SUMMARY STATEMENT

PROGRAM CONTACT: **(Privileged Communication)** ***Release Date:*** **06/17/2010**

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Application Number: **1 R01 AI092743-01A2**

Principal Investigator ***Formerly:*** **1R01HD061371-01A2**

RATNER, ADAM JONATHAN MD

Applicant Organization: COLUMBIA UNIVERSITY HEALTH SCIENCES

Review Group: **HIBP**
Host Interactions with Bacterial Pathogens Study Section

Meeting Date: **06/11/2010** ***RFA/PA:*** **PA10-067**
Council: **OCT 2010** ***PCC:*** **M37C**
Requested Start: **12/01/2010** ***Dual PCC:*** **PP -UR**
Dual IC(s): **HD**

Project Title: **Gardnerella vaginalis: toxin production and pathogenesis**

SRG Action: **Impact/Priority Score: 10 Percentile: 2**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **30-Vertebrate animals involved - no SRG concerns noted**

Project Year	Direct Costs Requested	Estimated Total Cost
1	250,000	402,500
2	250,000	402,500
3	250,000	402,500
4	250,000	402,500
5	250,000	402,500
TOTAL	1,250,000	2,012,500

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

EARLY STAGE INVESTIGATOR, NEW INVESTIGATOR

1R01AI092743-01A2 RATNER, ADAM

EARLY STAGE INVESTIGATOR
NEW INVESTIGATOR

RESUME AND SUMMARY OF DISCUSSION: The investigator proposes to study the molecular mechanisms by which a human-specific toxin vaginolysin (VLY), produced by *Gardnerella vaginalis*, induces bacterial vaginosis (BV) disease. Understanding the role of this toxin interaction with host cells may lead to the identification of targets that may be highly significant in developing new strategies to combat BV, a devastating woman disease. The application addresses both the lack of animal model and the genetic tools in *Gardnerella vaginalis* research which, by itself, is a highly significant step forward in this field. Additional strengths are the capabilities of the investigators to accomplish the proposed objectives, the adequate response to previous critiques creating a stronger foundation for the proposed project, the innovative approaches which are reflected by a logical flow of the experimental plan, the availability of adequate transgenic mice guaranteeing a successful/positive outcome from this application, and the importance of this female bacterial vaginosis (BV) disease. Hence, this application is considered by the review panel to be very exciting with virtually no weaknesses.

DESCRIPTION (provided by applicant): Bacterial vaginosis (BV) is an exceedingly common disorder of the vaginal microflora affecting >30% of all women, with higher rates in pregnancy and among African- American populations. Women with BV are at substantially increased risk of preterm birth, which is a major cause of neonatal morbidity and mortality, as well as acquisition of sexually transmitted diseases including human immunodeficiency virus. Despite its public health importance, the pathogenesis of BV is not well understood. We have recently characterized vaginolysin (VLY), a cholesterol-dependent cytolysin from *Gardnerella vaginalis* (a bacterial species present on the vaginal mucosa in the setting of BV and thought to contribute to the pathogenesis of disease) that exhibits exquisite human specificity. We hypothesize that this species-specific toxin may be an important virulence factor of *G. vaginalis* with relevance to the pathogenesis of BV. In our preliminary data, we have characterized the receptor for VLY (human CD59) on genital tract epithelial cells. Introduction of this receptor into non-susceptible cells renders them sensitive to VLY. We have engineered a transgenic mouse expressing the hCD59 receptor and also constructed a VLY chimera that is hCD59-independent. These represent candidate *in vivo* models for BV. In addition, we have developed techniques for genetic manipulation of *G. vaginalis*, including transposon mutagenesis. In Aim 1, we will define genetic determinants of *G. vaginalis* virulence using new techniques for mutagenesis and assays of toxin production. In Aim 2, we will determine the role of VLY at the host-pathogen interface both *in vitro* and *in vivo* with a focus on unique aspects of the VLY-hCD59 interaction. At the conclusion of these studies, we will have expanded our knowledge of *G. vaginalis* pathogenesis, evaluated new *in vivo* models of BV, identified candidate strategies to inhibit toxin-host interaction, and developed new tools for continued investigation into the pathogenesis of an important disorder.

PUBLIC HEALTH RELEVANCE: Bacterial vaginosis (BV) is a very common but not well-understood disease affecting women. Women with this disorder are at substantially higher risk of preterm birth and acquisition of other serious diseases, including HIV. This project investigates the role of a newly described toxin made by *Gardnerella vaginalis* in causing inflammation and damage to vaginal cells during BV and focuses on the development of new models and treatments for this important disease.

