

## Calix, Sample F31 Application with Reviews

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PI	Calix, Juan
Title	The role of wcjE disruption in pneumococcal serotype 11A humoral escape
Received	04/13/2010
FOA	PA10-109
Application ID	1 F31 AI093103-01

## Full Application

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## SF 424 R&R Face Page

[Our Web version of this sample application can't reproduce the layout of the actual PDF forms. Here is the summary that eRA Commons generated at the start of the sample app, which reflects many key fields from that form.]

PI	Calix, Juan
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<b>Title</b>	The role of wcjE disruption in pneumococcal serotype 11A humoral escape
<b>Received</b>	04/13/2010
<b>FOA</b>	PA10-109
<b>Application ID</b>	1 F31 AI093103-01
<b>Organization</b>	University of Alabama at Birmingham
<b>IRG/SRG</b>	ZRG1 F07-C (20)L
<b>AIDS</b>	No
<b>Expedited</b>	No
<b>Animals</b>	Yes
<b>Humans</b>	No
<b>Clinical Trial</b>	No
<b>Current HS Code</b>	10
<b>HESC</b>	No
<b>New Investigator</b>	No
<b>Early Stage Investigator</b>	No

## Performance Sites

[Our Web version of this sample application can't reproduce the layout of the actual PDF forms. The performance site is in Birmingham, Alabama.]

## Research & Related Other Project Information

[Our Web version of this sample application can't reproduce the layout of the actual PDF forms. Here are the key fields from that form and the applicant's responses.]

<b>Are Human Subjects Involved?</b>	No
<b>If YES to Human Subjects</b>	
<b>Is the Project Exempt from Federal regulations?</b>	NA
<b>If yes, check appropriate exemption number.</b>	NA
<b>If no, is the IRB review Pending?</b>	NA
<b>IRB Approval Date:</b>	NA
<b>Human Subject Assurance Number:</b>	NA
<b>Are Vertebrate Animals Used?</b>	Yes
<b>If YES to Vertebrate Animals</b>	
<b>Is the IACUC review Pending?</b>	Yes
<b>IACUC Approval Date:</b>	NA
<b>Animal Welfare Assurance Number</b>	A3244-01

## Project Summary

The success of *Streptococcus pneumoniae* (pneumococcus) as a human pathogen is largely due to its capability to employ a polysaccharide (PS) capsule to avoid host immunity. Recent discovery and

characterization of the pneumococcal serotype 11E strongly suggested that inactivation of the capsule synthesis gene *wcjE*, which encodes a putative O-acetyltransferase, plays an important role in escaping a host humoral immune response to the closely related serotype 11A. Since many epidemiologically prevalent serotypes also contain the gene, discerning the effects of *WcjE*-mediated variable O-acetylation on the serological properties of serotypes 11A and 11E will aid in understanding pneumococcal pathology, in determining trends of emerging pneumococcal serotypes and in designing effective PS-based vaccines.

Previously serotyped as 11A, the true epidemiological nature of 11E is unclear. Clinical isolates originally serotyped as 11A will be readdressed for the expression of 11E capsule using serospecific monoclonal antibodies. To understand the serological flexibility of these serotypes, the genetic properties that lead to seroswitching between 11A and 11E will be further studied.

Additionally, the role *wcjE* inactivation plays in avoiding a human humoral response will be evaluated using an in vitro opsonophagocytosis killing assay (OPKA) with sera from humans vaccinated with the 23-valent polysaccharide vaccine, which includes 11A PS. Independent and competitive OPKA survival of strains expressing 11A and 11E capsule will be compared, and the capacity of purified 11A and 11E PS to inhibit functional antibodies will be determined.

Finally, the capacity of serotype 11A to escape an 11A-specific humoral response through *wcjE* inactivation in vivo and the effects on pathology will be verified in a murine infection model. The survival of 11A and 11E strains will be compared under naïve conditions, with passive 11A-specific immunization, and with preimmunization against 11A and 11E PS. These assays will also provide preliminary information on the use of 11E as a potential vaccine against both serotypes.

## Public Health Relevance Statement (Project Narrative)

Understanding how *Streptococcus pneumoniae* serotypes escape an immune response is important for the design of interventional and preventative strategies against this significant human pathogen. This proposed research will study the effects of O-acetylation modification of *S. pneumoniae* capsule on antibody-dependent clearance of the bacteria, in relation to the prevalent serotype 11A and the closely related serotype 11E.

## Bibliography and References Cited

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3. Henrichsen J. Six newly recognized types of *Streptococcus pneumoniae*. *J Clin Microbiol.* 1995;33(10):2759-62.
4. Bratcher PE, Kim KH, Kang JH, Hong JY, Nahm MH. Identification of natural pneumococcal isolates expressing serotype 6D by genetic, biochemical, and serological characterization. *Microbiology.* 2009
5. Calix JJ, Nahm MH. A new pneumococcal serotype, 11E, has variably inactivated *wcjE* gene. *J Infect Dis.* 2010; In Print.
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10. Zartler ER, Porambo RJ, Anderson CL, Chen LH, Yu J, Nahm MH. The structure of the capsular polysaccharide of pneumococcal serotype 11A reveals a novel acetyl-glycerol that is the structural basis for 11A subtypes. *J Biol Chem*. 2009;284(11):7318-29.
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33. Jakobsen H, Sigurdsson VD, Sigurdardottir S, Schulz D, Jonsdottir I. Pneumococcal serotype 19F conjugate vaccine induces cross-protective immunity to serotype 19A in a murine pneumococcal pneumonia model. *Infect Immun.* 2003;71(5):2956-9

## Facilities and Other Resources

University: UAB has many investigators studying various aspects of pneumococcal pathogenesis and immunology. These include: Susan Hollingshead and Janet Yother for pneumococcal pathogenesis; David Briles for pneumococcal infection mouse models; Suzanne Michalek and Robin Lorenz for mucosal immunology; Kevin Dyvbig for bacterial genetics; David Pritchard for biochemistry of pneumococcal polysaccharide. The Medical Scientist Training Program provide exceptional support for the training of its students, and putting them in contact with potential mentors and collaborators on-site.

Laboratory: Dr. Nahm's laboratory occupies about 2500 square feet in the Bevill Biomedical Research Building (BBRB 614) on the UAB Campus. This building houses laboratories of many microbiologists in the UAB. Drs. Hollingshead, Briles, and Yother, who study pneumococci, have laboratories in the same floor as the PI and supervisor. Dr. Hollingshead specializes in the genetic evolution of pneumococcus; Dr. Yother specializes in bacterial genetics and physiology; and Dr. Briles is an expert in the application of animal models in the research of bacterial pathogen. Having this many specialized investigators makes for a specially suitable environment in which to execute

this research and training plan. All these investigators will be regularly consulted for guidance in executing this proposal.

Information technology: There are various personal computers in Dr. Nahm's laboratory and in his office. Dr. Nahm's laboratory has a flow cytometry data analysis computer station. These computers are connected to internet and files can be shared electronically with co-investigators via internet and departmental servers.

Animal facility: An animal facility is located in the basement of Bevill Biomedical Research Building and has a specially designed room for housing mice that are used for pneumococcal infections. The facility also has a staff that provides adequate veterinary care and living conditions to all experimental animals.

Clinical: UAB medical school is a part of a medical center that includes a university hospital, a veteran's hospital, a children's hospital, and a clinic. The hospital has more than 900 beds and has all the major clinical departments. The clinic has more than 800 physicians and handles 400,000 clinic visits per year. All the hospitals are involved in the clinical training of medical and MD/PhD students. The university also contains a Center for Clinical and Translational Research which sponsors and supports the training of physician scientists.

## Equipment

Dr. Nahm's laboratory is equipped for various microbiological, serological, and immunological work. His laboratory has various centrifuges, water bath, spectrophotometer, pH meter, analytical balances, isoelectric focusing gel station, tissue culture hoods, CO<sub>2</sub> incubators, ELISA plate readers, ELISA plate washer, SDS-PAGE apparatus, power supplies, various glassware adequate for polysaccharide purification, fraction collectors, lyophilizer, various microscopes (including a fluorescence microscope), PCR machine, bacteria colony counter for opsonization assays, liquid nitrogen freezers, and various freezers (-20°C, -70 °C). His laboratory has a FACSarray analyzer which permits us semi-automate pneumococcal serotyping (multibead serotyping assays).

One FACScalibur machine is available one floor above our laboratory for general flow cytometry and FACSbased serotyping assays. If any polysaccharide biochemical work becomes necessary, a GLC/MS (Varian 4000) system used for polysaccharide analysis is available on the same floor. In addition, UAB has several core laboratories including the fermentation facility, NMR center, and mass-spectrometry laboratory.

## List of Referees

[Redacted from this sample.]

## Sponsor and Co-Sponsor Information

[Redacted from this sample.]

## Research & Related Senior/Key Person Profile

[Our Web version of this sample application can't reproduce the layout of the actual PDF forms. See below for the biographical sketch attachments.]

# Biographical Sketch

<b>NAME</b>	Juan Jose Calix
<b>POSITION TITLE</b>	Graduate Student Trainee

<b>EDUCATION/TRAINING</b>			
<b>INSTITUTION AND LOCATION</b>	<b>DEGREE (if applicable)</b>	<b>YEAR(s)</b>	<b>FIELD OF STUDY</b>
Loyola University New Orleans (LOYNO)	B.S. (MCL)	05/2005	Biological Sciences
University of Alabama in Birmingham (UABSOM)	MD/Ph.D.	05/2014	Microbiology

## A. Personal Statement

I am currently seeking a MD/PhD degree with an emphasis on microbiology and am pursuing a career as an academic physician in pediatric infectious disease. My past academic training has prepared me to make significant contributions in the fields of infectious disease and vaccine development. For my undergraduate research I had the opportunity to work in two labs. I did a summer undergraduate research fellowship at Albert Einstein College of Medicine, where I worked in the lab of Dr. Jurgen Brojatsch studying the kinetics of anthrax-toxin mediated killing of macrophages. Later, I then spent a year in the lab of Dr. Patricia Dorn at Loyola University New Orleans, where I studied the genetic flow of the Central American vector for American trypanosomiasis, *Triatoma dimidiata*. These experiences provided a strong foundation in basic laboratory techniques and exposed me to the lifestyles of an academic scientist. They also provided opportunities to present research at the local, regional and national levels. I am currently pursuing my doctoral thesis in the laboratory of Dr. Moon Nahm, whose research focuses on the immunological and pathological implications of *Streptococcus pneumoniae* capsule biology. Dr. Nahm has served on various advisory WHO boards and his lab is currently the NIH Bacterial Respiratory Pathogen Reference Laboratory. His mentorship and the lab environment provide an advantage for someone seeking to work and make a contribution in my preferred field. My proposed project focuses on *S. pneumoniae*, a significant pediatric pathogen, and how its antigenic polysaccharide capsule can be modified to escape the host humoral response. The long term goal of this project is to gain a better understanding of the mechanisms that contribute to capsule diversity and aid in the design of future vaccines against the bacterium. This project is multidisciplinary and offers the chance to interact and collaborate with scientists at different institutions. I have also had the chance to mentor younger graduate students who have contributed to this project. The experience and expertise I obtain from this work will contribute to both the overall knowledge of the field of *S. pneumoniae* disease prevention and to my personal goals of being a physician scientist on the translational front of infectious disease intervention.

