Career Development Series 2020

K Awards: The Next Step

Presentation will begin at 12:00 PM (PT)

ITHS | Institute of Translational Health Sciences

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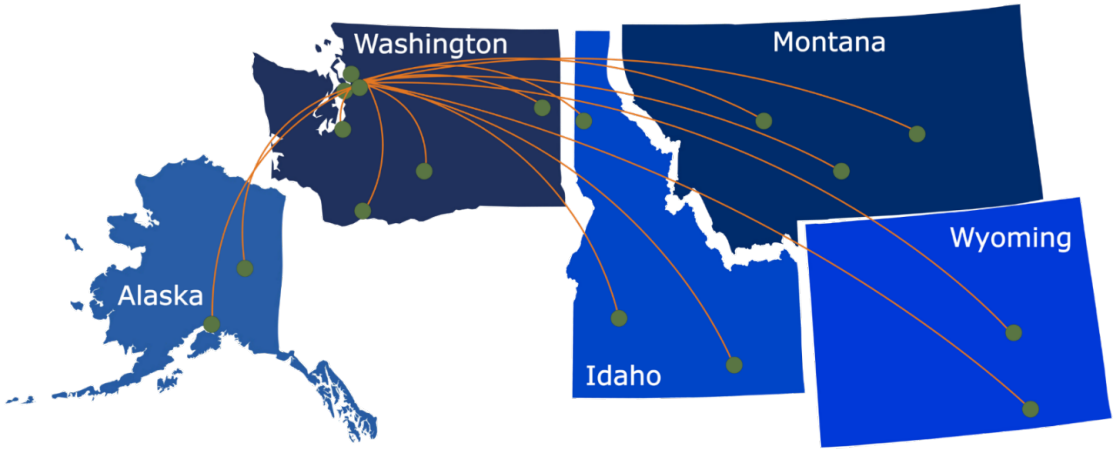

What We Offer:

1. **Research Support Services:** Members gain access to different research services, resources, and tools offered by ITHS, including the ITHS Research Navigator.

2. **Community Engagement:** Members can connect with regional and community based practice networks.

3. **Education & Training:** Members can access a variety of workforce development and mentoring programs and apply for formal training programs.

4. **Funding:** Members can apply for local and national pilot grants and other funding opportunities. ITHS also offers letters of support for grant submissions.

Contact our **Director of Research Development**

- **Project Consultation**
- **Strategic Direction**
- **Resources and Networking**

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ithsnav@uw.edu
206.616.3875

Feedback

At the end of the seminar, a link to the feedback survey will be sent to the email address you used to register.

Career Development Series 2020

K Awards: The Next Step

September 30, 2020

Presented by Sheila Lukehart, PhD

Emeritus Professor of Medicine and
Global Health at the University of Washington

ITHS | Institute of Translational Health Sciences

ACCELERATING RESEARCH. IMPROVING HEALTH.

Learning Objectives

1. Attendees will be able to select the correct K award mechanism for their circumstances.
2. Attendees will be able to develop a detailed career development section and research plan.
3. Attendees will be able to format an application to make it more attractive to reviewers.

NIH and Career Development Awards

- NIH Organization
- Types of career development awards
- Getting information about K's
- Components of a K application
- Tips on writing a great application
- Review process

NIH Structure

27 Institutes & Centers

- NIAID
- NCI
- NHLBI
- NINDS
- NIAMS
- Fogarty Int'l Center

Divisions / Branches
- DAIDS
- DMID
- DAIT

Office of Research Training

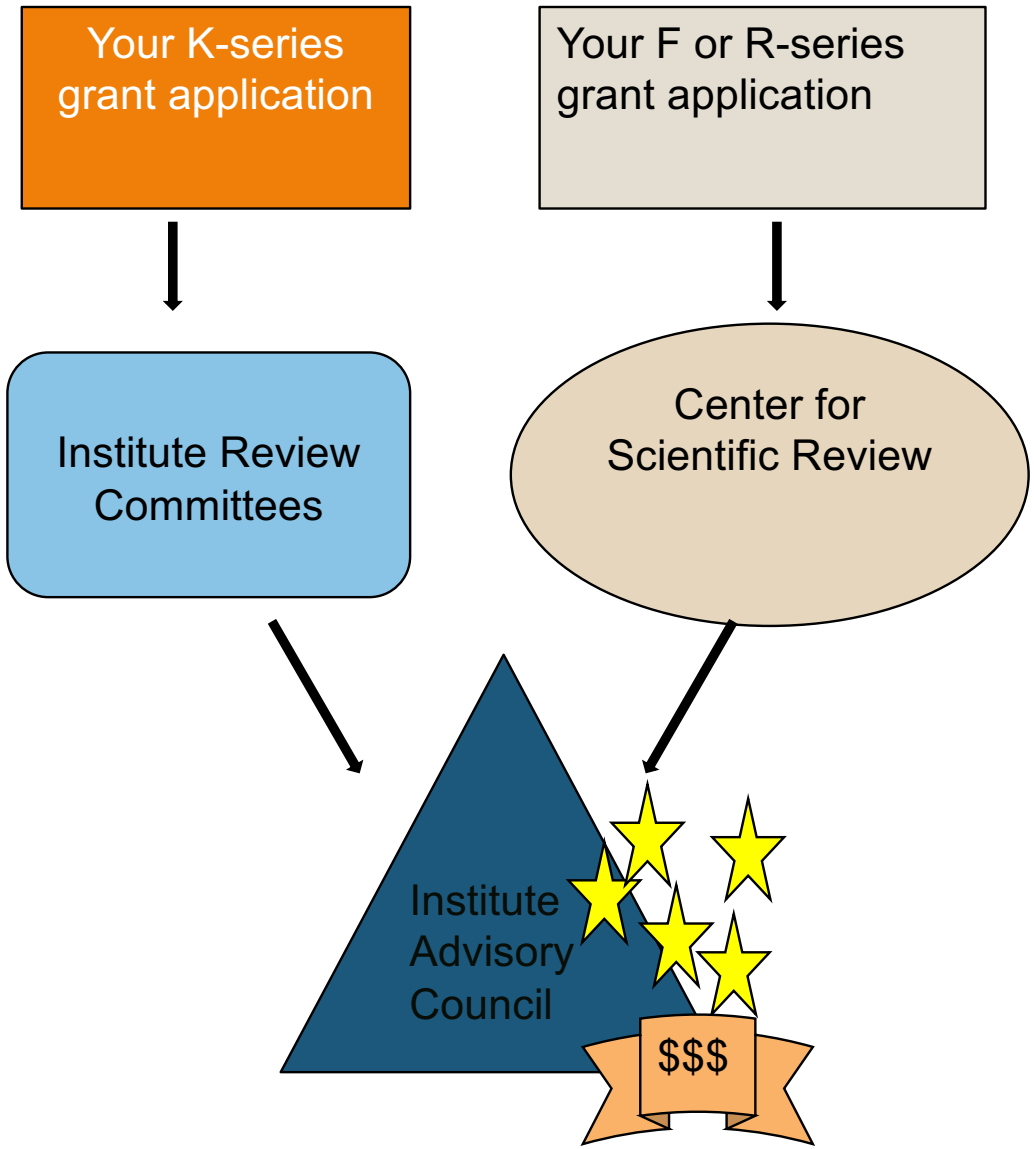
Scientific Review Program
- Review Committees--SRO

