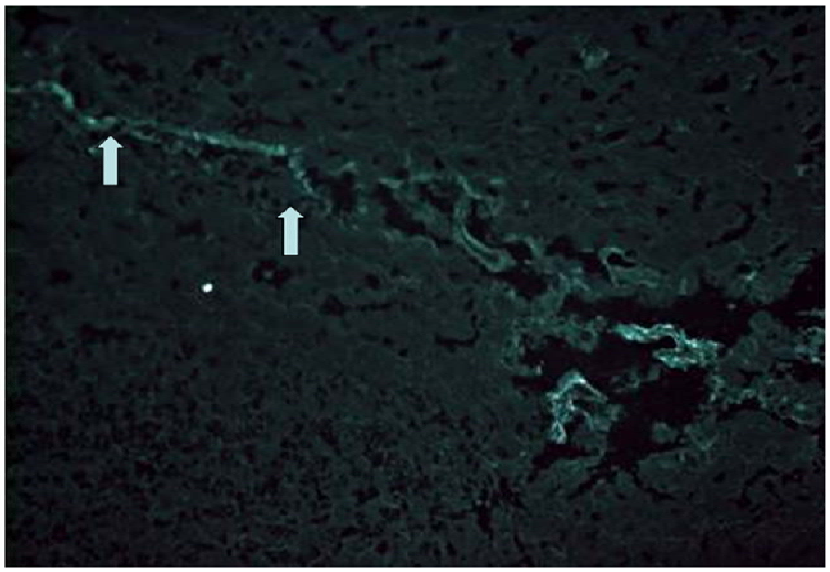

Figure 5: Evidence of neuronal death is observed along the catheter in infected mice (arrows), but is not observed in sterile mice (not shown).

***5. In vivo* bioluminescence imaging (IVIS).** Bioluminescence imaging is a powerful tool that allows for the direct visualization of bacterial growth over time in a single experimental animal (16,17). This technology takes advantage of photon emission from the bacterial luciferase gene, *lux,* which encodes an enzyme that constitutively emits photons. The power of IVIS lies in the fact that the same cohort of animals can be imaged over an entire experiment, providing a longitudinal assessment of bacterial growth or inflammatory gene expression. This information is not attainable with standard quantitative cultures or histology since mice must be sacrificed at specified time points to evaluate these parameters. This approach has been utilized in several models of biofilm infection (16,17) and in unpublished studies in Dr. Kielian's laboratory, demonstrating the presence of bioluminescent bacteria within the murine brain abscess model (Figure 6). Well-defined abscesses were readily apparent in infected animals as early as 24 h following *S. aureus* exposure despite the depth of this infection within the brain parenchyma, establishing the feasibility of this imaging modality for use with intracranial infections. An *In Vivo* Imaging System (IVIS) is available at the University of Nebraska Medical Center for the experiments outlined in this proposal. IVIS technology represents a valuable tool in the evaluation of longitudinal bacterial growth in an *in vivo* model.

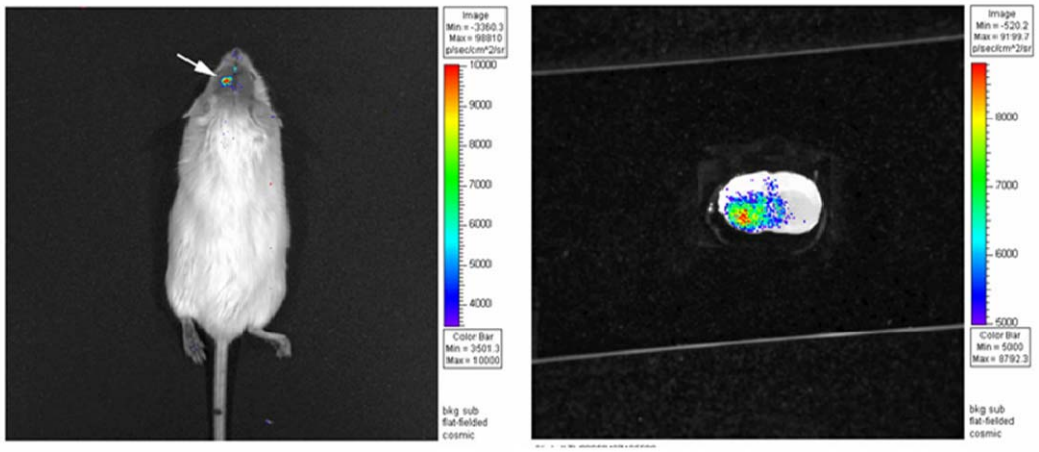

Figure 6. In vivo imaging (IVIS) of *S. aureus* in brain abscesses, demonstrating the utility of bioluminescent imaging within the CNS. (T Kielian, unpublished data)

Based on these results, we propose that our mouse model provides a valid tool for further investigations of catheter-associated CNS infections.

Summary of Background, Significance and Rationale

1. Infection is one of the most common and serious complications of CSF shunt placement for the treatment of hydrocephalus.
2. The organisms that cause most CSF shunt infections, *S. aureus* and *S. epidermidis,* form biofilms on the catheter surface, making treatment with antibiotics alone very difficult. Because of this, treatment of these infections currently requires removal of the catheter.
3. Very little is known about the interactions between the immune system and biofilm infections, particularly within the CNS.
4. Understanding the interactions between the neuroimmune system and the biofilms that form on infected catheters will allow us to explore novel management strategies for these infections.
5. Our preliminary studies demonstrate that the novel murine model generates a consistent catheter-associated infection.
6. Collectively, our preliminary findings indicate that the host immune response may be actively attenuated to favor bacterial persistence in the setting of a CNS catheter-associated infection.

Experimental Design and Methods

The studies proposed here will test the ***hypothesis*** that the host innate immune response in the brain is actively attenuated in response to biofilm colonization of a CNS catheter. The research plan will define the immune cells and mediators responsible for the innate immune response to a catheter-associated infection within the CNS compartment.

Specific Aim 1: To characterize the bacterial growth kinetics and innate immune response in a murine model of CNS catheter infection.

A. Rationale. Biofilms have the ability to evade the host immune system through several mechanisms that have yet to be fully defined. In particular, there is very little known about the interactions between the immune system within the CNS and biofilm infections. Studies in peripheral biofilm infection models and *in vitro* models have shown that immunity appears to be attenuated in response to a biofilm infection since organisms are not efficiently cleared (6,7,18). Given the inherent anti-inflammatory nature of the CNS, it is logical that the immune response may also be attenuated within the brain during biofilm infections (10). Our preliminary studies support this theory, demonstrating a decrease in chemokines and subsequent immune cell infiltrates as the biofilm matures. This possibility will be further examined in the experiments outlined in this Specific Aim by investigating the behavior of the bacteria within the biofilm as well as the evolution of the host innate immune response over time. Defining the nature of this immune response could provide valuable tools for screening patients at higher risk for these infections and for providing adjunctive therapy to current antibiotic treatment regimens.

B. **Hypothesis.** These studies will test the ***hypothesis*** that the host innate immune response in the brain is actively attenuated in response to biofilm colonization of a CNS catheter**.** The objectives of this specific aim are: (1) to define the bacterial growth and gene expression kinetics associated with CNS catheter infection and (2) to define the influx and activation status of innate immune cells associated with CNS catheter infection throughout the various stages of biofilm development.

C. Experimental Design and Methods

C.1 Define the bacterial growth and gene expression kinetics associated with CNS catheter infection.