## B. Positions and Honors

<b>POSITIONS AND HONORS</b>					
<b>ACTIVITY/OCCUPATION</b>	<b>BEGINNING DATE (mm/yy)</b>	<b>ENDING DATE</b>	<b>FIELD</b>	<b>INSTITUTION/COMPANY</b>	<b>SUPERVISOR/EMPLOYER</b>

		(mm/yy)			
Projects Coordinator	10/05	12/05	Non-profit administration	Catholic Charities New Orleans	Dr. Elmore Rigamer, Medical Director
Small Projects Coordinator	12/05	07/06	Non-profit administration	Catholic Community Services Baton Rouge	Deborah Roe, CEO

#### C. Publications

##### Research Publications

- Calix JJ and Nahm MH. A new pneumococcal serotype, 11E, has variably inactivated wcjE gene. J Infect Dis. 2010. In Press.
- Franco CM, Andrade AL, Andrade JG, Almeida e Silva S, Oliveira CR, Pimenta FC, Lamaro-Cardoso J, Brandão AP, Almeida SC, Calix JJ, Nahm MH, de Cunto Brandileone MC. Survey of nonsusceptible nasopharyngeal Streptococcus pneumoniae isolates in children attending day-care centers in Brazil. Pediatr Infect Dis J. 2010 Jan;29(1):77-9

##### Published Abstracts

- Calix JJ and Nahm MH. 2009. "Genetic Basis for a New Pneumococcal Serotype, 11E." Abstract for Poster Presentation. American Society for Microbiology 109th General Meeting. Philadelphia, PA.
- Calix JJ and Dorn P. 2006. "Gene flow among Triatoma Dimidiata populations across Central America and Mexico" Abstract for poster and oral presentation. 54th Annual American Society of Tropical Medicine and Hygiene Conference. Washington DC.

#### D. Scholastic Performance

[Redacted from this sample.]

## Biographical Sketch

<b>NAME</b>	Moon Hea Nahm
<b>POSITION TITLE</b>	Professor of Pathology

EDUCATION/TRAINING			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Washington University, St. Louis, MO	A.B. (SCL)	1970	Physics
Washington University, St. Louis, MO	M.D.	1974	Medicine
Washington University, St. Louis, MO	Post-doctoral	1977-1980	Microbiology/Immunology

#### *A. Personal Statement*

My laboratory has been studying diversity of pneumococcal capsule and pneumococcal antibodies. We investigated diversity of polyclonal and monoclonal antibodies to polysaccharide (PS) capsules of *Streptococcus pneumoniae* using their isoelectric points, peptide sequences, DNA sequences, binding affinity (avidity), cross-reactions, and peptide mimotopes. Using monoclonal antibodies specific to pneumococcal capsules, we have begun to investigate diversity of pneumococcal capsule. We recently discovered many new pneumococcal serotypes including serotypes 6C, 6D, and 11E. We have now studied the genetic and biochemical basis of these new serotypes using DNA sequencing, 2D-NMR and mass spectrometry techniques. Discovery of serotype 6C was important since the 7-valent pneumococcal conjugate vaccine did not provide cross-protection against 6C and its prevalence significantly increased in the past several years and future vaccines should provide protection against 6C. Our studies led us to develop ways to accurately measure vaccine-induced antibodies to pneumococcal capsule and also developed a multiplexed opsonophagocytosis assay. My laboratory currently serves as the reference laboratory for NIH and WHO.

#### *B. Positions and Honors*

1974-76 Internship and Residency, Department of Medicine, Jewish Hospital, Washington University Medical School, St. Louis, Missouri

1976-80 Resident, Laboratory Medicine Division, Barnes Hospital, Washington University Medical School, St. Louis, Missouri

1980-89 Assistant Professor, Division of Laboratory Medicine, Departments of Medicine and Pathology, Washington University School of Medicine, St. Louis, Missouri

1989-96 Associate Professor, Division of Laboratory Medicine, Departments of Medicine and Pathology, Washington University School of Medicine, St. Louis, Missouri

1996-01 Professor, Departments of Pediatrics, Pathology, and Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York

1996-01 Director of NIH Pneumococcal and H. influenzae Reference Laboratory University of Rochester Medical Center, Rochester, NY

2001- Director of NIH Bacterial Respiratory Pathogens Reference Laboratory University of Alabama at Birmingham, Birmingham, AL

2001- Professor, Departments of Pathology and Microbiology & Director of the Clinical Immunology Laboratory, University of Alabama at Birmingham, Birmingham, Alabama

#### *C. Selected peer-reviewed publications (Selected from more than 160 publications)*

##### **Most relevant to the current application**

1. Zartler ER, Porambo RJ, Anderson CL, Chen LH, Yu J, Nahm MH. Structure of the capsular polysaccharide of pneumococcal serotype 11A reveals a novel acetylglycerol that is the structural basis for 11A subtypes. *J Biol Chem*. 2009 Mar 13;284(11):7318-29. Epub 2008 Dec 29. [PMCID: PMC2652282]
2. Wernette CM, Frasc CE, Madore D, Carlone G, Goldblatt D, Plikaytis B, Benjamin W, Quataert SA, Hildreth S, Sikkema DJ, Käyhty H, Jonsdottir I, and Nahm MH: Enzyme-linked Immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. *Clin Diagn Lab Immuno* 2003;10:514-519 (Describes specific pneumococcal antibody ELISA)
3. Bratcher PE, Kim KH, Kang JH, Hong JY, Nahm MH. Identification of natural pneumococcal isolates expressing serotype 6D by genetic, biochemical and serological characterization. *Microbiology*. 2010 Feb;156(Pt 2):555-60. Epub 2009 Nov 26. PubMed PMID: 19942663.
4. Park IH, Pritchard DG, Cartee R, Brandao A, Brandileone MCC, Nahm MH: Discovery of a new capsular serotype (6C) within serogroup of *Streptococcus pneumoniae*. *J Clin Microbiol*. 2007;45:1225-1233.
5. [Redacted publication in press]

## Additional recent publications of importance to the field

1. Shin JS, Yu J, Lin J, Zhong L, Bren KL, Nahm MH: Peptide mimotopes of pneumococcal capsular PS of 6B serotype. A peptide mimotope can bind to two unrelated antibodies. *J Immunol* 2002;168:6273-6278
2. Burton R and Nahm MH: A four-fold multiplexed OPA for pneumococcal antibodies, *Clin Vaccine Immunol* 2006 Sep;13(9):1004-1009.
3. Jackson LA, Neuzil, KM, Nahm MH, Whitney CG, Yu O, Nelson JC, Starkovich PT, Dunstan M, Carste B, Shay DK, Baggs J, Carlone GM: Immunogenicity of varying dosages of 7-valent pneumococcal polysaccharide-protein conjugate vaccine in seniors previously vaccinated with 23-valent pneumococcal polysaccharide vaccine. *Vaccine* 2007;25:4029-4037.
4. Park IH, Park S, Hollingshead SK, Nahm MH: Genetic basis for the new pneumococcal serotype 6C. *Infect Immun* 2007;75:4482-4489.
5. Seo HS, Michalek SM, Nahm MH: Lipoteichoic acid is important in innate immune responses to grampositive bacteria. *Infect Immun*. 2008;76(1):206-13. [PMCID: PMC2223632] (The article was spotlighted by the journal. *Infect Immun*. 2008;76(1):21).
6. Seo HS, Cartee RT, Pritchard DG, Nahm MH: A new model of pneumococcal lipoteichoic acid structure resolves biochemical, biosynthetic, and serologic inconsistencies of the current model. *J. Bacteriol*. 2008; 190:2379-2387. [PMCID: PMC2293179]
7. Schenkein JG, Park S, Nahm MH: Pneumococcal vaccination in older adults induces antibodies with low opsonic capacity and reduced antibody potency. *Vaccine* 2008;26:5521-5526. PMID: 18706464 (PMCID: PMC2574975).
8. Park IH, Moore MR, Treanor JJ, Pelton SI, Pilishvili T, Beall B, Shelly MA, Mahon BE, Nahm MH, the Active Bacterial Core Surveillance Team: Differential effects of pneumococcal vaccines against serotypes 6A and 6C. *J Infect Dis*. 2008 Dec 15; 198(12):1818-22. [PMID: 18983249]
9. Nahm MH, Lin J, Finkelstein JA, Pelton SI. Increase in the prevalence of the newly discovered pneumococcal serotype 6C in the nasopharynx after introduction of pneumococcal conjugate vaccine. *J Infect Dis*. 2009;199(3):320-325. PMID: 19099489 (PMCID: PMC2743180)
10. Park S, Parameswar AR, Demchenko AV, Nahm MH. Identification of a simple chemical structure associated with protective human antibodies against multiple pneumococcal serogroups. *Infect Immun*. 2009 Aug;77(8):3374-9. Epub 2009 May 18. PubMed PMID: 19451241; PubMed Central PMCID: PMC2715663.

### *D. Research Support*

#### **Ongoing Research Support**

R01-AI-31473

NIH/NIAID

Nahm, Moon H. (PI)

02/01/07 – 01/31/12

**Title:** Impact of a new group 6 serotype on pneumococcal vaccines

The purpose of the grant is to study biochemical, genetic and serological basis of the newly identified pneumococcal serotypes 6C and 6D.