Advisory Council

Center for Scientific Review

Divisions
- Study Sections--SRO

Application Pathway



Types of Career Training Awards

- US citizen, permanent resident
  - K08
  - K23

    MD, DVM, DDS, other Clinical Doctorate
  - K01
  - K22
  - K25

    MD or PhD
- US Citizen/PR or Non-citizen
  - K99/R00 Pathway to Independence: MD or PhD
  - K99/R00 Physician/Scientist (NIAID only): MD only

Some institutes don't offer all grant mechanisms

Overview of Relevant K Awards

NIH Research Career Development Awards

https://researchtraining.nih.gov/programs/career-development

Mentored Career Development: K Awards

- **K08** Mentored Clinical Scientist Research Career Development Award
  - Laboratory focused research
  - May use human samples
- **K23** Mentored Patient-Oriented Research Career Development Award
  - Patient oriented research
    - Clinical trial not allowed
    - Clinical trial required
    - Independent basic experimental studies with humans required

Clinical doctoral degree: MD, DVM, PharmD
US citizen, permanent resident

Mentored Career Development: K Awards

- **K01** Mentored Research Scientist Development Award
  - Institute-specific purposes (e.g. NIAID limits to epidemiology, modeling techniques, and outcomes research)
- **K25** Mentored Quantitative Research Career Development Award
  - Quantitative or engineering degree moving to health-related topics

PhD or MD (or other doctorate)
US citizen, permanent resident

Mentored Career Development: K Awards

- Require 75% protected time for research and training
- 3-5 years duration
- Stipend ($50-100K per year)
- Very modest funds for research

K Awards: Transition to Independent Career

- **K99/R00** NIH Pathway to Independence Award
  - Mentored/Independent phases
- **K22** Career Transition Award
  - Not mentored

Participation varies by institute—check with your institute!!

K99/R00 Pathway to Independence

- Transition award for finishing postdoc and moving to Assistant Professor
- No more than 4 years of postdoctoral research* experience at the time of submission (or resubmission)
- 3-5 years of support
- Non-citizens eligible

*Eligibility extended by two cycles for JunJul 2020 – FebMar 2021 due dates

Uses and rules vary by institute—check with your institute

K99/R00 Pathway to Independence

K99 years

- Apply for K99 phase with specific postdoc career development and research plan, include broad description of independent phase
- Provides salary support ($50-100K) and benefits
- Modest research support ($20-50K/year)
- 1-2 years

Uses and rules vary by institute—check with your institute

K99/R00 Pathway to Independence

R00 years

- Total cost cannot exceed $249,000* per year
- Includes salary, fringe, research costs, **and indirect costs**
- Up to 3 years

***~$160 direct costs**

Uses and rules vary by institute—check with your institute
May have special areas of focus

K22 Career Transition Award

Two phases

1. Submit application while at postdoc institution
   - Applicant accomplishment/potential and scientific merit
   - No institution or $$ yet
2. Assistant Professor (2-3 years)
   - Protected research time (≥75%)
   - $150K/$100K DC (NIAID)
   - $150K/year (NCI)
   - Limit of $50-100K per year for salary

Participation varies by institute—check with your institute

K22 Research Scholar Development

- Participation varies by institute
- Eligibility
  - Must not have >5 years* of postdoc training at time of application or resubmission(NIAID) OR
  - Have 2-8 years of postdoc training (NCI)
  - Must not have held an independent research position anywhere
  - Must not have been PI on another K award, R01 or equiv, or Project leader on P01 or U19.
  - PI of R03 (or R21) is OK if specific aims are the same as in K22 application
  - Must not have another K application pending