Biofilms are known to periodically release planktonic bacteria, making the spread of organisms from the infected catheter to immediately adjacent tissues very likely, particularly during the early phases of infection as the biofilm matures (6). The course of *S. aureus* catheter infection observed in my preliminary studies (Figure 2) indicates that a biofilm infection is established within the first week of infection after a period of minimal planktonic spread. To confirm this trend and further define the nature of *S. aureus* growth as the biofilm matures, bacterial burdens associated with the catheter and surrounding brain parenchyma will be assessed at days 1, 3, 5, 7, 10, 14 and 21 following catheter implantation by quantitative culture using blood agar plates. To examine the possibility that bacteria may detach from the biofilm matrix and disseminate systemically, *S. aureus* colonization will also be evaluated at several peripheral sites including the blood, spleen, spinal cord, heart, and liver. It is expected that sterile catheters and associated surrounding tissues will remain negative for

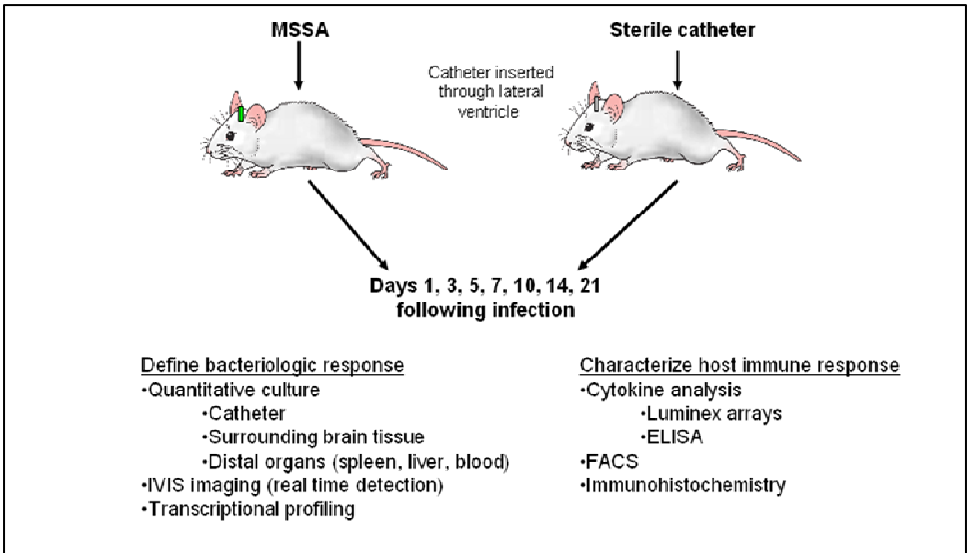

Figure 7: Schematic of *in vivo* studies using mouse models of CNS catheter infection. C57BL/6 mice will have sterile or MSSA coated catheters implanted through the lateral ventricle, with tissues collected at multiple time points for analysis. Detailed methodologies are given in the General Methods section.

bacterial growth throughout the course of this study based on my preliminary data. Planktonic and systemic spread from infected catheters may occur within the first week, if at all, and be minimal thereafter, while catheter-associated bacterial growth is expected to stabilize after the first week of infection as the biofilm matures. Alternatively, with prolonged biofilm infection, there may be a release of planktonic bacteria at later time points as the bacteria continue to propagate in a protected environment. However, this possibility appears unlikely based on my preliminary studies demonstrating steady increases in catheter-associated bacteria with a concomitant decline in planktonic organisms located in the surrounding brain parenchyma.

To visualize the longitudinal growth of *S. aureus* along the catheter in the same animal, a luciferase-expressing variant of the *S. aureus* strain used in the above studies will be used in conjunction with IVIS (*In Vivo* Imaging System). The power of bioluminescence imaging is that it enables an assessment of luminescence markers in the same animal over time, such that the growth of bacteria can be discerned longitudinally throughout the course of infection. This approach has been utilized in several models of biofilm infection (16,17) and in unpublished studies in Dr. Kielian's laboratory (Figure 6), demonstrating the presence of bioluminescent bacteria within the murine brain abscess model. Well-defined abscesses were readily detected by IVIS in infected animals as early as 24 h following *S. aureus* exposure despite the depth of this infection within the brain parenchyma, establishing the feasibility of this imaging modality for use with intracranial infections. This technology will also allow us to confirm optimal catheter placement in the lateral ventricle as well as assess bacterial dissemination from the CNS biofilm to systemic tissues. Images will be taken at days 1, 3, 5, 7, 10, 14 and 21 following catheter implantation, to correlate with the quantitative bacterial cultures performed in the experiments outlined above. **Importantly, the IVIS approach provides unique information not attainable with traditional colony counts since the same cohort of infected animals can be imaged throughout the course of the experiment, whereas the latter requires terminal end point analysis (i.e. euthanasia), obviously precluding animals from further study.** Mice will be implanted with catheters coated with the *lux*-expressing *S. aureus* strain through the lateral ventricle, as described in the General Methods. This strain will be engineered by Dr. Mark Smeltzer at the University of Arkansas for Medical Sciences (please refer to letter of support), using the *S. aureus* clinical isolate utilized in my preliminary studies. Mice receiving infected catheters, as well as sterile catheter controls, will be anesthetized with isoflurane within the IVIS imaging chamber and placed prone for imaging of the entire animal, as well as cross-section images of the brain. When scanning, a black and white image of each mouse will be taken for overlays with luminescent images to orient the position of infection to the mouse. Mice will be imaged for 3-5 minutes with the CCD camera set at the highest sensitivity. For signal quantification, photons emitted from the catheter site will be quantified using LivingImage software (Caliper Life Sciences), where photons will be obtained from a region of interest that is maintained constant in area and positioning between all experiments. Importantly, the same cohort of animals will be monitored at each time point, effectively reducing the numbers of mice required for these studies. If biofilm growth is established within the first week after implantation, as is suggested by the preliminary data described in Figure 2, we would expect to observe an initial period (days 1-4) of diffuse luminescent signaling, followed by localization of the signal to the area immediately surrounding the catheter in the later days of infection (days 5-21). During the early phase of infection (days 1-4), we may also observe areas of luminescent signal at disseminated sites (liver, spleen), suggesting bacterial colonization beyond the CNS. This level of dissemination should be minimal as the biofilm matures during the end of the first week of infection. Alternatively, we may observe evidence of dissemination at later time points (days 14-21) as the biofilm begins to disperse after a period of protected growth. The growth kinetics observed in these studies will be reinforced by the quantitative bacterial cultures performed as described above. Collectively, the use of *lux*-expressing *S. aureus* and IVIS imaging provides an innovative and powerful approach to assess temporal changes in bacterial growth since the same cohort of animals can be imaged over time.

C.1.1. Use of transcriptional profiling to characterize changes in *S. aureus* gene expression profiles during biofilm growth and maturation in the CNS.

