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SUMMARY STATEMENT
(Privileged Communication)

Release Date: 04/06/2017
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Application Number: 1 F31 AI133926-01

PUTNAM,NICOLE
Vanderbilt University
[REDACTED]

Review Group: ZRG1 F13-C (20)
Center for Scientific Review Special Emphasis Panel
Fellowship: Infectious Diseases and Microbiology

Meeting Date: 03/16/2017

Council: MAY 2017

Requested Start: 07/01/2017

PCC: M38 B

Dual PCC: 6 A

Dual IC(s): AR

Project Title: The impact of innate immune recognition of Staphylococcus aureus
on bone homeostasis and skeletal immunity

Requested: 2 Years

Sponsor: Cassat, James E

Department: Pathology and Microbiology Edu

Organization: VANDERBILT UNIVERSITY

City, State: NASHVILLE TENNESSEE

SRG Action: Impact Score: [REDACTED]

Next Steps: Visit https://grants.nih.gov/grants/next_steps.htm

Human Subjects: 10-No human subjects involved

Animal Subjects: 30-Vertebrate animals involved - no SRG concerns noted

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1F31AI133926-01 Putnam, Nicole

RESUME AND SUMMARY OF DISCUSSION: An exceptional predoctoral fellowship applicant, in this application, seeks training in translational research with an interdisciplinary project studying the effect of *S. aureus* infection on osteoclast precursor cell biology and bone remodeling. The highly qualified applicant possesses an MPH, strong academic record, laudatory letters of support and two co-author papers completed since joining her current research group. The sponsor is excellent with a strong record of publications and funding. A senior co-sponsor and additional mentor, both with outstanding training records, are part of the collaboration. The institutional environment is highly supportive of basic science and clinical training. The research plan is hypothesis driven, supported with strong preliminary data and a well thought out experimental design. Overall, the extraordinary applicant, outstanding sponsor and mentors, exceptional environment and thoughtful research plan provide a tremendous opportunity for an excellent foundation for a future independent research career.

DESCRIPTION (provided by applicant): *Staphylococcus aureus* is a ubiquitous human pathogen, resulting in superficial, invasive, and disseminated infections. One of the most common invasive manifestations of *S. aureus* disease is osteomyelitis, a frequently occurring and debilitating infection of bone. Osteomyelitis triggers dramatic alterations in bone architecture, leading to severe complications such as bone destruction, pathologic fractures, and growth defects. An emerging body of literature suggests that both local and systemic inflammation trigger altered interactions between bone-forming osteoblasts and bone-resorbing osteoclasts to impact bone homeostasis. Skeletal cells are known to express innate pattern recognition receptors (PRRs), but the contribution of innate sensing towards bone homeostasis and antibacterial immunity during *S. aureus* osteomyelitis has not yet been explored. The overarching objective of this proposal is to characterize how innate sensing of bacterial pathogens by skeletal cells triggers alterations in bone physiology. In order to define the impact of skeletal cell PRRs on bone homeostasis, we first focused on the critical signaling adaptor protein, MyD88, which is necessary to transduce signals through toll-like receptors (TLRs) and IL-1 receptors (IL-1R). Our preliminary data demonstrate that MyD88 is necessary to control *S. aureus* replication and dissemination in vivo and that osteoclast differentiation can be stimulated by bacterial components in a MyD88-dependent manner in vitro. Therefore, the central hypothesis of this proposal is that *S. aureus* modulates osteoclast precursor cell biology and bone remodeling through ligation of osteoclast PRRs and the induction of inflammation. To test this hypothesis, I will use a newly developed murine *S. aureus* osteomyelitis model from our laboratory. This model is advantageous compared to other osteomyelitis models because it allows us to utilize genetically modified animals, high-resolution quantitative imaging analysis, and unique histologic techniques for quantifying perturbations in bone remodeling. Experiments proposed in Aim 1 will investigate the roles of TLR and IL-1R signaling on osteoclast differentiation by monitoring osteoclastogenic signaling cascades, transcription factor activity, expression of mature osteoclast markers, and functionality of osteoclasts formed in vitro. Aim 2 will explore how MyD88 signaling in skeletal cells impacts clearance of *S. aureus* and bone remodeling. Collectively, these data will define signaling crosstalk between canonical osteoclast differentiation and innate immune pathways to activate osteoclast differentiation and maturation programs. Additionally, these findings will describe how MyD88 signaling in skeletal cells contributes to immune defenses and affects the kinetics of bone remodeling. This proposed work will have broad implications for how innate skeletal cell sensing contributes to the development of an effective immune response and influences bone homeostasis.