*Eligibility extended by two cycles for JunJul 2020 – FebMar 2021 due dates

Participation varies by institute—check with your institute

Finding information and contacts at NIH

Go to NIH Career Development Award page

https://researchtraining.nih.gov/career-path

Program Announcement

Department of Health and Human Services

Part 1. Overview Information

Participating Organization(s)	National Institutes of Health (NIH)
Components of Participating Organizations	National Heart, Lung, and Blood Institute (NHLBI) National Human Genome Research Institute (NHGRI) National Institute on Aging (NIA) National Institute on Alcohol Abuse and Alcoholism (NIAAA) National Institute of Allergy and Infectious Diseases (NIAID) National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) National Institute of Biomedical Imaging and Bioengineering (NIBIB) *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) National Institute on Deafness and Other Communication Disorders (NIDCD) National Institute on Drug Abuse (NIDA) National Institute of Mental Health (NIMH) National Institute of Nursing Research (NINR) National Center for Complementary and Alternative Medicine (NCCAM) Division of Program Coordination, Planning and Strategic Initiatives, Office of Research Infrastructure Programs (ORIP) Office of Behavioral and Social Sciences Research (OBSSR) Office of Dietary Supplements (ODS) **Special Note**: Because of the differences in individual Institute and Center (IC) program requirements for this FOA, prospective applicants strongly e the Table of IC-Specific Information, Requirements and Staff Contacts, to make sure that their application is responsive to the requirements of one NIH ICs.
Funding Opportunity Title	**Mentored Research Scientist Development Award (Parent K01)**
Activity Code	K01 Research Scientist Development Award - Research & Training
Announcement Type	Reissue of PA-11-190
Related Notices	• June 4, 2014 - Notice NOT-14-074 supersedes instructions in Section III.3 regarding applications that are essentially the same. • May 2, 2014 - See Notice NOT-OD-14-088. Notice of Clarification of Career (K) Award Eligibility. • February 27, 2014 - See Notice NOT-EB-14-003. Notice of Change to the Duration of Career Development Awards Supported by the NIBIB. • February 3, 2014 - See Notice NOT-HG-14-018. Notice of NHGRI Participation.
Funding Opportunity Announcement (FOA) Number	**PA-14-044**
Companion Funding Opportunity	None
Number of Applications	See Section III. 3. Additional Information on Eligibility.
Catalog of Federal Domestic Assistance (CFDA) Number(s)	93.242; 93.856; 93.855; 93.846; 93.213; 93.279; 93.839; 93.838; 93.837; 93.233; 93.361; 93.273; 93.286; 93.866; 93.351; 93.173; 93.865
Funding Opportunity Purpose	The purpose of the NIH Mentored Research Scientist Development Award (K01) is to provide support and "protected time" (three, four, or five years) f supervised career development experience in the biomedical, behavioral, or clinical sciences leading to research independence. Although all of th Institutes and Centers (ICs) use this support mechanism to support career development experiences that lead to research independence, some ICs us individuals who propose to train in a new field or for individuals who have had a hiatus in their research career because of illness or pressing family ICs utilize the K01 award to increase research workforce diversity by providing enhanced research career development opportunities. Prospective ca encouraged to contact the relevant NIH staff for IC-specific programmatic and budgetary information: Table of IC-Specific Information, Requiremen Contacts.

READ THIS CAREFULLY!!

- Purpose
- Eligibility
- Deadlines
- Page limits
- Links to forms
- Required sections
- Review criteria
- Animal, human subjects info
- Contacts

Things to do ahead of time

- Obtain preliminary data to support hypotheses
- Publish papers
- Develop a good mentoring team

Preparing to submit an application

- Read the instructions!
  - Program Announcement—has link to forms
  - SF424 Instructions

  https://grants.nih.gov/grants/how-to-apply-application-guide.html

  Item K: Specific instructions for K applications
- Be aware of page limits
- Look at grant tutorials online
- Read a successful application (or two!)

How to Get Started

- Administrative Issues—Division Administrator
- Timeline for preparing the application
- Mechanics: Putting Your Best Foot Forward
- Business pages
- Components of K Applications
- Understanding the review process

Administrative Issues: Their Rules and Yours

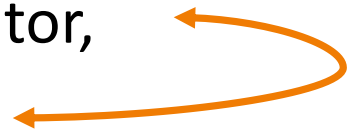
- Figure out what kind of application you will be writing—discussion with mentor
- Read the Program Announcement and Instructions—and read them again!
- Talk with a NIH Training Officer
- Talk with your dept'l or division administrator

Timeline: Writing the application

- Start planning and writing very early
- Talk with the administrator who will assist with application
- Talk with your mentor
- Have your mentor and others read the full application early

Timeline for Writing a Grant Application

>4 months ahead	Read NIH website about grants Talk with NIH official Decide on grant mechanism Discuss with your mentor and grants administrator
Week -12 to -14	Think, read, cogitate about career development and research plans
Week -10	Draft Specific Aims, give to mentor, meet to discuss, revise
Week -6	Give full draft of application to mentor and others; request letters



Timeline for Writing a Grant Application

Week -6	Work on business pages (biosketch, equipment, facilities/biohazards, HS, VA, authentication of reagents, etc)
Week -5	Revise draft
Week -3	"Final" draft to mentor
	Begin to route business pages
Week -2	Finished full text sent to Institutional Grants Office
Week -1	Submit to agency
Due Date	It's there on time!!!

Mechanics: Writing the application

- Use formal language—no slang or jargon
- Use correct grammar, punctuation
- No typos!
- Pay attention to required fonts, margins, page limits
- Leave white space on the pages-not solid text

Boring—and causes tired eyes……

these two lipoproteins as adhesins. The strain expressing both DbpA and DbpB acquired the ability to bind epithelial cells while only DbpB showed specificity for glioma cells *in vitro* (5). Later studies with the neuroborreliosis patients validated our results since antibodies mainly against DbpB were present in CSF after colonization by Lyme spirochetes (4, 12). Therefore, we anticipate that our *in vitro* experiments in the initial screen using non-infectious *B. burgdorferi* will identify surface localized *T. pallidum* adhesins. This non-adherent strain offers a cleaner background to study binding mechanisms since it does not express *B. burgdorferi* adhesins. Candidate adhesins identified from this experiment will help us select 3-4 surface proteins to express in the infectious, bioluminescent *B. burgdorferi* strain. ¶

We will first select the best luciferase reporter system and most useful promoter to express this reporter for *in vivo* imaging in the small animal model. Then, we will express and characterize the promising *T. pallidum* proteins, identified from the initial screen, in the infectious, sequenced *B. burgdorferi* strain to assess adherence to placental and neuronal cell lines *in vitro*. These results will form a foundation for our *in vivo* assessment of *T. pallidum* proteins in colonization of placenta and neuronal tissues. Hence, using the gain of function approach *in vitro* will allow us to test its validity also in the mouse model of infection. ¶