In addition to defining bacterial growth kinetics following implantation of infected CNS catheters, changes in gene expression profiles in *S. aureus* biofilms will also be examined, as changes may likely parallel alterations seen in the immune response. For these studies, gene expression profiles of biofilm-associated bacteria will be determined using commercially available Affymetrix GeneChips® by Dr. Paul Dunman at the University of Nebraska Medical Center (please refer to letter of support). The *S. aureus* GeneChip® to be used represents >3,300 *S. aureus* open reading frames and >4,800 intergenic regions from *S. aureus* strains N315, Mu50, NCTC 8325, and COL. Bacteria will be analyzed at days 3 and 14 following infection to identify the changes in

gene expression that occur at the biofilm matures within the CNS. These time points were chosen to represent primarily planktonic growth (day 3) versus predominantly biofilm formation (day 14). Additional time points can be evaluated if results warrant. GeneChip® signal intensity values for each treatment group (n≥3) will be normalized to the median signal intensity values for each GeneChip® and averaged using GeneSpring 7.2 software. Transcripts demonstrating 1) at least two-fold change in expression; 2) greater than background signal intensity value and determine to be "Present" by Affymetrix algorithms; and 3) significant by Student's *t*-test (ANOVA; *p* value=0.05) will be considered to be differentially expressed. We expect that biofilm maturation within the CNS (day 14) will lead to a distinct profile of differentially expressed genes compared to the early period of bacterial growth (day 3). Although it is difficult to predict the types of genes that will be differentially regulated, some candidates would include genes involved in immune deviation and/or bacterial adherence. Future avenues of investigation may involve evaluation of the genetic profiles of infiltrating innate immune cells associated with infected catheters, as these cells may demonstrate decreased expression of pro-inflammatory genes and other modifications in response to a biofilm infection. **Knowledge of bacterial genes that are expressed during catheter-associated CNS infection may identify novel targets for therapeutic agents or screening modalities for patients to offer tailored prophylaxis at the time of CSF shunt implantation, decreasing the incidence of these infections overall.**

C2. Define the influx and activation status of innate immune cells associated with CNS catheter infection throughout the various stages of biofilm development.

Understanding the dynamics between host immunity and *S. aureus* biofilms is an important issue that warrants further investigation as it may lead to immune therapies to supplement the antimicrobial regimens currently used to treat CSF shunt infections, thereby decreasing patient morbidity and the number of CNS catheters that must be removed. **Although other groups have investigated the interactions between *in vitro* biofilms and individual components of the immune system (7,8), no studies have yet examined these interactions *in vivo,* particularly within the CNS.** An *in vivo* model of these CNS immune interactions is imperative given the many factors involved in the complex interplay between the host and the biofilm. To evaluate the nature of inflammatory infiltrates during CNS biofilm infection, catheter-associated cells will be recovered from the brain parenchyma, subjected to collagenase/DNAse digestion to facilitate immune cell retrieval, and subjected to centrifugation on a discontinuous Percoll gradient as previously described to remove cell debris and RBCs (1,11). In our initial studies, samples will be obtained at days 1, 3, 5, 7, 10, 14 and 21 following catheter insertion to characterize the kinetics of innate immune cell influx and their activation status. Upon isolation, catheter-associated cells will be stained with fluorochromes specific for neutrophil (Ly-6G^{+}, CD11b^{+}, CD45^{high}), macrophage (Ly-6G^{-}, CD11b^{+}, CD45^{high}), microglia (Ly-6G^{-}, CD11b^{+}, CD45$^{low\text{-}intermediate}$) and dendritic cell markers (Ly-6G^{-}, CD11c^{+}, CD45^{high}) (1,19). Cells will be analyzed using a BD FACSAria with compensation set based on the staining of single fluorochromes alone and correction for autofluorescence using unstained cells. Controls will include cells stained with directly-conjugated isotype control antibodies to assess the degree of non-specific staining. This approach will allow us to quantitatively assess the degree of cellular influx into infection sites and simultaneously recover cell populations for downstream cytokine analysis, giving us *ex vivo* information about their activation status when present in the neuroinflammatory milieu *in vivo*. To do this, cells will be sorted into distinct populations as outlined above by FACS and immediately cultured in 96-well plates without any additional stimulation. After 24 h, cell-conditioned supernatants will be collected to assess secretion profiles using the Luminex multi-analyte bead arrays as described in the General Methods. It is important to note that we will not re-stimulate the various cell types with *S. aureus* during the subsequent 24 h *in vitro* culture period in an attempt to more accurately capture their activation state *in situ.*

In addition to defining the kinetics of innate immune cell infiltration into CNS biofilm infections as described above, the presence and cytokine expression profiles of T-lymphocytes can also be assessed in this model by intracellular cytokine staining, a technique with which I have first-hand experience (1). An *in vitro* study performed with human leukocytes and *S. aureus* biofilms reported that in early biofilm formation, a Th1-type response occurs (8), with IFN-γ present at day 2 of infection, but decreasing by day 7. This suggests that the typical anti-bacterial, pro-inflammatory Th1-T lymphocyte response seen with planktonic infection is shifted toward an anti-inflammatory profile, favoring bacterial persistence, as the biofilm matures. This is supported by my preliminary data presented in Figures 2 and 4, demonstrating reductions in parenchymal bacteria and chemokine levels as the biofilm is established. To further explore the role of infiltrating T-cells in this immune response, catheter-associated T-lymphocytes will be collected and evaluated by intracellular cytokine staining. The tissue surrounding the catheter will be collected and processed using a Percoll gradient method, as

described above (1,11), whereupon T-lymphocytes will be enriched using CD90-conjugated magnetic beads (Miltenyi Biotec). These cells are stimulated overnight with a mixture of PMA plus ionomycin (leukocyte activation mixture) in the presence of GolgiPlug for 12 h, then incubated with Fc block to minimize non-specific antibody binding (1). The cells are then stained with directly conjugated antibodies against CD4, CD8 or γδ TCR. They are next permeabilized with a CytoFix/CytoPerm kit and stained for intracellular IFN-γ or IL-13, to reflect Th1 versus Th2 activation status, respectively (1). It is expected that at early time points following infection, when there is still a relatively high degree of parenchymal involvement, a Th1 phenotype will predominate. Alternatively, the predominant T-lymphocyte population in early infection may be a Th17 phenotype, which is also known to play a significant role in pro-inflammatory immune responses. To investigate this possibility, intracellular cytokine staining may also be performed utilizing IL-17 antibody, as opposed to IFN-γ, using the same methods as described above. At later time points, when the biofilm is established and parenchymal involvement diminishes, I would expect to see a shift to an anti-inflammatory, Th2 phenotype as the biofilm attenuates the host immune response.

Flow cytometry will provide a quantitative assessment of immune cells in catheter-associated tissues, but does not allow visualization of the location of these cells within the CNS parenchyma. Our preliminary data shows evidence of early cellular infiltrates into the tissues surrounding the catheter (Figure 3); however, H&E staining cannot definitively identify the cell types present. Using immunohistochemistry, we may better define the immune cell types surrounding the infected catheter, as well as their ability to interact with the bacteria within the parenchyma versus the biofilm. In these studies, frozen brain tissues will be stained to demonstrate the location of immune cells in the area surrounding the catheter. Brain tissues from mice with infected and uninfected catheters will be removed at days 1, 3, 5, 7, 10, 14 and 21 and immediately flash frozen on dry ice. Serially interrupted (50 μm) 10 μm cryostat sections of brain tissues will be prepared and incubated with a panel of antibodies including: Ly-6G (neutrophils), CD11c (dendritic cells), GFAP (astrocytes) and Iba-1 (macrophage/microglia). Upon completion of the staining protocol, slides will be cover-slipped using the Prolong anti-fade reagent and imaged using a Zeiss laser scanning confocal microscope. This will allow visualization of innate immune cells in relation to the catheter over time. It is expected that flow cytometry and immunohistochemistry will demonstrate increased infiltration of neutrophils and macrophages during the early stages of infection, coinciding with the period of maximal planktonic growth, and subsiding at later time points. During early intervals post-infection, at which maximal planktonic growth is observed, I would expect infiltrating immune cells to be diffusely distributed in the parenchymal tissues surrounding the catheter. However, as the biofilm ages, the immune cell infiltrate is expected to subside in the tissues immediately surrounding the catheter and become more localized along the biofilm proper. The role of microglia and astrocytes has not been previously described in this setting, but these glia are likely activated in response to the catheter-associated infection given their behavior in parenchymal CNS infections. An alternative approach may include the use of fluorescently-labeled *S. aureus,* which would allow a better assessment of the intimate associations between the bacteria and various immune cell populations (2).