PUBLIC HEALTH RELEVANCE: Normal bone remodeling is a tightly regulated process that is dramatically altered by infection and both systemic and local inflammatory conditions. The proposed research will investigate how skeletal cells sense and respond to the human bacterial pathogen *Staphylococcus aureus*, the most common cause of bone infections, and how these cellular responses disrupt normal bone remodeling. This work will therefore describe how bone is altered by the presence

of bacterial pathogens and resulting immune responses, providing critical information for the development of therapeutics that may reduce bone pathology triggered by infection or inflammation.

CRITIQUE 1

Fellowship Applicant: 1
Sponsors, Collaborators, and Consultants: 2
Research Training Plan: 2
Training Potential: 2
Institutional Environment & Commitment to Training: 1

Overall Impact: This is an F31 application to study the effect of *S. aureus* infection on osteoclast precursor cell biology and bone remodeling. The applicant is an outstanding student with an MS from Johns Hopkins school of public health. She will be mentored by a talented young investigator with expertise in *S. aureus* bone infection. Co-sponsor with significant mentoring experience will contribute to strong mentoring. The project centers on the effect on *S. aureus* infection on pre-osteoclast differentiation into osteoclast and the signaling pathways investigated in two strong *in vitro* and *in vivo* aims. The proposal is well written and supported by good preliminary data, and should lead to strong training in microbial pathogenesis. Overall training plan by the applicant and sponsor are detailed and comprehensive and includes both research related activities and career development opportunities. Overall, this is an outstanding application that should provide strong training.

1. Fellowship Applicant

Strengths

- Commitment to career path with an MS at Johns Hopkins School of Public Health, multiple abstract presentations, and several co-authorships in current and previous labs.
- Excellent grades (mostly A's) especially at Johns Hopkins and Vanderbilt.
- Letters are uniformly strong, endorse her hard work, enthusiasm and productivity which included already several coauthor papers at Vanderbilt since joining the program.

Weaknesses

- None noted.

2. Sponsors, Collaborators, and Consultants:

Strengths

- Outstanding mentoring team that consists of a talented primary mentor James Cassat and a senior co-mentor, Julie Sterling, with relevant expertise in bone biology to provide strong and complementary mentorship.
- Primary mentor is well funded with a KO8 and a Burroughs Wellcome grant. An R01 is pending.
- Primary mentor has been productive and has several papers in top tier journals including Cell Host Microbe (2), and PLoS Pathogens (as senior author).

Weaknesses

- Primary mentor has no previous sponsored trainees. But this is not considered a significant weakness as PI will have two experienced senior mentors in Eric Skaar and Julie Sterling, both with excellent track record of mentoring

3. Research Training Plan:

Strengths

- Proposal is supported by strong preliminary data
- The premise underlying the hypothesis is strong and the research could lead to important contribution to bone and *S. aureus* biology.
- Experiments are overall well designed with a thorough discussion of pitfalls and alternative strategies. The two aims are complementary.
- The project should provide excellent training for microbial pathogenesis research.

Weaknesses

- None noted.

4. Training Potential:

Strengths

- The proposed project is quite different from the applicant's master thesis which was focused on T cell response to measles in rhesus macaque. It will provide excellent additional training.
- The PI has provided a detailed description of training she will receive during her PhD, including specific meetings, and in house seminar series and career development opportunities.
- The sponsor and co-sponsor also provided very good training plans.