1A. Identification and characterization of *T. pallidum* adhesins with affinity for placental and/or neuronal tissues and other virulence factors. We have selected several genes of *T. pallidum* for the initial screen to determine them as candidate adhesins in this study. We will obtain clones containing these genes from Drs. Sheila Lukehart and Arturo Centurion at University of Washington at Seattle (please see their letters of support). We will also produce respective recombinant tagged proteins in *E. coli* and generate polyclonal antibodies against the proteins for which antisera are not available from our collaborators. ¶

We considered different features in selection of these proteins, such as; they (i) are known to be expressed during congenital syphilis or neurosyphilis on the basis of serological analysis, (ii) show specificity to a particular host receptor expressed in placenta and/or neuronal tissues, (iii) exhibit other potential activities important for pathogenesis, and (iv) were previously described membrane proteins with unknown function. Selected eight *T. pallidum* proteins, TP0171, TP0319, TP0435, TP0574, TP0954, TP0957, TP0971, and TP1037 have potential to contribute to neurosyphilis or congenital syphilitic manifestation. We will clone the genes along with their promoters in *B. burgdorferi* shuttle vector and transform the non-infectious *B. burgdorferi* B314 strain, which was also used to examine role of DbpA-DbpB, as described above (rationale). We will first assess the function of *T. pallidum* proteins expressed in *B. burgdorferi* as a surrogate system *in vitro*. Expression of *T. pallidum* genes in *B. burgdorferi* will be confirmed by Western blotting. Some of the selection criteria for candidate proteins are described here. ¶

(i) Several immunogenic proteins are identified but their functions not yet determined. TP0171 is a 15kD lipoprotein, which shows homology to proteins of *Listeria monocytogenes* and *L. innocua*, two pathogens causing adverse outcomes in pregnant women. TP0171 is a major membrane immunogen in *T. pallidum*. TP0435 (17kD) lipoprotein and TP0574 (previously known as TpN47) are two highly immunogenic proteins used in diagnosis of syphilis. However, their localization on the spirochete surface remains questionable and their roles have not been examined. This study will unequivocally determine their subcellular localization in the spirochete and will help us evaluate their roles. If one or more of these proteins are present on the spirochete's surface in our initial screen, they will be selected for further experiments. ¶

(ii) Based upon a comprehensive analysis of the available information, we anticipate that TP0954 protein may located on the outer membrane and may facilitate colonization of placenta and neuronal tissues by *T. pallidum*. If so proved, it will provide a model molecule to study molecular basis of congenital spirochete transmission and neurosyphilis. We anticipate that TP0954 encoded protein will be located on the surface of the *T. pallidum* since it possesses a potential signal peptide. In addition, the predicted 3D-structure of this protein using the Hiden Marko models (HMM) program with Protein Data Bank (PDB) shows similarity with several surface proteins in other organisms. These similar proteins include the PilF outer membrane lipoprotein of *Pseudomonas aeruginosa*, peroxisomal targeting signal 1 binding domain of *Trypanosoma brucei* Peroxin 5 protein, and yeast mitochondrial outer membrane translocon protein Tom70p. All possess tetratricopeptide repeats. Finally, one peptide of TP0954 showed 54% similarity with defined chondroitin sulfate A-binding variable domain of PfEMP1 *Plasmodium falciparum*. Furthermore, PfEMP1 of malaria parasite displayed on infected red blood cells (RBCs) promotes adherence of the RBC to placenta. Interestingly, we have previously shown that DbpB lipoprotein of *B. burgdorferi* shows affinity to chondroitin sulfates and mediates binding to the glial cells. Later analyses of cerebrospinal fluid from neuroborreliosis patients confirmed intrathecal (in situ) expression of DbpB by Lyme spirochetes (4, 12). This collective information strongly supports inclusion of this protein in this proposal. ¶

(iii) TP1037 encoded protein is designated as hemolysin III in the genome. Any organ can be affected due to *T. pallidum* dissemination after infection of the fetus by this spirochete. Anemia is common in congenital syphilis and non-hemolytic anemia can persist for weeks even after treatment (21). It will be useful to determine if hemolysinIII of *T. pallidum* is involved in this manifestation. Hemolysis on blood agar plates stimulated by *T. pallidum* hemolysin III will determine its enzymatic activity *in vitro*. These experiments will functionally establish its current predicted role on the basis of sequence homology with proteins of other pathogens. In addition, we will determine in our later experiments whether the expression of this hemolysin results in anemia in mice, similar to that seen in some syphilis patients and in congenital syphilis. ¶

(iv) We have selected three more proteins, which are known membrane proteins with unknown functions. First, Treponema-specific membrane lipoprotein (*tmpC* or TP0319) is an ABC-type nucleoside transport system that may transport purine nucleosides, which are essential for the survival of *T. pallidum* within its obligate human host. If it is not exposed to the surface of the spirochete in the initial analysis, it will serve as a negative control for all following experiments in the specific aim 2. Second, Dr. Norgard's group recently crystallized the membrane antigen (*tpd* or TP0971) of *T. pallidum*. It shows high affinity for human lactoferrin, suggesting its role as iron scavenger. These two proteins, TmpC and Tpd, are expressed at high levels in *T. pallidum* during infection (19) but their contribution to *T. pallidum* pathogenesis remains to be established. The current study will determine if they are located on the surface and potentially play a role in survival of the spirochetes in specific tissues during infection. Third, TP0957 encoded protein belongs to the extracellular solute-binding transporter superfamily that also includes sialic acid-binding protein in other bacteria. Sialic acids are found widely distributed in mammalian tissues. They are also components of gangliosides and are found attached to the glycosphingolipid (ceramide and oligosaccharide). Since gangliosides are predominantly found in the nervous system, TP0957 could be a potential adhesin for neuronal tissues. ¶