CRITIQUE 1:

Significance: 2
Investigator(s): 1
Innovation: 1
Approach: 2
Environment: 1

Overall Impact:

Strengths

- Bacterial vaginosis is of great importance to public health. The pathogenesis of BV is not well understood. There is compelling evidence that *G. vaginalis* is important to BV pathogenesis. Dr. Ratner has established techniques for genetic manipulation of Gv. This is a major advance for the Gv field, as it paves the way for using genetic approaches to study its pathogenesis. He has intriguing findings concerning the role of Gv cytolysin VLY in pathogenesis, and proposes novel approaches to determine its specificity for human cells, its binding and signaling to host cells, and its ability to lyse host cells. Dr. Ratner has consistently made progress in this project, and satisfactorily answered the concerns of the two previous reviews. This gives us confidence that the research program described here will make significant contributions to our understanding of the role of Gv in BV.

Weaknesses

1. Significance:

Strengths

- The investigator discovered that the host specificity of Gv is determined at least in part by the interaction of the Gv cytolysin VLY with CD59, a human specific complement regulatory protein.
- He identified the region of VLY that confers specificity for lysing human cells, and constructed a chimeric VLY – VLY:PLYD4 – that has lost specificity for human cells. This chimera cytolysin is a useful tool for studying BV in mouse models of the disease.
- The investigator has developed a mouse model to study Gv. He has constructed CD59 expressing transgenic mice. He has also obtained additional lines of mice expressing CD59 from his collaborator Peter Cowan in Australia, who is an expert on the biology of CD59 and other complement regulatory proteins.
- With his collaborator Dave Figurski, the investigator has devised a system to genetically manipulate Gv. This is a major technical advance in his field and will make possible genetics approaches to studying Gv pathogenesis.

Weaknesses

- The biofilm section appears to be irrelevant to the main goals of this application, which is to characterize the role of VLY in Gv pathogenesis.

2. Investigator(s):

Strengths

- The investigator is a well-published new investigator who has demonstrated his ability to advance this project. He has systematically made progress on the project.
- A team of collaborators will add strength to every aspect of the application - D. Figurski, an established bacterial geneticist, and D. Cowan, an expert in CD59 who will also make available his mouse lines defective in CD59.

Weaknesses

3. Innovation:

Strengths

- Several approaches are highly innovative: addressing the host specificity issue by making chimeric VLY that loses specificity for human cells, and using CD59 expressing transgenic mice.
- The construction of a system to genetically manipulate Gv is a major step forward in studying this pathogen.

Weaknesses

4. Approach:

Strengths

- Most of the approaches are well thought through. Many have already been tested by the investigator.

Weaknesses

- The investigator will determine how VLY kills cells by staining with trypan blue and assaying for LDH activity. LDH is a readout for necrosis. If the investigator wishes to consider all aspects of cell killing he should use markers for apoptosis and other cell death pathways.
- How VLY stimulates CD59 signaling (rather than cell death) is not described.

5. Environment:

Strengths

- Columbia U is a very supportive place for the investigator and this project.

Weaknesses

Vertebrate Animals:

Acceptable.

Biohazards:

Acceptable.

Resubmission:

The investigator has satisfactorily addressed the previous concerns, and consistently made progress on his Aims.

Budget and Period of Support:

Recommend as Requested.

CRITIQUE 2:

Significance: 1
Investigator(s): 1
Innovation: 1
Approach: 1
Environment: 1

Overall Impact:

Strengths

- Addressing a common and potentially important but understated medical condition in women: bacterial vaginosis and preterm birth.
- The cell culture model will allow the investigator to define the mechanisms of bacterial vaginosis pathogenesis by focusing on the virulence factor vaginolysin (VLY) interactions with host cells (human CD59 as receptor of VLY).
- The mouse model will allow the investigator to further investigate bacterial vaginosis pathogenesis, especially biofilm formation, and potentially develop inhibitors for blocking the toxicity of VLY.
- The investigator has generated strong preliminary data for supporting the proposed aims.

Weaknesses

1. Significance:

Strengths

- Investigating the pathogenic mechanisms of bacterial vaginosis will not only advance knowledge but also provide important information for potentially developing strategies to attenuate the *Gardnerella* pathogenicity by blocking the toxicity of VLY.