**Role:** PI

N01-AI-30021

NIH/NIAID

Nahm, Moon H. (PI)

06/30/03 - 6/29/10 (extended to 9/30/11)

**Title:** Bacterial Respiratory Pathogens Reference Laboratory

The major goal of this contract is to support the effort of NIH in studying the infections by respiratory

pathogens by providing new analytical solutions and/or standardizing the assays. Pneumococci are used as the model pathogen. Our laboratory has developed the 3rd generation pneumococcal antibody ELISA and a multiplexed opsonization assay for pneumococcal antibodies. We are now spearheading the international effort to standardize pneumococcal antibody opsonization assays. These pneumococcal antibody assays will be important in performing the studies described in the current proposal.

**Role:** PI

R01-AI-69509

Nahm, Moon H. (PI)

NIH/NIAID

04/01/06 – 03/31/10

**Title:** Pneumococcal conjugate vaccines and old adults

The goal of this grant is to study pneumococcal antibodies induced in old adults. We will compare the

opsonizing capacity and antibody avidities of pneumococcal antibodies to serotypes 14 and 23F induced with a

polysaccharide and a conjugate vaccine. The current proposal is a continuation of this study.

**Role:** PI

### Completed Research Support

[Outside research support redacted from sample.]

## PHS Fellowship Supplemental Form, Research Plan

[Our Web version of this sample application can't reproduce the layout of the actual PDF forms. The attachments are included below. In the original version, these attachments met the page limits.]

### Specific Aims

## The role of *wcjE* disruption in pneumococcal serotype 11A humoral escape

**Rationale:** Serotype 11E is a novel pneumococcal serotype, previously unidentified due to its serological similarity to the epidemiologically prevalent 11A, a significant serotype in both asymptomatic carriage and disease-causing strains. Genetic findings indicate that each 11E strain emerged independently in separate hosts. 11E differs from 11A due to a disruption of the *wcjE* capsule synthesis gene, which encodes an Oacetyltransferase that targets 1-phosphoglycerol in capsule polysaccharide. We hypothesize that disruption of the gene allows a strain initially expressing 11A capsule to avoid a host humoral response by changing its capsule structure, resulting in an 11E infection. Given that no previous studies have recognized 11E as a separate serotype, we aim to determine the extent of the role 11E plays following initial 11A infection, setting the stage for future studies addressing the prevention and control of disease caused by serotype 11A.

### **Aim 1. Examine nasopharyngeal (NP) isolates for the presence of 11E strains**

**(1A)** Develop a FACS-based assay for efficient detection and distinction of 11A and 11E strains.

**(1B)** Identify additional 11E clinical strains, focusing on NP isolates originally typed as serotype 11A.

**(1C)** Examine newly identified 11E isolates for heterogeneity of *wcjE* disruption.

**Aim 2. Determine whether a human humoral immune response can be selective for 11A and not 11E in vitro**

**(2A)** Generate isogenic 11A and 11E strains for comparative studies.

**(2B)** Determine antibody specificity for 11A or 11E PS in sera from individuals vaccinated with the pneumococcal vaccine PPV-23 (PPV-23 sera) by using ELISA.

**(2C)** Detect functional anti-11A and anti-11E antibodies in PPV-23 sera by using Single Opsonophagocytic Killing Assay (SOPKA) of 11A and 11E.

**(2D)** Determine competitive advantage of 11E by immunological escape in PPV-23 sera by using Multiplex Opsonophagocytic Killing Assay (MOPKA).

**(2E)** Verify the role of anti-capsular PS antibodies in 11A and 11E opsonization.

**Aim 3. Determine that 11E has a selective advantage in an immune response against 11A in vivo and whether 11E infection emerges from initial infection with 11A**

**(3A)** Develop an 11A and 11E mouse infection model.

**(3B)** Detect total and functional anti-11A and anti-11E antibodies in murine sera following 11A and 11E infection.

**(3C)** Determine in vivo survival of 11A and 11E in mice actively immunized against 11A and 11E PS.

**(3D)** Assess in vivo survival of 11A and 11E in mice passively immunized with 11A-specific monoclonal antibodies.

# Research Strategy

## *A. Significance*

*Streptococcus pneumoniae*, also known as the pneumococcus, is a commensal gram-positive diplococcus that ubiquitously colonizes the human nasopharyngeal (NP) tract. However, pneumococcus can spread to normally sterile sites and cause diseases such as pneumonia, meningitis and otitis media. Especially susceptible populations include infant, elderly and immunocompromised individuals. Despite the development of effective vaccines and antimicrobial therapies, the adaptable pneumococcus continues to have a widespread impact as a pathogen.

The bacterium's most significant virulence factor and a prerequisite for almost all pneumococcal disease is its polysaccharide (PS) capsule. The capsule is composed of chains of PS repeat units that surround and protect the bacterium against the host immune system, e.g. antibody-independent opsonophagocytosis (1). Host exposure to the capsule antigen following either natural infection or vaccination can elicit a capsule-specific antibody response. This selective pressure has contributed to the diversification of capsular structures into the 93 currently recognized pneumococcal serotypes (2-5). This diversity is directly reflected in comparison of the genetic contents in the capsule PS synthesis (*cps*) loci of different serotypes, which contain the genes necessary for the synthesis of each serotype's particular PS subunits (6). Despite the large diversity of capsule structure, only a few prevalent serotypes are responsible for most of pneumococcal disease (7).

Classically, serotypes were designated according to the Quellung reaction, in which a strain is tested for reactivity with polyclonal antisera. However, serological assays that make use of more antigenically specific monoclonal antibodies (mAb) have been developed and have led to the identification of new serotypes (2, 4, 8). Using a mAb assay, we identified two subtypes within strains typed as 11A according to classical Quellung methods (8, 9). Subsequent studies determined that the subtypes were serologically, structurally and genetically distinct serotypes, 11A and a novel serotype 11E (5, 10). An NMR spectroscopy study of the closely related serotypes identified the presence of an O-acetylated 1-phosphoglycerol (1-P-Gro) on 50% of the capsular PS repeat units on 11A, whereas all 11E 1-P-Gro are not acetylated (10). Serological and structural changes are attributed to disruption of the capsule synthesis gene *wcjE* in all 11E strains, indicating that the gene encodes an O-acetyltransferase (OAcT) (5). In each 11E strain examined the mutation in *wcjE* was different.

Serotypes that are prevalent in the population are the targets for the current polysaccharide-based vaccines. A 23-valent pneumococcal polysaccharide vaccine (PPV-23), which includes 11A PS, is administered to at-risk adult populations, but is ineffective in pediatric populations. A 7-valent conjugate vaccine (PCV-7), which is effective for children under the age of two and does not include 11A PS, has been in use since 2000 (11). The latter vaccine has been effective in lowering the incidence of invasive pneumococcal disease (12, 13). However, pediatric carriage rates have not declined, and there is growing concern that non- PCV-7 serotypes are filling the void left by widespread vaccination (14-16). Development and implementation of new, more inclusive vaccines (e.g. PCV-10 and PCV-13) is underway to address the concern over emerging serotypes. Recent epidemiological studies place serotype 11A among the top five occurring serotypes isolated from disease and colonized individuals (14-16). The 11A serotype appears to be filling the void left by widespread use of the pediatric vaccine in North America (15-17) and has been associated with greater rates of 30-day mortality relative to other serotypes (18). These findings make a strong case for the consideration of 11A in future vaccine design. However, all these studies were done before the discovery of 11E, and preliminary results suggest that 10-25% of invasive disease isolates serotyped as 11A may actually be 11E (8). Furthermore, we have not identified any 11E isolates associated with NP carriage.

The unrelated disruptive mutations observed in all 11E isolates indicate that each strain emerged independently. It also suggests a barrier to host-to-host spread of these strains. Because these

strains were capable of independently emerging and surviving in hosts, we speculate that serotype 11E emerges from an initial 11A infection, competitively survives in a state of co-infection, and goes on to cause disease. We hypothesize **that in certain individuals that mount an 11A-specific humoral response, selective pressure to escape host's immunity favors loss of wcjE in 11A; this leads to disease caused by 11E.**

### *B. Innovation*

## Vertebrate Animals

Previous studies have shown that antibodies to one serotype can be non-reactive to another serotype with identical capsule structures except for the lack of O-acetylation (19, 20). However, these studies did not address competitive survival between counterpart serotypes and may have understated the effects of loss of O-acetylation. Furthermore, no study has shown *in vivo* seroswitching in response to an O-acetylation-independent immune response. Comparative studies of these closely related serotypes may reveal what roles capsular O-acetylation plays in pneumococcal pathology. Indeed, sequencing of the wcjE pseudogenes in multiple strains of serotype 9A and the vaccine serotype 33F (the wcjE-inactivated counterparts of serotypes 9V and 33A, respectively) shows the existence of heterogeneous disruption of the wcjE gene (data not shown), suggesting that loss of O-acetylation may be playing a similar role in serotypes beyond serogroup 11. Half of the known serotypes, including 8 current vaccine targets, contain a putative OAcT in their respective cps locus (21). Identifying the conditions that contribute to the adaptation or loss of capsular O-acetylation is important for anticipating serotype emergence and can be translated into future vaccine design. This study also begins to validate the use of de-O-acetylated PS in pneumococcal vaccines for disease prevention, a similar strategy to what is being developed against other bacterial pathogens (22).

### *C. Approach*

**Aim 1. Examine nasopharyngeal (NP) isolates for the presence of 11E strains.** To confirm whether previous results apply to the entire population of clinical isolates previously serotyped as 11A, we must readdress further clinical isolates from NP carriage states. Furthermore, we will define the genetic characteristics of wcjE inactivation in additional strains. These results will indicate whether inter-host transmission of 11E occurs, and whether 11E emerges in the NP tract.