Although some of these selected proteins were initially predicted to be periplasmic proteins, Hazlett and coworkers (2005) showed that several periplasmic proteins of *T. pallidum* can get exposed due to outer membrane destablization facilitated by outer membrane protein encoded by TP0453 (7). Therefore, it is useful to determine exact location of these proteins and assess their roles in colonization of neuronal and/or placental tissues. ¶

1B. Evaluation of *T. pallidum* proteins in adherence to cell lines derived from human placenta and neuronal tissue. Colonization of specific tissues *in vivo* often can be predicted on the basis of *in vitro* binding experiments conducted with relevant cell lines and the pathogen. The focus of this study is to identify proteins important in colonization of placental and/or neuronal tissues. Therefore, we will use the human epithelial cell line obtained from placental choriocarcinoma, CCL-98, and fibroblast cell line, CRL7464 as model for placental colonization, while neuronal cell line, PC12, and C6 glioma cell lines will be used to depict colonization of the central nervous system (CNS) during infection. Radiolabeled *B. burgdorferi* will be used in the binding experiments to assess the contribution of *T. pallidum* proteins in adherence with the gain of function approach. The wells without the cell line monolayers, and *B. burgdorferi* strain transformed with the shuttle vector alone will provide negative controls for specific mammalian cells and expressed *T. pallidum* protein, respectively. A significantly higher level of adherence by *B. burgdorferi* expressing specific *T. pallidum* protein(s) on their surface to these cell lines, as compared to *B. burgdorferi* control will identify them as adhesin(s). In addition, these results will suggest potential role of these proteins in colonization of specific tissues by *T. pallidum* during infection of humans. We have extensive experience in conducting these experiments with *B. burgdorferi* and found them to be very useful in identifying the bacterial adhesins and host receptors, and predicting their contribution in specific tissue colonization *in vivo*. ¶

Visual Appeal

- Open space
- Clear organization
- Use of Bold, CAPITALS, underlining to define sections

EXPECTED RESULTS AND INTERPRETATION Based upon our experience with TprK,[62] we expect that antibody specificity will be detected among different sequences for a given DR, and that the number of AA changes necessary to abrogate antibody binding will be few. We expect that antibodies will bind to sequences in the predicted loops, but these loops also contain conserved sequence in addition to the DR, so we cannot predict now whether there will be cross-reactive antibodies that bind the conserved regions of these loops. If so, this may have implications for the specificity of opsonization and neutralization, and may argue against a major role for TprC and D subspecies- and strain-specific immunity. The role of the conserved regions (within loops and separate from loops) in functional immunity, including cross-protection, will be explored formally using a complementary approach in Aim 4. Those results, along with results from Aims 2 and 3, will be evaluated together to reach conclusions or to develop further hypotheses.

LIMITATIONS AND ALTERNATIVE APPROACHES Completion of Aim 2 will require successful production and purification of a large number of recombinant proteins and peptides. OM proteins can be quite difficult to express in *E. coli*. We have been expressing Tpr proteins and other putative OM proteins from *T. pallidum* for ~15 years. The laboratory has used a number of different vectors, host strains, and growing conditions in order to optimize expression for individual molecules. We routinely express such proteins without the signal sequence to avoid toxicity to *E. coli*. Even so, the protein is often found in inclusions, which requires solubilization in urea or other agents before it can be purified (we typically use 6XHIS-tags for purification). Depending upon its intended use, the quality of the antibody that is produced following immunization with recombinant proteins is dependent upon the correct folding of the immunizing protein: If one wants an antibody simply to identify a protein in an immunoblot, correct folding is not necessary; if one wants antibody to recognize a 3-dimensional structure on an intact bacterium, however, correct folding may be critical. Lack of appropriate attention to this issue may be the reason that functional assay results obtained in one laboratory may not be successfully reproduced in another lab. For the proteins that are produced in this project, conditions for optimal folding will be determined, and the degree of correct folding will be evaluated by circular dichroism. Figure 5 shows an example of purified recombinant TprK (predicted to have a structure very similar to TprC and D) that has been optimally refolded in our lab; the spectrum is typical of a molecule rich in β-sheets, consistent with β-barrel structure. Purity of our recombinant proteins and peptides will be assessed by SDS-PAGE and immunoblotting (using anti-6xHIS and infection-immune rabbit serum). If further purification is needed, size exclusion chromatography will be used. Synthetic linear and cyclic peptides will be obtained commercially. We have considerable experience with performing ELISA and lymphocyte proliferation assays using whole recombinant proteins and synthetic peptides as antigens; we don't anticipate any problems with these assays.[56, 57, 59-61]

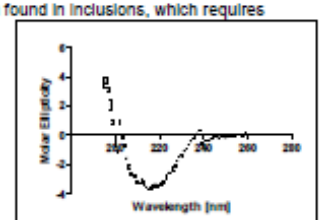


Figure 5. CD spectrum for purified refolded recombinant TprK, another likely OM protein of *T. pallidum*. The spectrum indicates abundant β-sheet composition.

Aim 3. Determine the role of the distinct regions of TprC and D in functional immunity, using homologous and heterologous *T. pallidum* strains as the targets of the functional assays.

RATIONALE AND PRELIMINARY DATA
Antibody can facilitate the killing of *T. pallidum* in two ways: opsonization for phagocytosis by macrophages,[63] and complement-mediated neutralization.[64] It is now widely believed that the major mechanism of clearance of *T. pallidum* from early lesions is by opsonophagocytosis, so the identification of the targets of opsonic antibody has been long-sought. Such targets are also surface-exposed antigens, so opsonization of *T. pallidum* has been used as a functional assay for surface-exposure of an antigen of interest. Several proteins have been reported to be opsonic targets in *T. pallidum*, including TprK,[44] although acceptance of these results has not been universal.[65] Data presented above indicate that several of the Tpr proteins, including TprC and TprD are also targets of opsonic antibody, and 3D

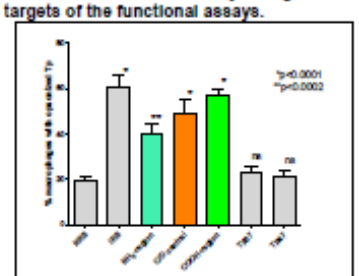


Fig. 6. Opsonization of *T. pallidum* Nichols strain by antisera directed to recombinant peptides of TprC/D.