The inflammatory mediator profiles observed in response to infection provide important information about the types and activation status of immune cells in the area surrounding the catheter. A shift in cytokine and chemokine levels from a pro-inflammatory (IL-1β, IL-6, IL-12, TNF-α, IFN-γ, KC, MCP-1) to an anti-inflammatory profile (lower levels of pro-inflammatory mediators or elevated IL-13 and/or IL-10 expression) can provide valuable evidence to support our hypothesis that the immune response is attenuated as the biofilm matures (20,21). In the initial days following catheter infection, there is a significant amount of parenchymal involvement, as observed in my preliminary data (Figure 2). Given this, elevation of pro-inflammatory mediators such as IL-1β, TNF-α, IFN-γ, KC and MCP-1 are expected, as is seen in the brain abscess model (12, 22). This is supported by both *in vitro* data that demonstrates elevated IL-1β, IL-12 and IFN-γ at day 2 following biofilm infection (8), as well as my preliminary data presented in the CNS catheter infection model (Figure 4), demonstrating elevations in KC and MCP-1 early in infection. However, as the biofilm is established and the amount of parenchymal involvement dissipates, I would expect that the robust pro-inflammatory mediator response seen during the early stage of infection will diminish, with the potential elevation of anti-inflammatory cytokines such as IL-4, IL-10 and IL-13 (20,21). This is supported by both *in vitro* data that demonstrated a decrease in IL-1β, IL-12 and IFN-γ by day 7 of biofilm formation (8), as well as by my preliminary chemokine data shown in Figure 4, which revealed a reduction in KC and MCP-1 expression after day 3 of infection.

In these studies, the brain tissue surrounding the catheter will be collected and homogenized as outlined above for bacterial titering and inflammatory mediator measurements. After homogenization, samples are centrifuged and mediators measured in the resultant supernatant using the Biosource multi-analyte microbead array. The Biosource multi-analyte microbead array allows for the simultaneous detection of 20 individual inflammatory molecules in a single 75 μl brain homogenate sample and has been used successfully to analyze inflammatory mediators from brain tissues in prior studies the Kielian lab (2,11). This technology offers several advantages, including the large amount of data that can be obtained from a single tissue specimen, which is not possible with traditional ELISAs, and the array of mediators tested. This array includes molecules typical of pro- and anti-inflammatory pathways, providing a more comprehensive survey on the nature of the host response to biofilm infection. In addition, commercially available ELISA kits will be used to quantitate the neutrophil chemoattractant MIP-2, which is not included in the multi-analyte array, and TNF-α, which may be produced at levels below the detection limit of the multi-analyte bead array. These samples will be analyzed at days 1, 3, 5, 7, 10, 14 and 21 to track the changes in immune mediator profiles occurring throughout the course of infection. Importantly, control animals will receive sterile catheters to assess the CNS foreign body response, which is anticipated to be minimal based on my preliminary studies (Figure 4). Inflammatory mediator expression will be normalized to the amount of total protein collected obtained to correct for differences between tissue sampling size. Based on my preliminary studies, it is expected that chemokines such as MIP-2, MCP-1 and KC will be elevated early during infection, when there is a greater degree of planktonic bacterial growth. However, as the biofilm matures, these inflammatory chemokines will likely decrease, which is anticipated to occur after the first week of infection. This profile should correlate with the reduction in neutrophils and macrophages seen with the FACS and immunohistochemistry studies outlined above. The presence of T-cell derived anti-inflammatory cytokines, such as IL-4, IL-10 or IL-13, later in the course of infection, will suggest the continued presence of T-cells infiltrating the tissues surrounding the catheters as the biofilm matures. This data will be confirmed with the intracellular cytokine staining assays outlined above. In the sterile catheter implantations, no elevation in chemokines is expected. This approach will also allow further evaluation of the role of other inflammatory cytokines, including IL-1β and IL-17 which were elevated in pilot studies, and factors that may play a role in the response the foreign material within the CNS, such as FGF. This cytokine data will provide valuable information about the activation status of the immune cells infiltrating the CNS, which can be correlated with the cellular influx noted on flow cytometry and immunohistochemistry.

D. Potential Pitfalls and Alternative Approaches. The studies outlined above represent a best first approach to the investigation of these aims. Prior experience with similar methodologies in a mouse brain abscess model suggests that these approaches will be technically feasible (1,2,11,12,22). Regarding the proposed IVIS studies, the utility of bioluminescent imaging within the CNS has shown promise in preliminary studies performed in Dr. Kielian's laboratory, as noted above. The sensitivity of this imaging modality may be limited within the CNS, but given its success in the brain abscess model and the high bacterial burden achieved on infected catheters as demonstrated by my preliminary studies, I anticipate that this will not be a significant issue and that bioluminescent imaging will prove to be a very useful tool in the investigation of CNS catheter infections. Another caveat is that the bacteria may down-regulate genes driving luciferase expression during biofilm growth, depending on the promoter used to drive *lux* expression. Dr. Smeltzer, who will be creating this *lux*-expressing *S. aureus* strain, will work closely with us to minimize this occurrence. In the proposed FACS studies, the yields of catheter-associated cells recovered from infected and sterile animals can be limiting. However, in prior studies in the brain abscess model, adequate numbers of cells could be obtained when tissues from at least 5 animals were pooled, hence the rationale for the group sizes in these experiments (1,11).

Specific Aim 2: To define the role of bacterial regulatory factors in the development of CNS catheter infection.

A. Rationale. In these studies, I will describe the impact of mutation of the staphylococcal accessory regulatory gene (*sarA*) on the bacterial growth kinetics and immune response during CNS catheter infection. The *sarA* locus encodes a DNA-binding protein (SarA) that has a global impact on gene expression in *S. aureus* (23,24,25). Studies in the laboratory of our collaborator, Dr. Mark Smeltzer, have demonstrated that mutation of the *sarA* locus limits, but does not abolish, the ability of most *S. aureus* strains to form a biofilm and results in greater susceptibility to anti-staphylococcal antibiotic treatment (23,24,25). With decreased biofilm formation, there is likely a greater degree of planktonic growth in the infected tissues, which would provide both

antibiotics and the immune response more physical access to bacteria compared to a fully formed biofilm. These studies will define the role of *sarA* in CNS catheter infections and determine if inhibitors of *sarA* expression may be viable therapeutic agents in the treatment of CSF shunt infections. In addition, by limiting biofilm formation on the catheter, these experiments will define the impact of the biofilm itself on the immune response when compared with the findings obtained with the biofilm-competent strains utilized in Specific Aim 1. Understanding the role of *sarA* in CNS catheter-associated infections may have a significant impact on the treatment of CSF shunt infections.

B. Hypothesis. These studies will test the ***hypothesis*** that the *sarA* regulatory locus engenders *S. aureus* more resistant to the CNS immune response based on its ability to regulate robust biofilm formation. This mechanism may represent one way in which the biofilm actively attenuates the host innate immune response to favor bacterial persistence. The objectives of this specific aim are: (1) to define the bacterial growth and gene expression kinetics associated during CNS catheter infection with a *S. aureus sarA* mutant and (2) to define the influx and activation status of innate immune cells associated with a *S. aureus sarA* CNS catheter infection throughout the various stages of biofilm development.