Weaknesses

2. Investigator(s):

Strengths

- Although the investigator is a new in the field, he has have acquired more than enough knowledge and expertise required for accomplishing the proposed work.
- The investigator has brought onboard the relevant experts, which has further strengthened the application.

Weaknesses

3. Innovation:

Strengths

- Although the overall experimental designs are not particularly novel, it is expected that new/novel information be acquired from the proposed experiments.

Weaknesses

4. Approach:

Strengths

- Investigating the role and mechanisms of vaginolysin in the pathogenesis of bacterial vaginosis is important.
- The experiments proposed in aim 1 may provide important information on how *G. vaginalis* regulates VLY expression and production and what genes are involved in *G. vaginalis* biofilm formation. The experiments on creating and testing a mutant *G. vaginalis* expressing a non species-specific VLY will provide the investigator the tools for in depth studying of the toxin function in animal models.

- The cell culture experiments in Aim 2 will allow the investigator to learn about the effects of VLY-mediated host cellular bleb and VLY-increased cellular susceptibility to complement lysis on *G. vaginalis* pathogenesis.
- The animal model experiments in Aim 2 will allow the investigator to further evaluate the roles and mechanisms of VLY-CD59 interactions in *G. vaginalis* pathogenesis and to potentially develop inhibitors for attenuating the toxin-mediated pathogenicity.

Weaknesses

5. Environment:

Strengths

- Columbia University Health Sciences has adequate institutional infrastructure and expertise support.
- The investigator's lab is equipped with the tools and expertise required for accomplishing the proposed research.

Weaknesses

Protections for Human Subjects:

Not Applicable (No Human Subjects).

Vertebrate Animals:

Acceptable.

Biohazards:

Acceptable.

Resubmission:

Addressed most previous concerns.

Resource Sharing Plans:

Acceptable.

Budget and Period of Support:

Recommend as Requested.

CRITIQUE 3:

Significance: 2
Investigator(s): 2
Innovation: 1
Approach: 1
Environment: 1

Overall Impact:

Strengths

- This application from a new investigator was well received in the previous review and remarkably, the investigator has improved it since that submission.
- The investigator has provided a plethora of supporting data for the proposed studies that include a transgenic mouse model expressing human CD59.
- The proposed studies will likely provide significant new insight into *G. vaginalis* bacterial vaginosis (BV) and the role of the human cell specific vaginolysin in establishing and promoting disease.

Weaknesses

1. Significance:

Strengths

- BV is a major cause of preterm births and other complications.
- The proposed studies are the first to tackle a difficult but significant problem.

Weaknesses

2. Investigator(s):

Strengths

- Dr Ratner is well trained in pathogenesis and the contribution of VLY-like toxins to pathogenesis.

Weaknesses

3. Innovation:

Strengths

- A major complication to the study of this organism is the fact that VLY is human cell specific due to its use of human CD59 as its receptor: the investigator has developed two innovative approaches to aid in the development of an animal model, the use of human CD59 transgenic mice and if that should fail he has constructed a VLY-PLY chimera that allows binding directly to cholesterol rather than CD59.

Weaknesses

4. Approach:

Strengths

- Development of a mouse model of infection.
- Demonstration that a close relative of VLY, pneumolysin, when co-administered with *G. vaginalis* can significantly increase bacterial colonization of the mouse model.
- Well developed hypotheses driven aims.
- Complementation of animal studies with cell studies.
- Investigation of the host cell response to nonlytic levels of VLY and the relevance to disease.

Weaknesses

5. Environment:

Strengths

- The investigator is in the department of Microbiology & Immunology, Columbia which has robust pathogenesis and model systems programs, which provides an excellent environment with faculty that have strengths that complement his application's aims.

Weaknesses

Protections for Human Subjects:

Not Applicable (No Human Subjects).

Vertebrate Animals:

Acceptable.

Biohazards:

Not Applicable (No Biohazards).

Resubmission:

The investigator has addressed all of the previous critiques and strengthened the application with new data.

Budget and Period of Support:

Recommend 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:

VERTEBRATE ANIMAL (Resume): ACCEPTABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

MEETING ROSTER

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Infectious Diseases and Microbiology Integrated Review Group
CENTER FOR SCIENTIFIC REVIEW
HIBP
June 11, 2010

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* Temporary Member. For grant applications, temporary members may participate in the entire meeting or may review only selected applications as needed.

Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.