Weaknesses

- None noted.

5. Institutional Environment & Commitment to Training:

Strengths

- The environment at Vanderbilt is outstanding for biomedical sciences.
- Top notch group of *S. aureus* researchers

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

YES, all four points addressed

Biohazards:

Acceptable

Training in the Responsible Conduct of Research:

Acceptable

Comments on Format (Required):

- acceptable

Comments on Subject Matter (Required):

- acceptable

Comments on Faculty Participation (Required):

- acceptable

Comments on Duration (Required):

- acceptable

Comments on Frequency (Required):

- acceptable

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2

Fellowship Applicant: 1

Sponsors, Collaborators, and Consultants: 2

Research Training Plan: 2

Training Potential: 1

Institutional Environment & Commitment to Training: 1

Overall Impact: This is a strong, well-written and innovative proposal to investigate molecular mechanisms underlying bone inflammation, destruction and aberrant bone formation during murine osteomyelitis caused by *Staphylococcus aureus*. The proposal is based on a solid body of preliminary data, the aims are logical, with hypotheses and pitfalls clearly laid out. The first aim, based on preliminary studies that suggest osteoclasts are affected *in vitro* by bacterial components via a MyD88-mediated mechanism, is to investigate whether *S. aureus* modulates the cell biology of osteoblast precursors through TLR recognition or IL1R signaling upstream of MyD88. The second aim uses a mouse osteomyelitis model developed in her lab to investigate pathogen clearance and dissemination and alterations in bone remodeling in both WT and MyD88 null mice. The applicant is outstanding; her academic performance has been strong and she has already been quite productive for someone early in her PhD studies. The primary sponsor is a new faculty member with a limited training record, but the training plan is well laid out – and based on the quality of the proposal and the progress the applicant has made, there is little concern about the training she is receiving. The co-mentor, while still fairly junior, does have a solid “early” training record. Furthermore, the chair of her dissertation committee is Dr. Eric Skaar, an established investigator with extensive training experience. The applicant is also part of the competitive Vanderbilt Program in Molecular Medicine (VPMM) which provides her with a unique opportunity to supplement her basic research with exposure to the clinical care of patients, under the supervision of both basic science and clinical mentors. The project has high clinical relevance and will provide excellent doctoral training for a very promising young scientist.

1. Fellowship Applicant

Strengths

- Very strong academic performance
- Outstanding productivity. She is already a contributing author on two papers from her PhD advisor's lab, plus another one from one of her lab rotations. Work she did as a MS student is also currently being revised for resubmission.
- Letters speak to her independence of thought, superior critical thinking and analytical abilities, fearlessness and tenacity when it comes to acquiring new skills, and effectiveness as a teacher. She is uniformly characterized as an outstanding applicant.

Weaknesses

- None noted.

2. Sponsors, Collaborators, and Consultants:

Strengths

- Sponsor, James Cassat, is a highly productive young MD/PhD investigator with a solid background in host-pathogen interactions during invasive bacterial infections, and particularly with *S. aureus*. He has already published extensively in high quality journals and is well-funded through the period of this project.
- Co-sponsor Julie Sterling provides expertise in bone disease. She has also been highly productive.

Weaknesses

- The only possible weakness is the lack of a training record by the sponsor, Dr. Cassat. This is compensated for, however, by the training team – which includes not only Dr. Sterling but also several collaborators (letters provided) and the chair of her thesis committee, Eric Skaar (who also collaborates with Dr. Cassat).

3. Research Training Plan:

Strengths

- Clear, hypothesis driven proposal
- Pitfalls and alternatives clearly laid out
- Two independent and complementary aims
- Solid preliminary data
- Impressive range of techniques
- Innovative mouse osteomyelitis model
- Clinically relevant problem

Weaknesses

- None noted.