C. Experimental Design and Methods

C.1 Define the impact of *sarA* on biofilm formation within the CNS.

A mutation in the *S. aureus sarA* locus has been shown to cause reduced biofilm capacity (23,24). Therefore, a *sarA* mutant would be expected to exhibit greater planktonic spread and decreased biofilm formation in the CNS catheter infection model. To investigate this possibility, my collaborator Dr. Mark Smeltzer will engineer the MSSA strain used in the experiments outlined in Specific Aim 1 to eliminate *sarA* expression (please see letter of support). This *sarA* mutant will be used in conjunction with the isogenic wild type parental strain to generate catheter-associated CNS infections as described in Specific Aim 1 and the General Methods (Figure 8). Catheters and the surrounding tissue will be recovered at days 1, 3, 5, 7, 10, 14 and 21 following implantation to enumerate bacterial burdens and evaluate the impact of sarA on bacterial growth and persistence. Given the decreased ability of *sarA*-deficient *S. aureus* to form biofilms (23,24,25), it is expected that there will be less catheter-associated bacterial growth in those animals infected with the *sarA* mutant, as opposed to the isogenic parental strain, which is known to form a robust catheter-associated infection based on my preliminary results (Figure 2). In addition, a greater amount of bacterial growth may be seen in the tissues surrounding the catheters infected with the *sarA* mutant, as opposed to the isogenic parental strain, reflecting a higher degree of planktonic spread in the absence of biofilm formation in the former. Alternatively, given the global role of *sarA* in staphylococcal gene regulation, the *sarA* mutant may be less fit and have decreased growth along both the catheter and within the tissue (23,24,25). To determine the role of sarA in bacterial dissemination from the CNS, cultures will also be obtained from systemic sites including the peripheral blood stream, spleen, liver, spinal cord and heart. It is expected that a sterile catheter and its associated brain parenchyma will remain negative for bacterial growth throughout the course of this experiment. Enhanced planktonic growth and systemic dissemination from infected catheters will likely occur throughout the course of infection to a greater degree in the *sarA* mutant compared to the isogenic strain, with potential abscess formation in the former at

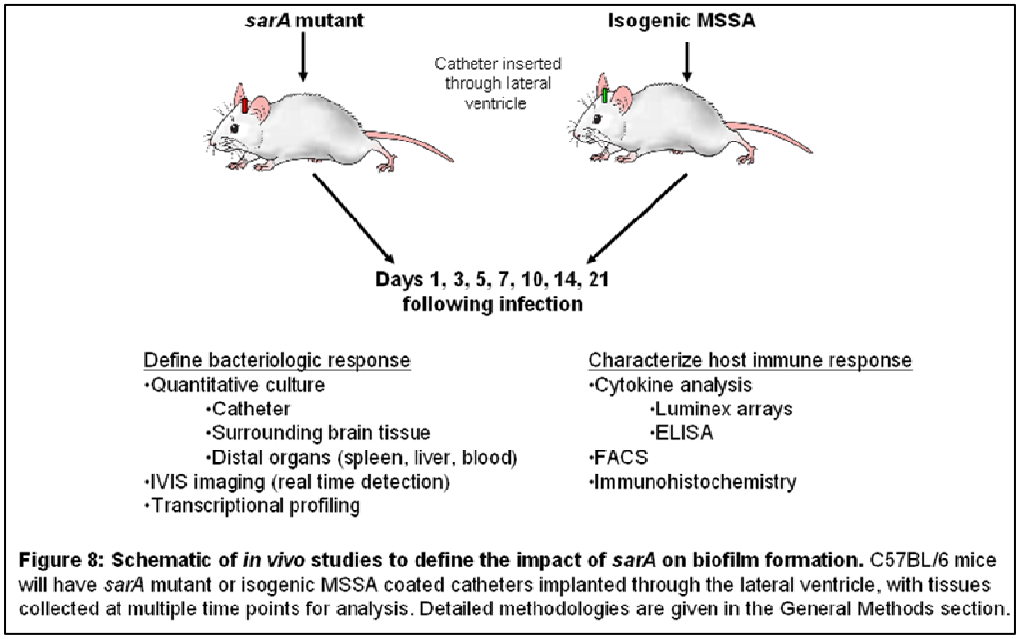

Figure 8: Schematic of *in vivo* studies to define the impact of *sarA* on biofilm formation. C57BL/6 mice will have *sarA* mutant or isogenic MSSA coated catheters implanted through the lateral ventricle, with tissues collected at multiple time points for analysis. Detailed methodologies are given in the General Methods section.

later time points. As time and my experimental results dictate, transcriptional profiling (as described in Specific Aim 1) may also be performed using this *S. aureus sarA* mutant to identify changes in gene expression over time within the CNS using a biofilm-impaired bacterial strain.

To visualize the growth kinetics of the *sarA* mutant along the catheter and its potential to disseminate systemically, a luciferase-expressing variant of the *sarA*-deficient *S. aureus* strain used in the above studies will be used in conjunction with IVIS (*In Vivo* Imaging System). Bioluminescent imaging will enable us to longitudinally track bacterial growth throughout the course of infection in a single animal (16,17). This approach, and the benefits for using this technology, has been described extensively in the Background section as well as in Specific Aim 1. IVIS images will be taken at days 1, 3, 5, 7, 10, 14 and 21 following implantation of *sarA* mutant or isogenic infected catheters. Importantly, the same cohort of animals will be monitored throughout the course of IVIS experiments, effectively reducing the numbers of mice required for these studies. Based upon its impairment in biofilm formation, a greater degree of planktonic spread is anticipated with the *sarA* mutant throughout the course of infection, as opposed to the localization of luminescent signal that is expected around the catheter in the isogenic parental strain by the end of the first week of infection (23,24,25). Additionally, we may also observe areas of luminescent signal in systemic sites (i.e. liver, spleen) in animals infected with the *sarA* mutant, suggesting dissemination of the infection beyond the CNS in the absence of biofilm formation. The growth kinetics observed in these IVIS studies will be reinforced by the quantitative bacterial cultures performed as described above and will support the hypothesis that the activity of *sarA* engenders *S. aureus* more resistant to the CNS immune response based on its ability to regulate robust biofilm formation.

C2. Define the impact of sarA-regulated factors on the host innate immune response during CNS biofilm infection.

Understanding the role of *sarA* expression in the interactions between the host immune response and *S. aureus* biofilms may lead to future treatment strategies integrating *sarA* inhibition with immune adjuvant therapies. The *in vivo* influx of innate immune cells into tissues surrounding a catheter infected with biofilm-impaired *S. aureus* has not yet been described. Given the fact that *sarA*-deficient *S. aureus* strains exhibit impaired biofilm formation (23,24), it is expected that the intensity and duration of inflammatory infiltrates will be significantly enhanced following CNS infection with this mutant compared to the isogenic biofilm-competent strain. In addition, decreased expression of *sarA* has been associated with a reduction in poly-N-acetyl-glucosamine (PNAG) (7,24,26). PNAG plays an important role in protecting planktonic *S. aureus* from phagocytes, as well as promoting biofilm maturation (7,24,26). A decrease in PNAG, as a result of *sarA* mutation, could result in increased anti-microbial activity by the host immune system. Therefore, both the increased planktonic spread and the decreased PNAG interference suggest that the influx of host immune cells will be more robust in response to a *sarA* mutant infection. To evaluate inflammatory infiltrates in the tissues surrounding the catheter, catheter-associated cells will be recovered and enumerated by FACS as described in Specific Aim 1 and the General Methods. This approach will allow us to quantitatively assess the degree of cellular influx into infection sites and simultaneously recover cell populations for downstream cytokine analysis, giving us *ex vivo* information about their activation status when present in the neuroinflammatory milieu *in vivo*.