Visual Appeal

- Figures and flow charts to explain experimental approach

structural·predictions·support·the·notion·of·surface·exposure·of·10·loop·structures·in·these·proteins.··Eight·of·these·loops·contain·regions·(DR)·in·which·the·predicted·AA·sequence·differs·among·strains·and·subspecies·and·2·contain·conserved·AA·sequences.··Preliminary·data·(**Figure·6**)·demonstrate·that·antisera·raised·against·shorter·purified·recombinant·peptides·from·Nichols·TprC/D·are·able·to·opsonize·the·Nichols·strain;·these·antisera·are·roughly·equivalent·to·the·antibodies·to·the·amino-,·central,·and·carboxyl·regions·of·TprC/D·that·will·be·prepared·in·**Aim·2**.··Negative·and·positive·controls·include·normal·rabbit·serum·(NRS)·and·infection-immune·rabbit·serum·(IRS).·Additional·negative·controls·include·rabbit·antibodies·to·the·endoflagellar·sheath·FlaA·(Tp37)·and·the·47kd·lipoprotein·antigen,·both·of·which·are·abundant·but·are·not·surface-exposed·in·the·intact·treponeme[66-68].··Lack·of·opsonization·by·these·antisera·indicate·the·specificity·of·the·assay·for·surface-exposed·components·and·confirm·that·the·treponemes·in·this·assay·are·intact·throughout·the·incubation·period.¶

Although·our·laboratory·has·conducted·a·very·large·number·of·opsonization·assays·over·the·years,·we·have·always·used·the·Nichols·strain·as·our·target·strain.··In·this·proposal,·we·will·examine·the·ability·of·antibodies·directed·toward·TprC·or·TprD·proteins·from·different·subspecies·and·strains·(or·peptides·derived·from·these)—each·with·distinct·DR·sequences—to·opsonize·homologous·or·heterologous·strains.··Because·these·distinct·sequences·are·fixed·within·a·strain,·and·do·not·change·within·the·course·of·infection,·it·is·hypothesized·that·the·DR·confer·a·subspecies-·or·strain-specificity·to·opsonization·and·neutralization,·and·perhaps·contribute·to·the·lack·of·cross-immunity·among·strains·and·subspecies.¶

EXPERIMENTAL·APPROACH¶

Antisera·produced·in·Aim·2·will·be·tested·in·a·checkerboard·fashion·with·different·*T.·pallidum*·strains·in·opsonization·and·neutralization·assays·(**Figure·7**),·in·the·following·order:¶

1)→ Antisera·resulting·from·infection·with·each·of·the·7·strains¶
2)→ Antisera·raised·against·each·full-length·recombinant·TprC·or·TprD·protein.¶
3)→ Antisera·raised·against·the·sequences·found·in·amino-,·central,·and·carboxyl-·regions·of·these·proteins¶
4)→ Antisera·raised·against·selected·cyclic·peptides¶

Experiments·will·first·be·conducted·using·the·Nichols·strain·and·SamoaD·strains·as·the·target·strains—these·two·are·chosen·because·they·have·least·amount·of·similarity·at·the·DR·and·throughout·the·central·region·of·TprD.·Comparison·of·results·with·these·two·strains·should·give·us·the·most·robust·indication·of·the·effect·of·the·DR·sequences·on·cross-reactivity·in·functional·assays.··Other·strains·will·be·tested,·based·upon·the·initial·findings,·to·determine·the·effects·of·specific·amino·acid·differences·on·antibody·function.¶

Opsonization·assays·will·be·conducted·as·previously·described.[34]·After·incubation·of·treponemes·with·control·or·test·antisera·plus·macrophages,·the·uningested·treponemes·are·washed·off,·and·the·cover·slips·containing·macrophages·are·fixed·and·stained·for·*T.·pallidum*·(see·color·photo·**in·Fig.·7**).··The·percentage·of·macrophages·containing·ingested·*T.·pallidum*·is·determined·on·triplicate·slides·per·condition.··Past·studies·have·indicated·that·counting·the·%·of·macrophages·containing·ingested·*T.·pallidum*·is·proportional·to·scoring·the·actual·number·of·*T.·pallidum*·ingested·(not·shown).·We·choose·to·count·macrophages,·rather·than·individual·treponemes,·because·it·is·significantly·less·labor-intensive.··Importantly,·the·cover·slips·are·randomized·on·slides·and·coded·so·that·the·scorer·is·unaware·of·the·experimental·condition·represented.··Negative·controls·including·incubation·of·treponemes·+NRS·+·macrophages,·and·macrophages·incubated·without·treponemes;·the·positive·control·will·be·infection-induced·antibodies·raised·against·the·target·strain.·Each·test·antiserum·is·tested·in·triplicate·per·macrophage·donor,·and·with·at·least·3·macrophage·donors.¶

Neutralization·will·be·performed·as·described·by·Bishop·and·Miller.[64]··Treponemes·will·be·incubated·with·heated·control·or·test·sera,·with·and·without·added·active·rabbit·

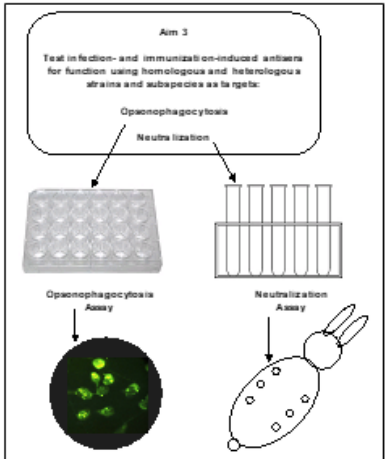


Fig.·7.··Schematic·showing·the·experimental·design·for·Aim·3.¶

Components of K Applications

- Specific Aims (1 page)
- Candidate Section*
- Training in Responsible Conduct of Research (1 page)
- Mentor's statement, Co-Mentors (6 pages)
- Environment & Institutional Commitment to Candidate (1 page each)
- Research Plan*
- Human Subjects
- Vertebrate Animals