**a. Develop FACS-based serotyping method for efficient detection and distinction of 11A and 11E.** To facilitate the study of serotypes that readily seroswitch, we must develop methods that can detect and distinguish clones expressing closely related serotypes within a culture. We have previously shown the validity of using monoclonal antibodies (mAb) to serotype clinical isolates (8). However, these assays are based on the capacity for soluble capsular PS to inhibit binding of mAbs to PS-coated beads or plates, thus, indirectly detecting the presence of a serotype. This method does not readily detect subpopulations within a culture. We have developed FACS-based methods to distinguish between the clinically significant 9V and the closely related serotype 9A, which genetically differ only by wcjE disruption in 9A. The assay takes advantage of the fact that most mAb made against 9V bind exclusively to 9V capsule, but one mAb (Hyp9VM5) is cross-reactive to both 9A and 9V (Fig 1). Given that preliminary ELISA results show that some mAb display crossreactivity to both 11A and 11E serotypes and some are selective for 11A (data not shown), we will develop a method similar to the 9A/9V protocol, for detection of 11A and 11E individual clones in culture. To validate the specificity of the method, mAb reactivity to the closely related serotypes 11F, 11B, 11C, and 11D will be evaluated. A successful method to distinguish the two serotypes will aid in studies outlined below.

Credit: NIAID

**b. Identify additional 11E clinical strains, focusing on NP isolates originally typed as serotype**

**11A.** Serotype 11A has recently been among the top five serotypes isolated from NP colonization strains (15, 17), however, no epidemiological study has distinguished 11E from 11A isolates. From previous studies we can extrapolate that 11E may represent a significant portion of isolates typed as 11A by Quellung (5). Furthermore, to date all 11E strains have been isolated from blood and cerebral spinal fluid, i.e. have been invasive disease strains. If 11E is capable of emerging and being selected for in the NP, we expect to identify 11E strains in carriage isolates previously typed as 11A. Through collaboration with groups studying NP isolates, we will screen using methods established in our lab (8, 9). We are currently collaborating with Dr. Stephen Pelton in the Department of Pediatrics at Boston University School of Medicine to identify NP isolates from pediatric patients and originally serotyped as 11A (15). If no 11E isolates are detected, we can interpret that 11E is either a rare serotype or it only emerges following infection of sterile sites (i.e. lung, blood or cerebral spinal fluid).

**c. Examine newly identified 11E isolates for genetic heterogeneity.** We showed that all 11E isolate identified to date contain disruptive mutations unrelated to each other (5). This result strongly suggests that a) the 11E serotype is capable of emerging independently in different hosts and b) there is a barrier to the epidemiological spread of an 11E strain. However, only a few invasive disease isolates have been examined. To support or dismiss initial conclusions that 11E is not transmissible, 11E carriage isolates must be analyzed genetically in search for evidence of clonal expansion between hosts, i.e. two separate isolates with identical disruptive mutations. Using PCR and sequencing methods as previously published (5), we will genetically characterize isolates identified in Aim 1B. If the degree of previously observed genetic heterogeneity applies to further isolates, then the hypothesis of 11E emergence in separate individuals is supported.

**Aim 2. Determine whether a human humoral immune response can be selective for 11A and not 11E in vitro.** Our hypothesis presumes that a humoral response made against serotype 11A is more effective at opsonizing an 11A strain than an 11E strain. Indeed, evidence shows that individuals may be capable of mounting an immune response that is not cross-reactive to serotypes where the only difference is O-acetylation, i.e. 15B and 15C, and 9A and 9V (19, 20). Our study is designed to confirm whether 11E strains have a competitive advantage over 11A strains against opsonization by sera of humans vaccinated against 11A, and whether this response is PS specific.

**a. Generate isogenic 11A and 11E strains for comparative studies.** To closely study the effects of 11A and 11E PS expression, we will create two isogenic strains that can be readily selected for by means other than serological differences. We will transform the *rpsL* gene from the streptomycin-resistant strain TIGR4 into a clinical isolate of 11A (i.e. MNZ270); *rpsL* from TIGR4 has been shown to confer resistance to streptomycin. Alternatively, MNZ270 will be passaged in sequentially higher concentrations of streptomycin to isolate a streptomycin-resistant 11A strain by artificial selection. However, this method may introduce additional non-related mutations that may affect further studies.

Next, we will transform the streptomycin resistant strain with a Janus cassette containing a kanamycin resistance gene and a streptomycin susceptibility gene, to disrupt *wcjE*, as previously

described (5). This method will result in a streptomycin resistant 11A and a kanamycin resistant 11E strain, respectively referred to as 11Astr and 11Ekan for the remainder of this proposal. Both strains will be validated for correct serology using ELISA. Studies in this lab have verified that in vitro disruption of *wcjE* results in loss of O-acetylated 1- P-Gro according to NMR analysis (data not shown).

To control for any other confounding factors that may influence results in the following assays, independent and competitive in vitro growth, average bacterial chain length and average capsule production will be examined in these strains. Growth will be monitored using OD600 measurements and serial dilutions on antibiotic selective plates. Average bacterial chain length will be determined by microscopy. Average capsule production will be determined by measuring capsule thickness according to electron microscopy and by comparing purified PS capsule chain length on SDS gel as previously determined (23). Preliminary studies of 11A and 11E clinical co-isolates of identical multilocus sequence types suggest that independent or competitive growth are not affected by serotype expression (Fig 2). If differences are detected in capsule production, we will design additional experiments to examine protective features, such as complement deposition, etc. However, small capsule expression differences will have minimal effect on antibody-mediated opsonophagocytosis.

In addition to 11Astr and 11Ekan, we will transform TIGR-JS (a TIGR-4 background with its *cps* locus deleted through recombination with a Janus cassette (24)) with 11A *cps* to create the streptomycin resistant TIGR11A. Following the same procedures for the creation of 11Ekan, we will create the kanamycin resistant TIGR11E. These two strains will be used interchangeably in the assays mentioned in this proposal to verify that capsule alone can confer any of the observed results, independent of a strain's genetic background.

Credit: NIAID

**b. Determine antibody specificity for 11A or 11E in sera from individuals vaccinated with pneumococcal vaccine PPV-23 by using ELISA.** Total antibody concentrations will be determined according to a previously established ELISA protocol (25). Briefly, sera will be absorbed with cell wall polysaccharide and serotype 23F PS. Resulting sera will then be incubated in ELISA plates coated with equal concentrations of purified 11A or 11E PS and detected with anti-human IgG, IgM and IgA alkaline phosphatase conjugated secondary antibodies. PS will be purified from 11Astr and 11Ekan using ethanol precipitation. A standard assay has been developed to detect absolute concentrations of 11A-specific antibody (25). Since no corresponding assay exists for 11E specific antibodies, anti-11E concentrations will be determined as binding signal relative to 11A.

Polyclonal sera may display similar reactivity to both PS. To analyze the crossreactivity of this sera to 11A and 11E PS, sera will be inhibited by soluble PS of either serotype previous to being analyzed by ELISA. This will determine the amount of 11A- or 11E- specific antibody in the samples. By using different concentrations of inhibitory PS, we can also determine total affinity of sera to 11A or 11E PS. Though PS may be altered, i.e. de-O-acetylated or misfolding of epitopes, by the purification process or adhesion to ELISA plates, these assays will identify any major specificity differences of vaccinated sera to these serotypes.

**c. Detect functional anti-11A and anti-11E antibodies in PPV-23 human sera using Single Opsonophagocytic Killing Assay (SOPKA) of 11A and 11E.** We hypothesize that individuals may mount a functional immune response specifically to serotype 11A, but in which strains with 11E capsule have a competitive advantage over 11A. To test this hypothesis, we will examine the capacity of sera from PPV-23 vaccinated patients to induce opsonophagocytosis of 11A and 11E in the well established SOPKA (26). In this assay, bacteria, rabbit complement, serial dilutions of an individual's serum and activated HL60 granulocytic cells are incubated to induce bacterial killing. Following incubation, tissues cells are lysed and the bacteria are plated to determine amount of viable colony forming units (CFU). If the serum contains functional antibodies against the bacteria, killing of bacteria is increased. Bacterial killing is measured as a function of titers, here defined as the inverse of the concentration of sera needed to obtain 50% maximum killing in vitro. Since these sera are obtained from patients vaccinated with a polysaccharide vaccine, titers are dependent on the capsule structure the bacteria. If our hypothesis is correct, some individuals will display higher titers against 11A than against 11E. Various sera may need to be tested, since previous studies have shown that not all patients mount O-acetylation-dependent responses (20). Additionally, surviving 11A CFU will be routinely screened serologically and genetically to verify that escape from OPK was not due to in vitro seroswitching.

**d. Determine competitive advantage of 11E by immunological escape in PPV-23 human sera by using Multiplex Opsonophagocytic Killing Assay (MOPKA).** A limitation of SOPKA is that we will use sera from patients immunized with vaccines containing purified 11A PS. O-acetyl groups are susceptible to mild hydrolysis and other factors, such as storage time, so the degree of O-acetylation of 11A may have decreased during preparation, storage and administration of the vaccine. Thus, these sera may not be representative of an individual whose first exposure to an 11A antigen was natural, potentially resulting in difference between 11A and 11E OPKA that are too small to detect when tested in separate systems. Furthermore, to support our hypothesis, we need to detect 11E competitively surviving against 11A in the same OPKA system.

To better visualize the effects of seroswitching in a system more closely resembling 11A and 11E competition within a host, we will adopt the MOPKA that has been established in our lab (27). The procedures will resemble those in aim 2c, except that 11Astr and 11Ekan will be co-incubated in the OPKA systems with increasing dilutions of human sera. After incubation, surviving bacteria will be spotted on plates containing antibiotic selective media, so that surviving 11Astr CFU will be enumerated on streptomycin plates, and 11Ekan CFU on kanamycin plates (Fig 3). Opsonization with 11A specific monoclonal antibodies will serve as a positive control. This multiplexing will allow us to detect the effect of the two serotypes within a single host, and if our hypothesis is correct, we expect 11E survival to be significantly greater than 11A. Conversely, 11E titers will be lower than 11A titers. To our knowledge, the competitive survival of closely related serotypes has never been examined in the same system.

Credit: NIAID

**e. Verify the role of anti-capsular PS antibodies in 11A and 11E opsonization.** We hypothesize that seroconversion of 11A to 11E is advantageous because de-Oacetylated PS has lower affinity to functional antibodies against 11A. To confirm this, we will attempt to inhibit OPKA with absorption of sera with whole 11A and 11E bacteria and with soluble 11A and 11E PS (see aim 2b). Human sera at optimal dilutions (according to Aim 2c) will be absorbed with increasing concentrations of purified PS, and we will detect for decreases in killing. Inhibition curves by 11A and 11E PS, and concentrations of PS that inhibit 50% killing will be compared. If an individual produces a predominantly 11A-specific humoral response (Aim 2b and 2c), we expect that 11A PS inhibit killing of both 11Astr and will 11Ekan at lower concentrations than 11E PS.