4. Training Potential:

Strengths

- Letters speak to the strength of her mentoring team, described as “highly funded and exemplary”; “wholeheartedly committed to her success and who have the necessary expertise to facilitate her development as a young scientist”
- Unique opportunity provided by her participation in the Vanderbilt Program in Molecular Medicine (VPMM). This competitive program, into which she has been accepted, is designed for graduate students and postdoctoral fellows who conduct basic research in areas with clinical, disease-based implications. Under the guidance of a clinical mentor and a basic science mentor, this program is designed to provide exposure to the clinical care of patients in the areas of her research, along with didactic and experiential courses and seminars.
- The project is innovative, sophisticated, and clinically relevant.

Weaknesses

- None noted.

5. Institutional Environment & Commitment to Training:

Strengths

- Outstanding research and training environment

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

YES, all four points addressed

- Well justified and described in detail

Biohazards:

Acceptable

- Facilities and training for BSL-2 work are noted

Training in the Responsible Conduct of Research:

Acceptable

Comments on Format (Required):

- 2 hour course during orientation plus 9 hour annual symposium

Comments on Subject Matter (Required):

- Standard list of topics

Comments on Faculty Participation (Required):

- Face to face faculty teaching

Comments on Duration (Required):

- Acceptable; see Format

Comments on Frequency (Required):

- In addition to the 11 hours of formal training, she receives ongoing training in RCR with lab meeting presentations covering each of the 9 areas of RCR every 1-2 months.

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3

Fellowship Applicant: 1

Sponsors, Collaborators, and Consultants: 1

Research Training Plan: 2

Training Potential: 1

Institutional Environment & Commitment to Training: 1

Overall Impact: This application is from an academically talented, motivated candidate who received a master's degree in the immune response of macaques to measles virus. The proposed studies center on bone remodeling during osteomyelitis caused by *Staphylococcus aureus*. The research project will expand the applicant's expertise to a bacterial pathogen and mouse models of infection, and the thoughtful description of the planned experiments and abundant preliminary data (generated by the applicant) instill confidence that significant results will be obtained. The sponsor is in the early stages of his career but is productive and well-funded, and a co-sponsor with good mentoring experience and appropriate scientific expertise has been enlisted. The training plan is robust and includes participation in numerous local seminar series, journal clubs, and group meetings; regional conferences; training/mentoring experiences; and review of manuscripts and grant applications. The support provided by Vanderbilt is extraordinary. The applicant has already received institutional funding for training in the co-sponsor's lab and generation of preliminary data. Moreover, she is participating in two programs that provide clinical experience and knowledge to basic researchers; these dovetail with her interest in pursuing a career in translational research. In summary, this is an outstanding application with considerable strengths and no substantial weaknesses.

1. Fellowship Applicant

Strengths

- The applicant earned a master's degree in public health, working on immune responses to measles virus in rhesus macaques. During this time, she earned co-authorship on three publications, and a first-authored publication based on her thesis work is under revision.
- The applicant has a solid academic record and has earned nearly straight A's in her doctoral program at Vanderbilt.
- The applicant has received several honors and is involved in professional societies. Notably, she is part of the organizing committee for the 2017 Southeastern Immunology Symposium.
- Five supportive letters of recommendation are included; these stress the applicant's enthusiasm, devotion to research, and drive.

Weaknesses

- None noted.

2. Sponsors, Collaborators, and Consultants:

Strengths

- The sponsor is an MD/PhD, which aligns nicely with the applicant's desire to pursue translational research.
- The sponsor has expertise in the field of osteomyelitis, has an excellent record of publication in high-impact journals, and is well funded. He is highly qualified to supervise the proposed research project.
- The sponsor has received numerous awards. Particularly relevant to this application is an institutional teaching award bestowed in 2015.
- Dr. Julie Sterling, an expert in bone biology, has been brought on board as a co-sponsor and to provide additional mentoring. Dr. Sterling's past and present trainees include nine graduate students and a postdoc. Examples listed have gone on to good positions related to biomedical sciences.
- A strong dissertation committee has been assembled to guide the applicant.
- The applicant is part of Vanderbilt's Program in Molecular Medicine, which provides a clinical mentor, in this case a specialist in pediatric infectious diseases. This mentor should do much to further the applicant's goal of a career in translational research.