Flow cytometry will provide a quantitative assessment of immune cells in catheter-associated tissues, but does not allow visualization of the location of these cells within the tissue. Using immunohistochemistry, as described in Specific Aim 1 and the General Methods, frozen brain tissues can be stained to demonstrate the location of neutrophils, macrophages/microglia, dendritic cells and astrocytes in the areas surrounding the catheter. This will allow visualization of CNS resident as well as infiltrating innate immune cells in relation to the catheter over time. It is expected that innate immune cell accumulation will be augmented following infection with the *S. aureus sarA* mutant compared to the biofilm-competent isogenic parental strain. In addition, given the reduced PNAG interference in the absence of *sarA* expression (7,24,26), the immune cells should be capable of forming more intimate associations with tissues immediately adjacent to the catheter.

Additional information about the types and activation status of immune cells recovered from CNS biofilm infections by immunohistochemical staining and FACS, respectively, can be obtained by measuring the inflammatory mediators produced in response to infection. As described above, several of the bacterial mechanisms thought to protect *S. aureus* from the immune system, such as robust biofilm infection and PNAG production, are decreased in the absence of *sarA* expression (7,24,26). Inflammatory mediators will be

quantitated at days 1, 3, 5, 7, 10, 14 and 21 post-infection using the multi-analyte bead array as described in Specific Aim 1 and the General Methods. Throughout the course of infection, the inflammatory response to the *S. aureus sarA* mutant is expected to resemble a pro-inflammatory profile typical of parenchymal infection in the CNS, with elevations in mediators such as IL-1β, IL-6, IL-12, TNF-α, IFN-γ, KC and MCP-1. This profile should correlate with an increase in neutrophil and macrophage infiltration seen with the FACS and immunohistochemistry studies outlined above, since the chemokines KC and MCP-1 specifically target these innate immune cell populations, respectively. This is in contrast to the isogenic parental strain, which is anticipated to shift to an anti-inflammatory profile (lower levels of pro-inflammatory mediators or elevated IL-13 and/or IL-10 expression) as the biofilm matures (20,21). This inflammatory mediator data will provide valuable information about the altered activation status of the immune cells infiltrating the CNS in the absence of the regulation provided by *sarA.* Collectively, the data collected in the flow cytometry, immunohistochemistry and inflammatory mediator experiments outlined above will reinforce the importance of *sarA* in rendering *S. aureus* more resistant to the CNS immune response. This information may reveal novel targets for future treatment of CSF shunt infections, as the role of *sarA* and the innate immune system is further elucidated.

D. Potential Pitfalls and Alternative Approaches. The studies outlined above represent a best first approach to investigate the impact of *S. aureus sarA* on the CNS anti-bacterial immune response during biofilm growth. One potential pitfall in this Aim involves the current method of pre-coating the catheter with bacteria prior to implantation. Using a strain deficient in biofilm formation (i.e. *sarA* mutant), there may be diminished attachment of the bacteria to the catheter, resulting in ineffective infections. Should this become an issue, the surgical approach can be altered such that the catheter is implanted first, followed by the injection of bacterial suspensions into the catheter lumen. Given the greater degree of planktonic spread expected with the *sarA* mutant, the infectious dose may also need to be reduced to allow adequate survival of the mice for the planned time points in these experiments. In addition, since *sarA* is a regulatory gene with broad effects on *S. aureus* gene expression, many of which have yet to be defined, the consequences of *sarA* mutation on bacterial growth in the CNS cannot be fully anticipated. However, the expertise of Dr. Smeltzer, who has a long-standing interest in *sarA* regulation, will be invaluable as we modify the experimental design as needed to address these variables and answer these very important questions about the role of *sarA* in the host response to biofilms within the CNS.

Statistical Plan

My preliminary studies in the CNS biofilm infection model have found that 5 animals/time point/group and three independent experiments are required to obtain statistically significant findings due to the inherent variability between animals in *in vivo* studies. This group size was also independently confirmed based on a sample size calculation. Specifically, based on the fact that the majority of our *in vivo* studies will involve comparisons between two (2) treatment groups, we also independently arrived at a sample size of 5 mice/group by performing a one-way ANOVA sample size test using the Sigma Stat program. Estimating a minimal detectable difference in means of 100 (i.e. 100 ng/ml for proinflammatory mediator expression), an expected standard deviation of 50 (due to the inherent degree of variation in *in vivo* studies), 2 experimental groups, a desired power of 0.80, and an alpha value of 0.05, resulted in a sample size of 5 animals per experimental group. In addition, we anticipate an approximate mortality rate of 15% in this model system from complications arising from infection; therefore, our total number of animals requested has been adjusted by 15%.

Time Line

We are requesting a total funding period of five years to complete the proposed work which will be required due to the large number of *in vivo* experiments. It is anticipated that the experiments outlined in Specific Aim 1, utilizing a clinical strain of *S. aureus* to evaluate the active attenuation of the innate immune response in a CNS catheter infection, will be completed in **years 1 and 2**. The studies outlined in Specific Aim 2, defining the role of *sarA* in regulating the biofilm's response to the immune system, will be completed in **years 3 through 5**. In addition, in **years 4 and 5**, preparation of a proposal for independent R01 funding will begin. We are confident that the experiments proposed in this study can be successfully completed in the five-year funding period requested.

Future Studies

The possibilities for future studies utilizing this animal model are virtually endless, given its novelty and the scope of the clinical problem it is designed to explore, namely CSF shunt infections that occur frequently in the pediatric population. The roles of the adaptive immune system and how the neuronal parenchyma responds to CNS catheter infection have yet to be explored, in addition to the role of Toll-like receptor 2 in a biofilm infection, which is known to play an important role in response to parenchymal brain abscesses (1,27). Toll-like receptor 9, which recognizes bacterial DNA (13), may also play a role in this setting and will be investigated in future studies. This model can also be adapted to explore the other primary pathogen in CNS catheter infections, namely *Staphylococcus epidermidis*. I will also be able investigate the impact of systemic antibiotic therapy with various agents in this model in future studies. Alterations in immune markers, bacterial kinetics and pathology in response to novel antibiotic therapies have not been previously described but can be explored using this model.

Summary

To our knowledge, the experiments described here represent the first attempt to systematically define both the contributions from the bacterial and host perspective in a murine model of CNS catheter infection. This work represents an important initial step in improving the management of CSF shunt infections in children. These studies will provide valuable information about the immune response to this biofilm infection within the CNS, potentially leading to novel diagnostic and therapeutic tools for use in management of these infections. New treatment and diagnostic modalities are imperative given the high costs, in healthcare expense and patient morbidity, associated with CSF shunt infections.

General Methods

Mouse strain. Adult C57BL/6 mice (8-12 weeks of age) will be utilized for these studies since our long-term goal is to eventually utilize various genetically engineered knockout or transgenic mouse strains for candidate immune molecules that are bred on a C57BL/6 background. This age range facilitates the ease of catheter placement within the ventricle due to the larger size of the brain compared to younger animals. These animals will be purchased from Harlan Laboratories.