**Aim 3. Determine that 11E has a selective advantage in an immune response against 11A in vivo and whether 11E infection emerges from initial infection with 11A.** To verify our hypothesis, it must be shown that an initial 11A infection can result in an 11E disease isolate in vivo under the selective pressure of an 11A-specific response. To recreate this scenario, we will develop and employ a murine model of 11A infection. All protocols specified below will conform to local and national guideline and will be reviewed by the UAB internal review board for the correct usage of animal subjects. This aim will be performed in close collaboration with Dr. David Briles who specializes in the development and use of murine models of pneumococcal disease.

**a. Create and 11A and 11E mouse infection model.** Murine models of pneumococcal infection have been well established (28, 29). However, currently there is no in vivo model for infection with serotype 11A. Given the rise in prevalence of the 11A serotype, a murine model is pertinent for future studies. Preliminary infection of C57BL/6 mice will include nasopharyngeal (NP) and intravenous (IV) inoculations according to previous studies (30, 31). The 11A clinical isolate MNZ270 and its 11E coisolate MNZ269 will be used separately to assess the capability of the serotype to colonize and/or persist in the NP and blood. NP colonization and pneumonia can be achieved by NP inoculation with different CFU amounts (30). Successful infections will be assessed by sacrificing mice 5 days post-infection with variable concentrations of bacteria, and determining CFU counts from blood, lung, nasal wash and nasal tissue. Successful infections will then be followed over 14-21 days to detect time from infection until mice exhibit moribund behavior. Following optimization of an infection protocol, we will assess infection with 11Astr and 11Ekan. This latter method will allow us to monitor the competitive survival of each serotype using selective antibiotic plates.

Since there is no evidence of 11E host-to-host dissemination, it is uncertain whether an 11E strain can successfully colonize a host following NP infection, or whether 11E can survive in the blood without emerging from an 11A predecessor. In order to achieve infection, 11E may be infected intraperitoneally (31).

**b. Detect total and functional anti-11A and anti-11E antibodies in murine sera following 11A and 11E infection.** Sera from mice infected NP and IV with 11A and 11E will be assessed for reactivity to capsular PS. Total antibody concentrations will be determined akin to aim 2b, and functional antibody levels will be determined akin to aims 2c and 2d. Since any influence of capsule differences may be masked by a humoral response to non-capsule antigens, sera will be absorbed with a nonencapsulated strain (e.g. TIGR-JS). If 11A infection is capable of producing an O-acetylation specific response, we expect sera from 11A infected mice to preferably bind and opsonize 11A PS and bacteria. Since 11E epitopes are present on both 11E and 11A bacteria, we expect difference between the binding and opsonization of both serotypes by sera from 11E infected mice to be minimal. Humoral responses will be assessed from every mice used in this study to determine correlations between titers and bacterial growth in hosts.

**c. Determine in vivo survival of 11A and 11E in mice actively immunized against 11A and 11E PS.** If the above studies reveal that mice can develop a humoral response that selectively targets the 11A serotype over 11E, we will assess the survival of the bacteria in vivo following immunization. Purified 11A PS will be conjugated to ovalbumin through cyanlation with CNBr, and the resulting compound will be injected subcutaneously into mice three times at two week intervals. This process was capable to induce antibodies that are specific for 11A PS and not 11E PS (32), so we assume that O-acetylation is at least partially conserved in the immunization product. Successful immunization will be verified by collecting sera from vaccinated mice and testing for 11A specific antibodies by ELISA and OPKA. If functional antibody levels are not detected, different protocols for vaccine synthesis or IP administration will be used.

A week after final immunization, mice will be co-infected NP or IV with both 11Astr and 11Ekan according to optimal protocols determined in aim 3a. At 2 and 5 days after infection, CFU counts from lung, NP tissue, nasal wash and blood will be determined by growth on selective plates to determine 11Astr and 11Ekan levels. Alternatively, we will employ FACS based methods developed above, since antibiotic resistance may be lost during in vivo growth and 11E clones may emerge de novo from 11Astr. If our hypothesis is correct, we expect levels of 11E to be significantly higher than levels of 11A upon collection of isolates.

In a similar manner, 11E conjugated vaccine will be created and administered to mice prior to coinfection. Since 11E PS may contain epitopes found on both 11A and 11E strains, we hypothesize that in vivo growth will be affected in both serotypes. This preliminary data begins to validate the use of 11E as a vaccine target.

**d. Assess in vivo survival of 11A and 11E in mice passively immunized with 11A specific monoclonal antibodies.** Since aim 3c will be using conjugated vaccine, it is likely that subject mice will not develop an exclusively anti-11A response. To test the growth of 11A and 11E in an exclusively anti-11A environment in vivo, we will employ passive immunization with murine mAb prior to co-infection with 11Astr and 11Ekan. Antibodies will be assessed for exclusive or near exclusive opsonization of 11A in vitro using OPKA described above. Antibodies will be administered prior and during infection and CFU numbers will be determined in all the tissues mentioned above.

We will compare bacteria isolated from two groups of mice infected only with 11Astr, a control group and a passively immunized group. Special interest will be paid to streptomycin resistant strains with 11E serology according to FACS analysis, as this population would constitute strains that seroswitched from 11A to 11E during infection. We would expect a higher population of de novo 11E to emerge in the passively immunized mice compared to the control group. If so, this would confirm

our hypothesis that 11E emerges in following initial 11A infection in response to an anti-11A immune response.

#### *VERTEBRATE ANIMALS*

##### **1a) Carriage Model Description.**

We will determine whether 11A and 11E strains are capable of establishing nasopharyngeal carriage in mice. It has been established that nasal colonization of mice with pneumococcus is a reasonable model of colonization in man. Most of these mice will exhibit no life-threatening infections. They will be euthanized after about 7 days and the numbers of CFU in their nasal tissue and nasal wash will be determined by growth on plates. Although most remain healthy until the end of the experiment, a small number (about 5%) will become ill. Once they become moribund (as defined below) they will be euthanized and recorded as having reached the moribund state. Various doses of pneumococcus will be used to find the dose of the new serotypes resulting in carriage in 50% of cases.

##### **1b) Infection Model Description.**

We will also study the ability of the new pneumococcal types to cause bacteremia and sepsis. This is an important endpoint, because virtually all of the human deaths due to pneumococcal pneumonia are associated with sepsis. In this study some mice will be bled retroorbitally up to four times over 2 day period by removal of 35 ul blood at each bleeding. Various doses of pneumococci will be used to find LD50 CFU of the new serotypes. In some cases, protective antibody will be given passively. We will do quantitative determination of CFU in these samples by plating the pneumococci. In all studies following i.v. infection mice will be monitored until they become unresponsive to touch and scored as being moribund (as described below). The time at which they become moribund will be recorded.

##### **1c) Immunization Description.**

After establishing colonization and bacteremia models of infection, we will validate whether preimmunization with conjugated polysaccharide incurs a protective humoral response against infection. We will immunize mice three times with conjugated polysachharides and peripheral blood samples will be obtained before each immunization or two weeks after the final immunization. The candidate PS will be mixed with an adjuvant (quilA or alum). Serum from blood samples will be analyzed for pneumococcal antibody levels (determined by ELISA) and capacity to opsonize pneumococci (determined by opsonophagocytic killing assay). Preimmunized mice will also be analyzed for protection from infection using the protocols mentioned above.

Mice will be considered moribund when they reach the following clinical presentation: mice that hardly move, fail to right themselves, or fail to respond to touch will be examined to determine their surface temperature. The surface temperature of a normal mouse is 30° C. When the surface temperature falls below  $\leq 25^{\circ}$  C the mice will be considered "moribund" and will be euthanized. The surface temperature will be monitored by using an infrared thermometer (Model: 15-077-966 from Control Company [Friendswood, TX]). Temperature will always be recorded by scanning the back of the mouse in question.

The reason that some of the mice will be studied until they become moribund is that this is the most sensitive measure of protection against sepsis. Even amounts of antibody that give unconvincing changes in blood clearance of bacteria can be protective. A study (33) reported that passively given antibody did not affect the degree of bacteremia at 12 hours post infection, but it resulted in a median time to become moribund of 8 days whereas mice without antibody became moribund in one day. By 24 hours mice in the unprotected group were dying, thus greatly complicating comparisons of CFU between the groups since there were no longer enough control mice still alive for statistical analysis.

Mice are infected with pathogenic bacteria (*Streptococcus pneumoniae*), which probably causes discomfort, but no real pain as such. No pain relieving agents can be used to eliminate this

discomfort without interfering with the validity of the study. Pain relieving agents such as aspirin or ibuprofen reduce inflammation and thus decrease resistance to infection. For this reason, they cannot be used in these studies. Their use would compromise our ability to identify relevant mechanisms of disease and resistance to infections.

## **2) Justification.**

There are no ways to determine in vivo survival of these strains or to describe the humoral response to live infection or vaccination without using actual animal models. We propose to use mice for this study because mice have been widely used for this purpose and therefore are well established. We will make every effort to use the fewest animals compatible with obtaining reliable data.

## **3) Veterinary care.**

The animals will be housed in our school facilities, which are supervised by professional veterinarians.

## **4) Limiting discomfort.**

Immunization and phlebotomy without anesthesia are not thought to be painful procedures. Nevertheless, we will have trained and experienced persons to perform the procedure. If any animal show any sign of discomfort (e.g., due to infections), we will euthanize them.

## **5) Euthanasia.**

The mice will be euthanized with carbon dioxide as recommended by the UAB Comparative Medicine Department.

### *RESPECTIVE CONTRIBUTIONS*

This training plan was initially conceived by the Principal Investigator (PI, Calix) and Supervisor (Nahm) according to work done in the lab by the PI. Writing of the proposal was performed by the PI. After completion of the initial draft, the proposal was submitted to the PI's steering committee for comments. Minor editings were made by the PI and Supervisor according to suggestions by the committee.