***combined 12 page limit**

Components of K's, continued

- Authentication of Reagents
- Biohazards (in Facilities section)
- Select Agents
- Consortium/Contractual Arrangements
- Letters of Support (Collaborators)
- Resource Sharing Plan

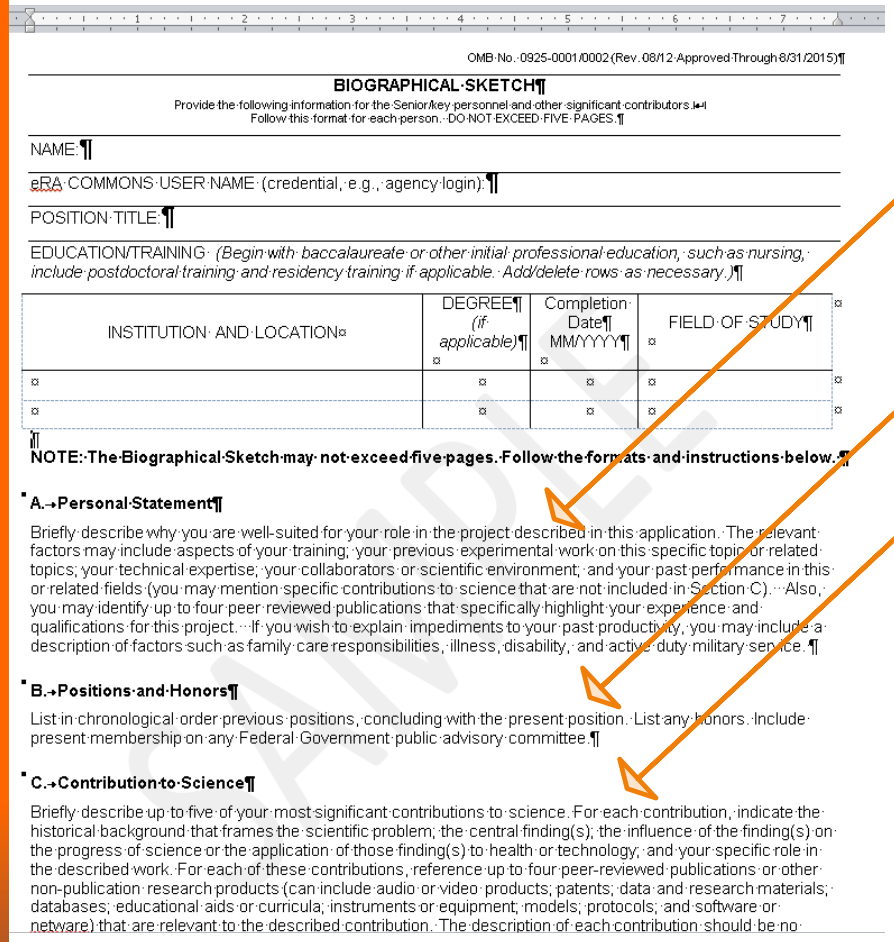
“Extra” Required Components for K’s

- Biosketches for Applicant, Mentor, Co-mentors
- Mentor’s Statement#
- Current & Pending Support for Mentor#
- Co-mentor statements#

 # Combined max 6 pages

- Letters of Reference
  - 3-5 letters from well-established scientists familiar with the candidate
  - May not be directly involved with the application

Biosketch



OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

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

NAME:

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE:

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

INSTITUTION AND LOCATION	DEGREE (*if applicable*)	Completion Date MM/YYYY	FIELD OF STUDY

NOTE: The Biographical Sketch may not exceed five pages. Follow the formats and instructions below.

A. Personal Statement

Briefly describe why you are well-suited for your role in the project described in this application. The relevant factors may include aspects of your training; your previous experimental work on this specific topic or related topics; your technical expertise; your collaborators or scientific environment; and your past performance in this or related fields (you may mention specific contributions to science that are not included in Section C). Also, you may identify up to four peer reviewed publications that specifically highlight your experience and qualifications for this project. If you wish to explain impediments to your past productivity, you may include a description of factors such as family care responsibilities, illness, disability, and active duty military service.

B. Positions and Honors

List in chronological order previous positions, concluding with the present position. List any honors. Include present membership on any Federal Government public advisory committee.

C. Contribution to Science

Briefly describe up to five of your most significant contributions to science. For each contribution, indicate the historical background that frames the scientific problem; the central finding(s); the influence of the finding(s) on the progress of science or the application of those finding(s) to health or technology; and your specific role in the described work. For each of these contributions, reference up to four peer-reviewed publications or other non-publication research products (can include audio or video products; patents; data and research materials; databases; educational aids or curricula; instruments or equipment; models; protocols; and software or netware) that are relevant to the described contribution. The description of each contribution should be no

Tips and Pet Peeves

- Keep the Personal Statement succinct
  - Why you? Impediments?
- Honors—nothing from high school!!
  - Phi Beta Kappa, AOA
  - Summa/magna cum laude
  - Poster or travel awards
- Contributions to Science—include publications
  - Up to 5 areas, with supporting pubs
  - Complete citations, all authors
  - Name changed? Make it clear!
  - List link to My Bibliography, with total number of publications, # as FA
- *Some leeway is OK for new investigators*
  - *OK to include manuscripts submitted and in press (clearly identify as such!!)*
  - *OK to add another heading for abstracts (e.g., Presentations)*

Text: Candidate Section

- Candidate's Background
  - How did you get where you are?
  - Why are you passionate about this topic?
  - Let the reviewers get to know you
- Career Goals and Objectives
  - Where do you want to be in 5, 10, 20 years?