Generation of CNS catheter infection. This method will be used to generate the catheter-associated infection in all of the experiments in this proposal. I will pre-coat silicone catheters with $2X10^4$ cfu/ml *S.aureus* for implantation into the CNS. This dose is calculated based upon the work to date in this model, described above, and my experience in experimental brain abscess models (1,2). The catheters will be placed while the mouse is under general avertin anesthesia, using a rodent stereotaxic apparatus for most accurate positioning. The coordinates for catheter placement into the mouse lateral ventricle were obtained from *The Mouse Brain in Stereotaxic Coordinates* by Paxinos and Franklin and include relative to bregma: + 0.02 mm rostral, + 1.0 mm lateral, and – 2.0 mm deep from the surface of the brain. Following catheter placement, the burr hole will be sealed with bone wax and the skin incision will be closed using glue, which is less irritating to animals compared to skin staples. Mice will be monitored three times daily (a.m., noon, and p.m.) during the first week following catheter implantation for signs of adverse effects from the biofilm infection.

Quantitation of viable bacteria from CNS parenchyma and catheters. Catheters and surrounding brain tissue will be collected from experimental animals at days 1, 3, 5, 7, 10, 14 and 21 following catheter implantation. The homogenization of brain tissues for bacterial titering and inflammatory mediator measurement is well-established in Dr. Kielian's laboratory and provides the opportunity to gather samples for culture, protein and RNA analysis from a single animal (27). The catheters will be sonicated in sterile PBS to remove bacteria adherent to the catheter, titered and inoculated onto blood agar plates. This method has been successfully utilized by Dr. Smeltzer's laboratory to characterize bacterial burdens in a subcutaneous catheter biofilm model and has been readily adapted to our CNS model (23).

In Vivo Imaging System (IVIS). For studies designed to monitor the degree of bacterial growth, mice will have catheters coated with *lux*-expressing *S. aureus* implanted through the lateral ventricle, as described in the "Generation of CNS catheter associated infection" above. Mice will be imaged in an IVIS Spectrum unit (Caliper Life Sciences) while maintained under isoflurane inhalation anesthesia. The imaging chamber is temperature controlled, to maintain the mouse's core body temperature while under anesthesia. Mice will be

placed prone for imaging of the entire animal, as well as cross-section images of the brain. When scanning, a black and white image of each mouse will be made for overlays with luminescent images to orient the position of infection to the mouse. Mice will be imaged for 3-5 minutes with the CCD camera set at the highest sensitivity. For signal quantification, photons emitted from the catheter site will be quantified using LivingImage software (Caliper Life Sciences), where photons will be obtained from a region of interest that is maintained constant in area and positioning between all experiments. For normalization of bioluminescent signals, baseline imaging will be performed using non-infected mice without catheters. Luciferase activity will be expressed as the photons emitted/sec.

***S. aureus* Affymetrix gene arrays.** Commercially available GeneChips® will be used representing >3,300 *S.aureus* open reading frames and >4,800 intergenic regions from *S. aureus* strains N315, Mu50, NCTC 8325, and COL. For defining differences in transcriptional profiles of innate immune cells exposed to biofilms, a GeneChip® Mouse Genome 430A 2.0 array will be utilized. GeneChip® signal intensity values for each treatment group (n≥3) will be normalized to the median signal intensity values for each GeneChip® and averaged using GeneSpring 7.2 software. Transcripts demonstrating 1) at least two-fold change in expression; 2) greater than background signal intensity value and determine to be "Present" by Affymetrix algorithms; and 3) significant by Student's *t*-test (ANOVA; *p* value=0.05) will be considered to be differentially expressed.

Quantitation of microglia, macrophages, neutrophils, and dendritic cells in CNS tissues. To evaluate the nature of inflammatory infiltrates during CNS biofilm infection, catheter-associated cells will be recovered from the brain parenchyma, subjected to collagenase/DNAse digestion to facilitate immune cell retrieval, and subjected to centrifugation on a discontinuous Percoll gradient as previously described to remove cell debris and RBCs (1,11). Upon isolation, cells will be stained with fluorochromes specific for neutrophil (Ly-6G^{+}, CD11b^{+}, CD45^{high}), macrophage (Ly-6G^{-}, CD11b^{+}, CD45^{high}), microglia (Ly-6G^{-}, CD11b^{+}, CD45$^{low\text{-}intermediate}$) and dendritic cell markers (Ly-6G^{-}, CD11c^{+}, CD45^{high}) (1,19). Cells are analyzed using a BD FACSAria with compensation set based on the staining of single fluorochromes alone and correction for autofluorescence using unstained cells. Controls include cells stained with directly-conjugated isotype control antibodies to assess the degree of non-specific staining. These cells will also be sorted into distinct populations as outlined above by FACS and immediately cultured in 96-well plates without any additional stimulation. After 24 hours, these supernatants will be collected to assess cell secretion profiles using the Luminex multi-analyte bead arrays as described in the below.

Intracellular cytokine staining. To further explore the role of infiltrating T-cells in this immune response, catheter-associated T-lymphocytes will be collected and evaluated by intracellular cytokine staining as previously described[Th17]. The tissue surrounding the catheter will be collected and processed using a Percoll gradient method, as described above (1,11). The T-lymphocytes will then be separated by incubated with CD90-conjugated magnetic beads and magnetic cells separation columns (Miltenyi Biotec). These cells are stimulated overnight with a mixture of PMA plus ionomycin for leukocyte activation and stained in the morning with directly conjugated antibodies against CD4+, CD8+ or $\gamma\delta$ TCR. They are next permeabilized with a CytoFix/CytoPerm kit and stained for intracellular IFN-γ or IL-13, to reflect Th1 versus Th2 activation status (1). Cells may also be stained with IL-17, instead of IFN-γ, to reflect Th17 activation status (1).

Immunohistochemical staining and confocal microscopy. Brain tissues from mice in each experimental group will be removed at days 1, 3, 5, 7, 10, 14 and 21 and immediately flash frozen on dry ice. Serially interrupted (50 μm) 10 μm cryostat sections of brain tissues will be prepared, mounted onto SuperFrost glass slides and air dried for 30 min at RT prior to storage at -80° C. To initiate staining, tissues will be equilibrated to RT for 10 min and fixed in ice-cold methanol. Catheter associated tissues will be incubated with the appropriate surface marker primary antibodies overnight at 4° C in a humidified chamber. Following numerous rinses in PBS, tissues will be incubated with the appropriate secondary Abs, when indicated, for 1 h at room temperature. The following antibodies will be used: neutrophils (Ly-6G), CD11c (dendritic cells), GFAP (astrocytes) and Iba-1 (microglia). Upon completion of the staining protocol, slides are cover-slipped using the Prolong anti-fade reagent and imaged using a Zeiss laser scanning confocal microscope.

Multi-analyte microbead arrays and ELISAs. Inflammatory mediators will be measured in the supernatants of homogenized catheter-associated CNS tissues as previously described (22,28,29). The brain tissue surrounding the catheter will be collected and homogenized as outlined above for quantitation of bacterial growth. After homongenization, samples are centrifuged and inflammatory mediators measured in the resultant supernatant. The Biosource multi-analyte microbead array allows for the simultaneous detection of 20 individual inflammatory molecules in a single 75 μl brain homogenate sample including IL-1α, IL-1β, TNF-α, IFN-γ, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12 p40/p70, IL-13, IL-17, IP-10, MIG, MCP-1, KC, GM-CSF, VEGF, and bFGF. Results will be analyzed using a Bio-Plex Workstation (Bio-Rad) and mediator levels adjusted

based on the amount of total protein extracted from CNS tissues for normalization as previously described by Dr. Kielian's laboratory (22,28,29). In addition, commericially available ELISA kits (R&D Systems) will be used to evaluate samples for the neutrophil chemoattractant MIP-2, which is not included in the multi-analyte array, and TNF-α, which is sometimes undetectable on these arrays.