### *SELECTION OF SPONSOR AND INSTITUTION*

**Sponsor:** Dr. Nahm is currently the director of the UAB Clinical Immunology Laboratory, of the WHO (World Health Organization) Pneumococcal Serology Reference Laboratory, and of the NIH Respiratory Bacterial Pathogens Reference Laboratory. He has served on multiple international advisory boards in matters of vaccine implementation, and his lab currently focuses on developing and monitoring the efficacy of vaccines. I selected Dr. Nahm as my research mentor because his expertise on both the medical and basic science components of research makes him an ideal mentor for students pursuing careers as medical scientist. Given that Dr. Nahm specializes in the immunological response to respiratory pathogens, his mentorship offers crucial support to the aims of this proposal. Furthermore, his laboratory, employed scientists and the facilities available to the Nahm lab offer a helpful environment for experimental work.

**Institution:** I decided to attend the University of Alabama Birmingham (UAB) because it is among the largest biomedical centers in the United States of America and is among the top ranked hospital systems in the country. As such, the UAB school of medicine offers advantageous opportunities for students seeking a career in academic science. Accordingly, the Medical Scientist Training Program (MSTP) is nationally recognized and was recently recommended for expansion according to a national MSTP review committee. The program and university has offered me many opportunities for both academic and personal growth.

Furthermore, UAB has a valued tradition of infectious disease research. Currently four laboratories (Dr. Moon Nahm, Dr. Janet Yother, Dr. David Briles and Dr. Susan Hollingshead) collaborate on

research in the field of *S. pneumoniae* pathogenesis and hold inter-laboratory meetings every month. The sharing of resources and ideas among these labs promotes interdisciplinary approaches to questions including pneumococcal genetics, vaccinology, in vivo pathology, etc. This is a special instance that an unusually large group of investigators focus on a same pathogen, and this makes for an ideal environment for me to address the hypothesis of this proposal.

#### *PLAN FOR INSTRUCTION IN THE RESPONSIBLE CONDUCT OF RESEARCH*

The University of Alabama at Birmingham (UAB) has a strong and ongoing commitment to the responsible conduct of research. The UAB campus offers many opportunities for such training including formal courses through graduate school programs and additional educational opportunities as detailed below.

MSTP trainees specifically take two courses:

(1) **Medical Ethics** is part of the new Patient, Doctor, and Society course taught by several faculty, including Dr. Gregory Pence, Ph.D., a Professor in the Department of Philosophy. Patient, Doctor, and Society (PDS) is a two week multidisciplinary required module for first year medical students. The PDS module is designed to introduce selected principles, behaviors and skills that are essential for a student's professional development as a physician-in-training. These fundamentals are expected of all physicians in practice and serve to complement his/her competency in medical knowledge and are necessary for effective patient care. The fact that PDS was chosen to be the first module in the revised curriculum serves to emphasize the importance of the concepts introduced in this course. Physicians today are being asked by the public to pay greater attention to issues that cross the boundaries of biomedical science into those of professionalism in clinical medicine, such as communication skills, compassion, honesty, cultural competency, medical decision making, ethics, patient safety, leadership and health policy. In addition, the effective physician will need to be reflective and attentive to his or her own needs for life-long learning, personal health and well-being.

(2) MSTP trainees, as part of their graduate training, take Principles of Scientific Integrity. This course was developed by Harold Kincaid, PhD, and provides systematic instruction about the responsible conduct of science. The three-credit hour, semester long course provides a survey of ethical issues and principles in the practice of science. It is offered twice a year (Fall and Spring). Among the topics discussed are: the nature, extent and causes of fraud in science; UAB policies on fraud; ideals of good science; the responsibilities of authorship and peer review; bias and sloppy practices; responsible use of the press; potential problems raised by the commercialization of research; scientists as public policy advisors; and ethical issues involved in animal experimentation and in clinical trials. Famous cases from the history of science as well as fictional case studies are used to involve students in discussion of the above issues including extensive use of video clips to engage students. This course is also required for all graduate students trained in the biomedical sciences.

The PI (Juan Calix) has taken both the Medical Ethics Course and the Principles of Scientific Integrity course in fall 2008. The PI has also completed online courses regarding HIPAA regulations, animal treatment and scientific integrity offered by UAB within the last four years. The Sponsor (Moon Nahm) has extensive experience on the topics of use of human samples, animal research and scientific properties. The laboratory has weekly meetings where these and other research topics are discussed among graduate students and senior scientists.

#### *GOALS FOR FELLOWSHIP AND TRAINING CAREER*

I aim to become a physician scientist in the field of pediatric infectious diseases whose research focuses on developing preventative treatments of disease. Having interacted with many professors who have found a balance between clinical and laboratory work, I, too, desire to be able to have significant interaction with patients and tailoring personal interventions against particular diseases. However, my major drive lies in academia, and the planning, partnership developing and teaching that accompany being a successful research faculty.

On pure scientific merit, this project will allow me a better understanding of the interplay between host immunity and bacterial genetics. Along with my additional background in medicine, this study, which deals with bacterial adaptation in during natural infection, will develop a knowledge foundation that will translate into better therapies as a clinician, and the formation of better research plans as a scientist. Understanding how to anticipate ways that bacteria may adapt to immune response is important in the design of effective vaccines. Furthermore, this training plan guides me through the further learning of two techniques important for the development of preventative therapies: evaluating human sera samples (opsonophagocytosis assays) and use of mouse infection models. Mastering these particular laboratory protocols will aid in the design of further methods to address future questions. Developing the expertise for addressing the interface between bacteria and host immunity is invaluable in my training as an effective infectious disease specialist.

I visualize my career developing collaborations with funding agencies, academic peers and students. In developing this study plan and application, I have already gained a better understanding of grant writing. I have also learned many of the intricacies of experimental design. Since many aspects of this research plan use previously established techniques for novel applications, this training plan will expose me to many troubleshooting challenges. Under the mentorship of Dr. Nahm and other experts in his lab, these experiences will be vital to my training.

I had the privilege to collaborate with extramural labs in earlier research opportunities, and I have learned that the resources and expertise that collaborating labs can provide are necessary for academic success. Certain aspects of this training plan and other related research projects require me to collaborate with labs in both industry and academia. As I further develop this project, I will also develop my relationship with these and additional scientists. These interactions will help develop the skills and knowledge that I will use in future collaborative research endeavors.

Lastly, I strive to become a good teacher and mentor while managing my research. This project has already afforded me the opportunity to work closely with a junior graduate student. Because this particular topic, i.e. role of O-acetylation in pneumococcal capsular biology, is under-addressed in the field of pneumococcal pathology, there is opportunity for the development of tangential research paths. However, as I prepare for next steps in my education and training, I may not be able to pursue these paths. This will provide a chance for me to teach others who will continue this work, and allow for the honing of my mentoring and teaching skills.

#### *ACTIVITIES PLANNED*

This activity plan was conceived by the PI (Calix) and supervisor (Nahm), and has been tailored for to train and establish the PI in the fields of host/microbe interactions, vaccine development, and academic medicine.

#### **Year 1 – Aug 2010-July 2011**

Research	90%
Coursework	10%

- a. Finish Aims 1 and 2 by the summer of 2011; we anticipate two publications from this work
- b. Attend and present at the Society for Advancement of Chicanos and Native Americans in the Sciences national conference, Sept-Oct 2010
- c. Attend and present at the Infectious Disease Society of America meeting, Oct 2010
- d. Attend and present at the American Society of Microbiology meeting, May 2011
- e. Take advanced microbiology courses: MIC718-Respiratory Tract Pathogens; MIC-Evolution of Virulence Factors in Streptococcus

#### **Year 2 – Aug 2011-July 2012**

Research 90%  
Coursework 10%

- a. Begin working on Aim 3; we anticipate one publication from this work
- b. Attend and present at the American Society of Microbiology meeting, May 2012
- c. Attend and present at the Society for Advancement of Chicanos and Native Americans in the Sciences 2011 national conference
- d. Begin writing thesis

### **Year 3 – Aug 2012-July 2013**

Research 50%  
Clinic clerkship 50%

This is the proposed year for defense of thesis work and return to the clinical phase of MD/PhD training. The clinical phase consists of 1.5 years of clerkships, including NBME (National Board of Medical Examiners) Shelf and USMLE (United States Medical Licensing Examination) STEP 2 examinations.

### **Year 4 – Aug 2013-May 2014**

Clinic clerkship 100%

This is the anticipated year of graduation.

**Additional coursework** will consist of symposia and journal clubs offered by the Department of Microbiology and Pathology, focusing on bacterial pathogenesis and host immunity, and topics of professional development:

- MIC710-Developing Communication Skills for Microbiologists
- MIC772-Bacterial Pathogenesis Journal Club PAT794-Comparative Medicine Seminar

### **Additional training opportunities:**

- This training plan will allow the PI (Calix) to develop networks with researchers at public health institutions, including the CDC and other Latin American collaborators; the supervisor (Nahm) will actively introduce the PI into the network of collaborators
- The PI and supervisor will work to have the PI attend and present at meetings focused on issues of vaccine development and public health, including the regional UAB Minority Health Research Center symposia
- Researchers in the pneumococcal field and based in the southeast (Mississippi, Alabama, Georgia and Tennessee) meet regularly to present work and discuss collaborations. The PI's work will be presented at these annual gatherings.
- We plan to present all the work from this study plan at the next International Symposia on Pneumococci and Pneumococcal Disease in Brazil, April 2012. This will be an invaluable training experience for the PI.
- The PI will continue to have a leadership role in supporting the administration of the MSTP, namely in recruitment efforts.

### *DOCTORAL DISSERTATION OR OTHER RESEARCH EXPERIENCE*

My first research experience was in a summer undergraduate research program fellowship during my junior year of undergraduate study. I was under the mentorship of Dr. Jurgen Brojatsch at Albert Einstein College Medicine. His lab focused on anthrax toxin-mediated cell killing of host immunological cells, including macrophages and dendritic cells. For eight weeks, my work focused

on describing the kinetics of organelle destruction in macrophages following treatment with toxin. Electron microscopy had shown the appearance of double-membrane vesicles within anthrax toxin-treated macrophages, so we hypothesized that type II cell death (i.e. autophagy) was induced in these cells by the toxin. To support this conclusion, I attempted to follow the destruction of cell structures in relation to the timing of cell death. I hypothesized that certain structures, such as the actin cytoskeleton, would remain intact until late in the death process, i.e. a marker of type II cell death. Since this was my first research experience, most of my eight week fellowship was developing basic laboratory techniques, e.g. tissue culture, etc. However, I was able to obtain enough data to be presented at a poster session at the end of the fellowship. I also used these findings to develop my undergraduate thesis, present at the Loyola University Undergraduate Biological Research Symposium (UBRS) and at the Association of Southeastern Biologists Regional Conference in 2005.