Text: Candidate Section

- Career Development & Training Activities
  - How will this award fill your training gaps?
  - Didactic coursework (req'd for 5 years)
  - Technical training
  - What will you be able to take with you to write an R01?
  - Time line

Candidate Section

- Career Development/Training Activities
  - Training in manuscript & grant writing, manuscript reviewing, budget management, lab/group management, directing staff/students
  - Attending scientific meetings, journal clubs
  - Presenting work orally, posters

Training in Responsible Conduct of Research

- Provide details for each section: format, topics, faculty participation, duration, frequency
- Future plans for RCR training
- 1 page (not counted in limit)

Mentor Statements (6 pages total)

- Primary Mentor's statement should include
  - Evidence of successful training history
  - Evidence of active productive research
  - Evidence of support for proposed research
  - Details about mentoring—e.g. frequency of meetings
  - Topic areas in which mentoring will occur
  - Plan for transitioning candidate to independence
- Co-Mentors' statements should be specific about the expertise that they bring to the mentoring team

Environment & Institutional Commitment to the Candidate

- Description of Institutional Environment (1 page)
  - Intellectual environment
  - Resources available
- Institutional Commitment to Candidate's Research Career Development (1 page)
  - Is usually letter from Chair/Division Head
  - Guarantees >75% protected time for research training
  - Lab space, office, academic appointment

The Science: Last, But Certainly Not Least!

- Schedule uninterrupted time to sit and think—days of time
- Think about the unknowns in the topic that you are studying
- Read the latest papers in your field as well as some well-written review articles
- Begin to see connections and patterns among your ideas
- *Follow your heart as well as your mind*

Research Plan

- Specific Aims—1 page (not in 12-page limit)
- Research Strategy
  - Significance
  - Innovation
  - Approach

Specific Aims

- The most critical page in the application
- Start with an intriguing statement
- One page summary of the application
  - What is the hypothesis(es), and what data support it?
  - What are the exciting new preliminary data that support your aims? Which data are YOURS?
  - What are you going to do? (numbered list)
  - What will your results mean for the field?

Specific Aims—1 page!!

- List your aims simply
  - 2-4 Specific Aims are sufficient
- Don’t be too ambitious!!
- Should not be dependent upon Aim 1
- Aims serve as the backbone of Research Plan

Significance (Background)

- Assume you are not writing for an expert
- Identify gaps in knowledge; state how you will fill those gaps
- Tie the background to each Specific Aim
- Avoid selective citation of the literature

Innovation

- How will your results affect the future of research in your field?
- Will it affect research in other fields?
- Simply using a new method is not innovative

Approach: Research Design and Methods

- Organize by Specific Aim
  - Rationale and Hypothesis
  - Preliminary studies
  - Experimental Approach
  - Expected Results & Interpretation
    - Include statistical analysis, sample size
  - Potential Pitfalls and Alternative Approaches
- Other Important Sections
  - Future Directions
  - Timeline

Approach: Preliminary Studies

- Show preliminary data relevant to each aim and clearly tie the data to the aim (highlight your data)
- Include control data
- About 3-5 readable figures or tables (K award)

Approach: Preliminary Studies

- Put figures on relevant pages
- Number figures; refer to figure number in the text in bold (**Fig.1**)
- Figures should be self-explanatory—legends, labeled axes, etc.

Approach: Research Design & Methods

- Describe experimental design
- Details of methods are unimportant (boring)
- Get collaborators and consultants- strong letters
- Timeline

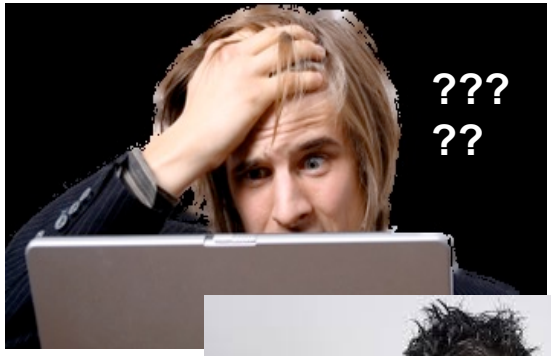
Aim	Description	YR 1	YR 2	YR 3	YR 4	YR 5
1A	Role of matrilysin in ischemia-reperfusion repair					
1B	Neutrophil activation *in vivo*					
2A	Neutrophil binding to KC/syndecan-1 complexes					
2B	Requirement of syndecan-1 shedding					
2C	Syndecan-1 association with integrins					
3A	Binding sites of KC:syndecan-1 interaction					
3B	Neutrophil activation with disrupted KC/syndecan-1.					
3C	Inhibit KC/syndecan-1 interaction *in vivo*					

Other Considerations

- Be thorough in addressing or stating “Not applicable” for all sections
  - Humans subjects
  - Vertebrate Animals
  - Biohazards
  - Authentication of reagents
  - Select agents, Resource Sharing, etc
- Bibliography
  - Correct format

Review Process

- Reviewed by 3 or more committee members
  - Write full reviews
- Entire committee scores each application
- Conflict of interest—not present for discussion
- ~15 minutes per application



Scored* Review Criteria-K Award

- Overall Impact
- Candidate
- Career Development Plan
- Research Plan
- Mentor(s), Consultants, Collaborators
- Environment & Institutional Commitment

*1 (best) to 9 (worst)

Additional Review Criteria*

- Training in Responsible Conduct of Research
- Protection for Human Subjects
- Inclusion of Women, Minorities & Children
- Vertebrate Animals
- Biohazards

* These criteria DO affect the score

Will your Application be Funded?

- Priority (Overall Impact) score will be posted on NIH Commons within a few days of the review meeting
- Summary Statement will appear 4-6 weeks later
- Paylines are posted by most institutes
- Final funding decisions are made by institute's Council

Good luck!!

Questions?

Thank You!

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Feedback Survey

A link to the feedback survey has been sent to the email address you used to register.

Please get out your device, find that email, and spend a few moments completing that survey before you leave today.

Tip: If on a mobile device, shift view to landscape view (sideways) for better user experience.