Weaknesses

- None noted.

3. Research Training Plan:

Strengths

- The research proposal focuses on osteomyelitis, specifically on how *Staphylococcus aureus* influences bone remodeling during infection. The proposal does an excellent job of highlighting the potential importance of the results. The high clinical relevance of the project speaks well to the applicant's interest in translational research.
- The project is supported by substantial *in vitro* preliminary data that support the hypothesis and *in vivo* data that support the feasibility of the planned approaches. These data were generated by the applicant.
- The project nicely combines *in vitro* approaches and experiments in mice that will train the applicant in a broad range of methodologies (including generation of novel skeletal cell-specific knockout mice).
- Overall, thoughtful consideration has been given to potential problems and alternative approaches.

Weaknesses

- Moderate: The applicant states that the sponsor will carry out the surgery for the osteomyelitis model, due to the advanced nature of the skills required (page 49). It may be beneficial for the applicant to work toward mastering those skills.
- Minor: Sensitivity is a potential problem for the experiments proposed in Aim 1B, since preliminary data indicate that only a few hundred cells per well differentiate into osteoclasts (Fig. 3).
- Very minor: A mouse model of osteomyelitis is key to many of the planned experiments. A full description is in the Vertebrate Animals section, but a brief summary in the proposal itself would have been helpful.

4. Training Potential:

Strengths

- The applicant's master's work involved a virus and immune responses to it in primates. The proposed research will nicely expand her expertise to a bacterium and a genetically tractable mouse model of infection.
- Both the applicant and the sponsor provide detailed training plans that include participation in local journal clubs and seminars; attendance at regional conferences; experiences in mentoring and teaching; and review of manuscripts and lab members' grants.
- The applicant received institutional funding to periodically train in the co-sponsor's lab, and it appears that the co-sponsor will continue to play a substantial role in mentoring the applicant.
- In addition to the Program in Molecular Medicine mentioned above, the applicant will be participating in Vanderbilt's ASPIRE Module for Clinical Laboratory Medicine. This program will further support the applicant's interest/expertise in translational research.

Weaknesses

- None noted.

5. Institutional Environment & Commitment to Training:

Strengths

- The institution provided funding for the applicant to undertake a 'mini-sabbatical' in the co-sponsor's lab.
- The institution provided pilot funds to generate preliminary data.
- The institution furnishes exceptional support through its Biomedical Research and Training (BRET) office, which has an IDP program, organizes RCR training, and sponsors a variety of career-related activities (including an annual career symposium).
- As already noted, Vanderbilt provides programs to strengthen the clinical knowledge of basic researchers who are interested in translational work.

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

YES, all four points addressed

- A very detailed, complete discussion is included that covers all four points.

Biohazards:

Acceptable

- A good description of the training and facilities for working with a human pathogen is provided.

Training in the Responsible Conduct of Research:

Unacceptable

Comments on Format (Required):

- Faculty lectures and small group discussions of case studies

Comments on Subject Matter (Required):

- Covers all relevant areas

Comments on Faculty Participation (Required):

- All sessions are conducted by faculty.

Comments on Duration (Required):

- Full-day symposium comprising nine hours

Comments on Frequency (Required):

- No mention is made of the need for refresher training after 4 years.

Resource Sharing Plans:

Acceptable

- Acceptable plans are in place for sharing data and mouse strains.

Budget and Period of Support:

Recommend as Requested

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

VERTEBRATE ANIMAL: ACCEPTABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

SCIENTIFIC REVIEW OFFICER'S NOTES: Training in the responsible conduct of research was adequately described.

Footnotes for 1 F31 AI133926-01; PI Name: Putnam, Nicole

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html>. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.