Upon beginning my senior year, I began working in the laboratory of Dr. Patricia Dorn at Loyola University New Orleans. Her lab focuses on Chagas disease (American trypanosomiasis), its epidemiology and the distribution of insect vectors. I worked in the laboratory part time and worked as her teacher's assistant. My research project attempted to detect genetic flow between different Latin American populations of the vector *Triatoma dimidiata*. By genotyping bugs collected from different sites and implementing population genetics models, we determined the existence of barriers to intermigration by bug populations. I was awarded the Loyola University Richard Frank Grant for undergraduate research to perform this project. I also presented my findings at the UBRS (only the second student in Loyola history to present twice at this event in the same year), and was rewarded a travel grant to present my research at the 54th Annual American Society of Tropical Medicine and Hygiene. Unfortunately, my research was interrupted by Hurricane Katrina, and I was unable to obtain enough data for publication.

During the medical portion of my MD/PhD training at the University of Alabama at Birmingham School of Medicine (UAB), I did three laboratory rotations. One rotation was in the lab of Dr. Charles Elson, during which I attempted to detect serological reactions to bacterial proteins in patients with Crohn's disease or ulcerative colitis. During my rotation in the lab of Dr. Suzanne Michalek, I helped quantify cytokine production in Toll-like receptor knockout mice, in response to infection with *Porphyromonas gingivalis*. I also rotated and eventually joined the laboratory of Dr. Moon Nahm, where I currently train.

Dr. Moon Nahm and his lab focus on the development and monitoring of effective vaccines against respiratory pathogens, namely *Streptococcus pneumoniae*. Accordingly, the lab studies host-pathogen interaction mediated by bacterial polysaccharide (PS) capsule. During my summer rotation, I attempted to describe the PS structure of a newly identified subtype of serotype 11A, currently known as 11E. Due to technical limitation of our methods, I was unable to determine the structure before resuming medical school classes. (Other efforts in the lab were able to eventually determine the structure through research collaboration with an extramural lab.) I became interested in the genetic basis for the serological and structural differences between the serotypes, and identified biological activity of an O-acetyltransferase, *wcjE*, as the only difference between serotypes 11A and 11E. These findings have been presented in national conferences, are published and are the basis of this proposal and my doctoral thesis, which focuses on determining the role *wcjE* in relation to strains expressing serotype 11A surviving within a host.

Other research-related activities I have done since joining the lab includes the presentation of my qualification examination. As part of the curriculum in the UAB Department of Microbiology, we must present and defend a grant proposal for a project unrelated to our work in lab, prior to proposing our thesis project. My grant proposal focused on *Escherichia coli* K1 capsule interactions with host lectins and the effects of capsular O-acetylation. For this work I won a departmental award for outstanding performance. Additionally, I have collaborated with an epidemiology study of pneumococcus in daycare centers in Brazil. During this opportunity, I became familiar with the monoclonal antibody serotyping methods developed and extensively used in this lab. In addition to

the work outlined in this project, I am working in another research collaboration to describe the PS structure of other *wcjE*-related serotypes. I have developed an interest in O-acetylation of bacterial surface structures and the effect of this modification on host interactions. It is these experiences that have prepared me for achieving the goals stated in this proposal.

# Summary Statement

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<b>SUMMARY STATEMENT ( Privileged Communication )</b>	
<b>PROGRAM CONTACT:</b>	Diane Adger-Johnson
<b>Release Date:</b>	07/19/2010
<b>Application Number:</b>	1 F31 AI093103-01
<b>Principal Investigator</b>	Calix, Juan
<b>Applicant Organization:</b>	University of Alabama at Birmingham
<b>Review Group:</b>	ZRG1 F07-C (20) Center for Scientific Review Special Emphasis Panel Fellowship: F07 Immunology Fellowship AREA
<b>Meeting Date:</b>	06/28/2010
<b>Council:</b>	OCT 2010
<b>Requested Start:</b>	N/A
<b>RFA/PA:</b>	
<b>PCC:</b>	X79A
<b>Project Title:</b>	The role of wjE disruption in pneumococcal serotype 11A humoral escape
<b>SRG Action:</b>	Impact/Priority Score: 19
<b>Human Subjects:</b>	44-Human subjects involved - SRG concerns
<b>Animal Subjects:</b>	44-Vertebrate animals involved - SRG concerns

## RESUME AND SUMMARY OF DISCUSSION:

This is a new application for an F31 Ruth Kirschstein National Research Service Award (NRSA) for an Individual Predoctoral Fellowship to Promote Diversity in Health-Related Research (Parent F31). The application proposes to study a capsule gene, wjE8, in *Streptococcus pneumoniae*, which

encodes a putative O-acetyltransferase and the role it plays in evading host defense in the serotypes 11A and 11E. The candidate proposes to complete an MD/PHD program while studying how the *wcjE8* gene and pneumococcal pathogens evade host defenses. Since this gene is generally found in the pneumococcal pathogens, thus further understanding how they affect the pathogenicity of the bacteria is highly clinically important. The candidate is exceptional, has outstanding academic grades, and has a long standing interest in infectious diseases. The Sponsor has trained many students and has significant expertise in this area of science to train a clinician/scientist although the current size of his laboratory might limit one-on-one time with this student. The Training Plan is thorough multi-pronged approaches to study the underlying microbial pathogenesis which is likely to lead to advancements in vaccine development. However there was concern that this was overly ambitious for an MD/PHD program. The environment is at an outstanding university to complete the proposed project. While this is an exceptional candidate and undoubtedly capable with the help of his sponsor to complete this project, there are concerns that since most of the studies will need to be developed, this will be outside the time limitations of an MD/PHD program. There was one comment on the presentation of the application and that is that the Human Subjects and Biohazards categories were not properly presented and this is a major concern. Nevertheless there was high enthusiasm for the candidate, sponsor, environment, Training Plan, and the significance of the project although tempered by concerns for the ambitious project.

**DESCRIPTION (provided by applicant):**

The success of *Streptococcus pneumoniae* (pneumococcal) as a human pathogen is largely due to its capability to employ a polysaccharide (PS) capsule to avoid host immunity. Recent discovery and characterization of the pneumococcal serotype 11E strongly suggested that inactivation of the capsule synthesis gene *wcjE8* which encodes a putative O-acetyltransferase, plays an important role in escaping a host humoral immune response to the closely related serotype 11A. Since many epidemiologically prevalent serotypes also contain the gene, discerning the effects of WcjE-mediated variable O-acetylation on the serological properties of serotypes 11A and 11E will aid in understanding pneumococcal pathology, in determining trends of emerging pneumococcal serotypes and in designing effective PS-based vaccines. Previously serotype as 11A, the true epidemiological nature of 11E is unclear. Clinical isolates originally serotype as 11A will be readdressed for the expression of 11E capsule using serospecific monoclonal antibodies. To understand the serological flexibility of these serotypes, the genetic properties that lead to seroswitching between 11A and 11E will be further studied. Additionally, the role *wcjE* inactivation plays in avoiding a human humoral response will be evaluated using an in vitro opsonophagocytosis killing assay (OPKA) with sera from humans vaccinated with the 23-valent polysaccharide vaccine, which includes 11A PS. Independent and competitive OPKA survival of strains expressing 11A and 11E capsule will be compared, and the capacity of purified 11A and 11E PS to inhibit functional antibodies will be determined. Finally, the capacity of serotype 11A to escape an 11A-specific humoral response through *wcjE*-inactivation in vivo and the effects on pathology will be verified in a murine infection model. The survival of 11A and 11E strains will be compared under naive conditions, with passive

11A-specific immunization, and with preimmunization against 11A and 11E PS. These assays will also provide preliminary information on the use of 11E as a potential vaccine against both serotypes.

**PUBLIC HEALTH RELEVANCE:**

Understanding how *Streptococcus pneumoniae* serotypes escape an immune response is important for the design of interventional and preventative strategies against this significant human pathogen. This proposed research will study the effects of O-acetylation modification of *S. pneumoniae* capsule on antibody-dependent clearance of the bacteria, in relation to the prevalent serotype 11A and the closely related serotype 11E.

**CRITIQUE 1:**

Fellowship Applicant: 2  
Sponsors, Collaborators, and Consultants: 2  
Research Training Plan: 4  
Training Potential: 4  
Institutional Environment & Commitment to Training: 2

*Overall Impact: Strengths*

- Training physician-scientists to conduct basic/translational research is critical to perform biomedical research.
- The findings from this investigation will be helpful to design new therapeutic strategies to minimize morbidity and mortality associated with pneumococcal.
- The fellow has an outstanding GPA.
- The sponsor, Dr. Nahm, is an accomplished expert in the field.
- The environment at the UAB is outstanding and collaborative.

*Weaknesses*

- Descriptive nature of the proposal dealing with molecular and cellular pathogenesis of pneumococcal (11A and 11E).
- It appears that the proposal is ambitious. In particular aim 3
- Aims 2 and 3 do not have preliminary data.

*1. Fellowship Applicant:*

*Strengths*

- He has clear objectives of the training necessary for translational research after obtaining MD/PhD.
- Has published one manuscript as the first author. In addition. Mr. Juan is a co-author in one more paper.
- The letters of recommendation are exceptional.

*2. Sponsors, Collaborators, and Consultants:*

*Strengths*

- Excellent mentor, Dr. Moon Nahm.

*Weaknesses*

- It would be better to have a mentoring team to monitor the progress of Mr. Juan.

*3. Research Training Plan:*

*Strengths*

**Weaknesses**

- Ambitious proposal.
- No preliminary data for Aims 2 and 3.

#### 4. Training Potential:

##### Strengths

- Outstanding applicant.
- Accomplished mentor(s).

##### Weaknesses

- The training plan/proposal needs to be revised.

#### 5. Institutional Environment & Commitment to Training:

##### Strengths

- Exceptional.
- Collaborative.