VERTEBRATE ANIMALS

1. Justification: We consider *in vivo* studies to be essential to our research proposal. The complex interplay of immunologic factors that are involved in the immune response to a catheter-associated infection cannot be adequately reproduced in *in vitro* experiments. Since these studies are ultimately aimed at finding therapeutic alternatives to treat biofilm infection in humans, the use of mice is required since many of their genes and protein products share a high degree of homology with the human. In addition, there are numerous immunologic reagents and tools available to study immune mechanisms in mice; therefore, this model offers the most promising means of identifying critical mediators during infection. We have chosen to use 8-12 week old C57BL/6 mice for the studies described in this proposal. C57BL/6 mice are commercially available (Harlan Laboratories) and were used in the development of this model. In addition, this strain was selected because C57BL/6 mice serve as the genetic background for many of the immunologically altered mice that may be used in future studies (Toll-like receptor knockout mice, etc) in the CNS catheter infection model. After extensive review of the current scientific literature, there is no adequate computer, mathematical or *in vitro* model to address the questions raised in this proposal.

2. Number of mice needed: The number of mice to be used in these experiments was determined from (1) our extensive experience using the experimental brain abscess model in Dr. Kielian's laboratory as well as pilot studies in the CNS catheter infection model and (2) statistical analysis. We have found that 5 animals/time point/group and three independent experiments are required to obtain statistically significant findings due to the inherent variability between animals in *in vivo* studies. This group size was also independently confirmed based on a sample size calculation. Specifically, based on the fact that the majority of our *in vivo* studies will involve comparisons between two (2) treatment groups (infected versus uninfected catheters), we also independently arrived at a sample size of 5 mice/group by performing a one-way ANOVA sample size test using the Sigma Stat program. Estimating a minimal detectable difference in means of 100 (i.e. 100 ng/ml for proinflammatory mediator expression), an expected standard deviation of 50 (due to the inherent degree of variation in *in vivo* studies), 2 experimental groups, a desired power of 0.80, and an alpha value of 0.05, resulted in a sample size of 5 animals per experimental group. In addition, based on pilot studies, we anticipate an approximate mortality rate of 15% in this model system from complications arising from infection; therefore, our total number of animals requested has been adjusted by 15%.

3. Housing and veterinary care: Mice will be housed under standard conditions in a barrier facility and fed standard rodent chow and water ad libitum. Mice housed at the Durham Research Center at UNMC will be monitored regularly by the UNMC laboratory animal veterinarian. Any animal that shows continued weight loss of greater than 20% from its pre-experiment weight or exhibits any adverse signs to CNS catheter infection (i.e. absence of startle reflex, lateral recumbency with loss of righting reflex, or extreme lethargy) will be euthanized. If an animal develops a ruffled coat, hunched posture or becomes moribund, it will be removed from the study and euthanized immediately.

4. Procedural discomfort and pain relief:
In these experiments, two groups of animals will be compared: those implanted with catheters coated in serum alone (control) and those implanted with catheters coated with *S. aureus*. The procedure itself will cause momentary pain with the scalp incision and burr hole placement, controlled with avertin anesthesia. Immediately following the emergence of animals from surgery and anesthesia, they are active and eat/drink normally. The mice implanted with control catheters appear to have no pain after the procedure. In the animals implanted with infected catheters, 24-72 hours following the procedure, they may exhibit clinical signs of infection, including lethargy and ruffled fur, as the bacteria grow along the catheter causing inflammation in the surrounding tissues. However, these symptoms are transient, and the majority of animals (85%) make a full recovery within 3-4 days following infection and resume normal activity and eating/grooming habits with no observable side effects from the procedure. These symptoms are not typically observed in the animals implanted with sterile catheters.

5. Euthanasia: Mice will be euthanized using an overdose of inhaled isoflurane. Isoflurane will be added to the bottom of a large, glass dessicator jar and animals separated from the irritating anesthetic by a 1 cm-thick porcelain base. At the point where respiration and heartbeat are no longer detectable for a minimum of 5 minutes, mice will be processed as indicated by the experimental objective.

BIOHAZARDS

1. Name of biohazardous material: The *Staphylococcus aureus* (*S. aureus*) strain to be used in these experiments is considered a Risk Group Classification 2 (RG2), meaning that it can be used under BSL2 conditions. This is due to the fact that it is sensitive to multiple antimicrobials that could be used for treatment in the unlikely event that any laboratory personnel acquire infection. Dr. Nichols has submitted a request for BSL2 approval at UNMC for the work outlined in this proposal.

2. Safety practices summary: *S. aureus* is associated with human disease, namely percutaneous injury, ingestion, or mucous membrane exposure being routes of infection/colonization. Because of this, the laboratory will have minimum traffic of personnel not directly applicable to the projects and the outside doors of all laboratory spaces where *S. aureus* is being used will be clearly marked with Biohazard signs. All manipulations of concentrated *S. aureus* stocks (i.e. < 108 cfu/ml) will be handled inside a Class II type A2 biological safety cabinet. In addition, all plasticware and other consumable items (i.e. gloves) that come into direct contact with *S. aureus* will be disposed of in designated biohazard containers for removal through UNMC Environmental Services. All liquids containing or coming into contact with *S. aureus* will be treated with a 10% bleach solution for a minimum of 30 minutes to ensure complete pathogen neutralization. All laboratory personnel that work with *S. aureus* will be required to wear appropriate personal protective equipment (PPE) including full-length laboratory coats, eye goggles, and gloves (personnel will be required to change gloves frequently and avoid handling items used by the general public such as doorknobs, elevator buttons, etc.). In addition, masks will be available for research personnel to wear if they desire when working with *S. aureus*; however, due to the nature of this pathogen this will not be an absolute requirement. Finally, a standard biosafety manual will be housed in the laboratory outlining common practices and procedures for working with *S. aureus*.

3. Risk for occupational exposure: The risk of *S. aureus* infection of research personnel is minimal when conducting experiments using good laboratory practice. All laboratory personnel will be required to wear the appropriate PPE as outlined above to minimize the risk of exposure, which could occur primarily via ingestion or exposure to mucous membranes. All research personnel in the laboratory, regardless of whether their project involves direct work with *S. aureus*, will be required to participate in the UNMC Biosafety Training program to be aware of standard procedures when working in a laboratory that utilizes infectious agents. In addition to this formal training course, Dr. Nichols will personally train and educate any new laboratory members that have not had previous experience working with infectious pathogens. The *S. aureus* strain to be used in our mouse studies is sensitive to several antibiotics; therefore, antibiotic resistance is not a concern.

RESOURCE SHARING PLAN

1. Data Sharing Plan: Not applicable.

2. Sharing of Model Organisms: We will adhere to the NIH Grant Policy on Sharing of Unique Research Resources. All 'model organisms' generated by this project, including the *lux*-expressing and *sarA* mutant *S. aureus*, will be available to the general scientific community following publication. In addition, we will provide relevant genetic and phenotypic data upon request.

3. Genome Wide Association Studies (GWAS): The genetic studies included in this proposal do not fall under the current Policy for Sharing of Data Obtained in NIH Supported or Conducted Genome-Wide Association Studies as they do not involve the human genome. However, the *S. aureus* microarray data obtained in these experiments will be posted on-line in a public accessible website following publication of the associated manuscript.