**Protections for Human Subjects:** Unacceptable

**Vertebrate Animals:** Acceptable

- Estimate for each experiment needs to be stated.

**Biohazards:** Acceptable

**Select Agents:** Acceptable

**Responsible Conduct of Research:** Acceptable

**Budget and Period of Support:** Recommend as Requested

**Resource Sharing Plans:** Acceptable

## CRITIQUE 2:

Fellowship Applicant: 1

Sponsors, Collaborators, and Consultants: 2

Research Training Plan: 4

Training Potential: 3

Institutional Environment & Commitment to Training: 2

##### Overall Impact:

##### Strengths

- A highly qualified applicant with longstanding interests in infectious diseases and academic medicine who has the potential to become a leader in the field.
- Sponsor is a successful clinician investigator with a strong history of mentoring postdoctoral fellows.
- Institutional commitment, sponsor's research activities and that of surrounding investigators provides a rich environment for training clinician scientists in infectious diseases.
- Research hypothesis is novel and research has important implications for vaccine development and public health.

## Weaknesses

- The project is highly ambitious.
- Technical concerns with proposed FACS assay and applicability to the desired use.

### 1. Fellowship Applicant:

#### Strengths

- Candidate's scholastic performance has been consistently high with mostly "A"s in his undergraduate science courses to Pass or "A"s in his MSTP coursework at UAB.
- He desires a career in academic medicine specializing in pediatric infectious diseases and his undergraduate research experiences (anthrax-toxin killing of macrophages, Chagas disease) demonstrate his longstanding interests in infectious diseases.
- He has passed his qualifying exam and received the Microbiology Department's award for the most outstanding qualifying examination.
- Completing his 2nd yr in the research portion of the MSTP program, he is 1st author on the publication (co-authored with Dr. Nahm, his major advisor) in J.Inf.Dis. that serves as the basis of his doctoral research and co-author on another publication in *S. pneumonia* epidemiology.
- Letters of recommendations describe a clear thinker and excellent communicator who are passionate about his research, very knowledgeable of the research literature and connect the basic sciences to clinical relevance; an individual who is committed to academic medicine and has the potential to be a leader in the field; among the best and the brightest.

## Weaknesses

### 2. Sponsors, Collaborators, and Consultants:

#### Strengths

- Dr. Moon Nahm has extensive record of mentoring postdoctoral scientists.
- The 3 PhD and 1 MD research associates in the lab provide ample opportunities for the applicant discuss technical and scientific issues with knowledgeable investigators.
- The research activities of the laboratory are an excellent match with the applicant's long term career focus in pediatric infectious diseases. The laboratory is productive with publications of both clinical and basic science relevance.
- The laboratory is also the NIH Reference Laboratory for respiratory bacterial pathogens and the ongoing collaborations with both basic science and clinician researchers provides opportunities for the applicant to participate in collaborative clinical and infectious disease research relevant to public health.

## Weaknesses

- The sponsor has limited experience training predoctoral trainees (total of 4).
- Including the applicant, there will be three graduate students in the 9-10 member laboratories over the training period. There is a concern whether the applicant will have sufficient access to Dr. Nahm.

### 3. Research Training Plan:

#### Strengths

- Project builds upon applicant's observations that a newly identified pneumococcal capsule serotype (11E) results from inactivation of an O-acetyltransferase gene in serotype 11A strains and that all isolates examined to date contain genetically unique mutations in this gene (*wcjE*) suggesting that emergence of serotype 11E strains were each independent events.
- Applicant's hypothesis is novel - 11E strains are not inter-host transmissible, but emerge during nasopharyngeal (NP) carriage of 11A strains due to selective pressure by the host humoral response (favoring loss of *wcjE*) and the competitive advantage of 11E over 11A strains within the host leads to invasive disease by 11E.
- Validation of applicant's hypothesis would describe a mechanism of pneumococcal pathogenesis that may be generally relevant to pneumococcal disease in vaccinated individuals and have important consequences for vaccine design and disease surveillance and control.

### Weaknesses

- The proposal overall is highly ambitious, bordering on over ambitious; The 3 aims test different aspects of applicant's hypothesis - Epidemiological study of 11A typed NP clinical isolates and sequence characterization of *wcjE* genes from additional 11E strains, Preferential recognition and killing in vitro of 11A vs. 11E strains by sera from vaccinated donors, selective growth/survival of 11E vs. 11A in vivo in a mouse infection model. - Each aim individually could provide the foundations for an interesting research project.
- Although feasible, each aim proposes development/adaptation of reagents or methods for the 11A/11E serotype (flow cytometry assay for stereotyping of 11A/11E strains, construction of isogenic, antibiotic-tagged 11A/11E strains, a mouse infection model). If reagent/methods development is not successful, the ability to complete the remaining sub aims or rigorously interpret the resultant data is compromised. Alternative strategies were not proposed.
- Value of the flow cytometric assay (Aim 1) is the potentially increased sensitivity to detect the presence of an 11E subpopulation in 11A NP isolates. Preliminary data shows differences in mab recognition of pure cultures of 9V/9A strains. However, it is doubtful that the assay as proposed will have sufficient resolution and sensitivity to detect a small 11E subpopulation in an 11A/11E mixed culture.
- Aim 3 - missing important experimental details (no. mice/group, frequency of sampling, infection optimization criteria etc).
- Aim 2 - uses sera from vaccinated individuals and Aim 1 includes 11A isolates from pediatric patients - thus specimens from human subjects are involved. Although the research likely qualifies as exempt, justification under protection of human subjects is missing.

#### 4. Training Potential:

### Strengths

- The multi-pronged approach proposed to test applicant's hypothesis will provide broad exposure to experimental methodologies used in microbial pathogenesis and vaccine research.
- Sponsor's training plan provides a nice balance of basic and clinical sciences.
- Plan provides opportunities for the applicant present his research at national/international meetings and to participate in collaborative studies that emerge from his research findings.

### Weaknesses

- The over ambitious character of the research plan reduces the exceptional training potential of this proposal.

5. Institutional Environment & Commitment to Training:

**Strengths**

- MSTP preclinical and research training are well integrated.
- Research environment in general and pneumococcal research at UAB is extremely strong and provides ample opportunities for the applicant to attend seminars, journal clubs, monthly multi- investigator laboratory meetings pertinent to his research and to expand his intellectual horizons.
- Research facilities are excellent.

**Weaknesses**

**Protections for Human Subjects: Unacceptable Risks and/or Inadequate Protections**

- Experiments will use sera collected from vaccinated individuals. Source is not described and justification for exemption is not provided.

**Inclusion of Women, Minorities and Children:**

G4U - Gender Unknown, Unacceptable

M4U - Minority Representation Unknown, Unacceptable

C4U - Children Representation Unknown, Unacceptable

- Research is likely using de-identified samples, composition of women, minorities and children unknown.

**Vertebrate Animals: Unacceptable**

- Most of the 5 points are adequately addressed except for point 1.

**Biohazards: Unacceptable**

- There should be a description of precautions and containment procedures in place for infectious pneumococcal strains.

**Training in the Responsible Conduct of Research:**

- MSTP 2 formal courses: Medical Ethics and Principles of Scientific Integrity; Principles of Scientific Integrity
- Online courses in HIPAA regulations, animal treatment and scientific integrity
- Survey of ethical issues and principles in practice of science - topics include: nature, extent and causes of scientific fraud, UAB policies on fraud; responsibilities of authorship and peer review; ideals of good science, bias and sloppy practices; responsible use of the press; commercialization of research; science and public policy; issues in animal experimentation and in clinical trials. Discussion of case studies and video clips illustrating topics.
- No description of faculty participation was provided by applicant.
- Scientific Integrity - 3 credit course, semester long.
- Frequency is appropriate.

**Budget and Period of Support: Recommend as Requested**

## CRITIQUE 3:

Fellowship Applicant: 1

Sponsors, Collaborators, and Consultants: 2

Research Training Plan: 2

Training Potential: 2

Institutional Environment & Commitment to Training: 1

*Overall Impact:*

### **Strengths**

- Letters of recommendation are unusually strong.
- Applicant already as a first author publication.
- Sponsor is leader in the field, provides an outstanding environment.
- Proposal is nice mix of human and mouse studies, clinically relevant.
- Overall environment is excellent.

### **Weaknesses**

*1. Fellowship Applicant:*

#### **Strengths**

- LORs are impressive. One referee compares him to Roger Perlmutter!
- Grades as MSTP student are strong.
- Clear commitment to research.
- Already has 1st author publication.

#### **Weaknesses**

*2. Sponsors, Collaborators, and Consultants:*

#### **Strengths**

- Outstanding environment.

#### **Weaknesses**

- Holds Saturday journal clubs.

*3. Research Training Plan:*

#### **Strengths**

- Well written, hypotheses clear and testable.
- Innovative.
- Nice mix of human and mouse studies, with some mechanistic focus.
- Clearly feasible in the environment provided.
- Some innovative approaches, such as FACS-based analysis of serotypes.

#### **Weaknesses**

*4. Training Potential:*

#### **Strengths**

- Particularly good statement of goals – believable, enthusiastic, sincere.
- Great grades, clearly works hard and doesn't shirk challenging projects and environments.

*Weaknesses*

- None noted.

*4. Institutional Environment & Commitment to Training:*

**Strengths**

- UAB is an outstanding environment for this topic, training students.

**Weaknesses**

**Protections for Human Subjects:** Unacceptable

**Inclusion of Women, Minorities and Children:** Unacceptable

**Vertebrate Animals:** Unacceptable

- Although thorough in its description, there is no listing of the # of mice to be used.

**Biohazards:** Acceptable

**Training in the Responsible Conduct of Research:** Acceptable

**Budget and Period of Support:** Recommended as Requested

**THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:**

**PROTECTION OF HUMAN SUBJECTS (Resume):** The studies will assess human humoral immune responses from serum although there is no mention on how they will obtain these samples or whether they will be identifiable or not. This is essential and can bar funding. **UNACCEPTABLE**

**VERTEBRATE ANIMALS (Resume):** Most of the 5 points are missing as are the numbers of animals to be used which can bar funding. **UNACCEPTABLE**

**TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH:** Acceptable

**COMMITTEE BUDGET RECOMMENDATIONS:** The budget was recommended as requested.

Content last reviewed on October 23, 2017