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SUMMARY STATEMENT
(Privileged Communication)

PROGRAM CONTACT:
Cristina Cassetti
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Release Date: **11/04/2010**

Application Number: **1 R15 AI094440-01**

Principal Investigator

HOLM, GEOFFREY H PHD

Applicant Organization: COLGATE UNIVERSITY

Review Group: **VIRB**
Virology - B Study Section

Meeting Date: **10/07/2010**
Council: **JAN 2011**
Requested Start: **04/01/2011**

RFA/PA: **PA10-070**
PCC: **M32A**

Project Title: **Mechanisms and Functional Consequences of Reovirus Innate Immune Activation**

SRG Action: **Impact/Priority Score: 11**
Human Subjects: 10-No human subjects involved
Animal Subjects: 10-No live vertebrate animals involved for competing appl.

Project Year 1	Direct Costs Requested	Estimated Total Cost
	[redacted]	[redacted]
TOTAL	[redacted]	[redacted]

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.

1R15AI094440-01 HOLM, GEOFFREY

RESUME AND SUMMARY OF DISCUSSION: This application proposes to explore the genetic basis of why similar reoviruses cause different diseases, with an emphasis on if type 1 strain Lang (T1L) produces a factor to modulate the innate immune responses and/or apoptosis in the cells. The application may generate results that have significant impact on reovirus-based therapeutics. The strengths are that the investigator has a strong publication record in molecular virology and an outstanding record in training undergraduate students; the observation that encephalitis causing strain causes inhibition of IRF3 but not NF-κB responses is novel; the approaches are straightforward. The environment is exceptional for the commitment to undergraduate training. Despite that the scope of research is large; the overall enthusiasm is extremely high.

DESCRIPTION (provided by applicant): Diseases caused by reovirus in mammalian hosts are strain-specific: some type 1 viruses are associated with myocarditis, whereas type 3 viruses cause lethal encephalitis. The molecular mechanisms by which genetically similar reovirus strains cause these disparate diseases are not fully understood. However, pathologic hallmarks of these diseases are recapitulated in cell-culture models of reovirus infection. Encephalitis induced by type 3 reovirus correlates with the capacity of the strain to induce apoptosis both *in vivo* and in cell culture, whereas reovirus-induced myocarditis is associated with the limited capacity of the strain to induce type 1 interferons (IFNs), a family of related antiviral cytokines. Both IFN induction and apoptosis following reovirus infection are linked to innate immune responses dependent on transcription factors IRF-3 and NF-κB. The central hypothesis of the proposed research is that prototype type 1 strain Lang (T1L) encodes an antagonist of innate immune response activation that alters regulation of cellular transcriptional networks that function to establish an antiviral state or induce apoptosis. Three integrated specific aims are proposed to test this hypothesis. In Specific Aim 1, mechanisms for reovirus strain-specific differences in innate immune activation will be defined. The role of capsid stability in the cytoplasmic exposure of genomic dsRNA, a known inducer of innate immune responses, will be assessed using viruses with known capsid-destabilizing mutations. The genetic basis for these differences will be assessed using single-gene reovirus reassortants generated by reverse genetics. In Specific Aim 2, cellular transcriptional networks responsible for a) apoptosis and b) antiviral signaling will be defined by microarray analysis, using virus reassortants incapable of activating one or both pathways. Specific Aim 3 will facilitate the other two aims and enhance the undergraduate research program at Colgate University by introducing a laboratory component to an elective Virology course, which will engage students in a semester-long project to generate and characterize reovirus reassortants, and by supporting a senior-level research course in which students conduct the independent research projects outlined in Specific Aims 1 and 2. Broadly, this research will enhance understanding of how genetically similar viruses can cause disparate diseases through manipulation of innate immune pathways. The proposed experiments also will provide insight into two long-standing questions in the dsRNA virus field: mechanisms of reovirus-induced apoptosis and mechanisms by which reovirus engages and counteracts cellular antiviral responses. As other pathogenic microbes employ similar strategies, this research program will uncover conserved mechanisms of infectious disease pathogenesis.

PUBLIC HEALTH RELEVANCE: Genetically similar viruses can cause widely disparate diseases through manipulation of innate immune pathways. This project uses mammalian reovirus, a powerful experimental model for studies of virus pathogenesis, to identify mechanisms for strain-specific differences in cellular responses to infection that influence organ-specific disease. As other pathogenic microbes employ similar strategies, this research program will uncover conserved mechanisms of infectious disease pathogenesis.

CRITIQUE 1:

Significance: 3
Investigator(s): 3

Innovation: 3
Approach: 4
Environment: 4

Overall Impact: This study proposes to elucidate the contribution of IRF3 activation and induction of apoptosis by reoviruses to the different disease manifestations of two strains (T1L and T3D). They will also investigate the presence of a potential IFN antagonist in reoviruses by comparing these two strains that induce differential activation of IRF3 in infected cells. The biggest impact of this application is that it will provide access to research in molecular virology and innate immunity to undergraduate students.

1. Significance:

Strengths

- This application studies two different prototype strains of reoviruses that show different pattern of IRF activation and induction of apoptosis and are associated with either myocarditis or lethal encephalitis. They will investigate the implication of differential transcriptional profiles responsible for IRF3 activation and/or induction of apoptosis with the ability of those viruses to cause different diseases. The inhibition of IRF3 but not NF-κB responses by the encephalitis causing strain is quite interesting.

Weaknesses

- The experiments proposed will give valuable information about the connections between the apoptosis pathway and induction of IFN by viruses. But the information is not likely to result in a significant advance in the IFN or reovirus fields.

2. Investigator(s):

Strengths

- Dr. Holm is a junior investigator with excellent training in molecular virology and innate immunity. He has also trained 20 undergraduate students in these disciplines in the past 5 years, which makes him a good candidate for an R15 application.

Weaknesses

- Not noted

3. Innovation:

Strengths

- The studies on the role of mutations in the capsid in relation to induction of innate immunity responses are quite innovative. The main innovation is the exposure of molecular virology and innate immune techniques to undergraduate students in a liberal arts college.

Weaknesses

- The application is not very innovative. It proposes to use classical techniques in molecular virology and innate immunity analysis.

4. Approach:

Strengths

- The application will provide students “hands on” experience in molecular virology and innate immunity assays. The systematic approach is likely to yield results. The generation of viruses

and cellular immunity studies are straightforward. The undergraduate student component is well thought.

Weaknesses

- Aims 1 and 2 propose a systematic analysis of the induction of IFN by two different strains of reoviruses that cause different diseases (myocarditis and encephalitis). They will also generate reassortant viruses to identify the protein or proteins responsible for the inhibition of the IFN pathway.
- The proposed studies are quite systematic and straightforward and not very innovative.
- Other viruses known to inhibit either NF-κB or IRF3 could be tested in their system to validate it.
- The microarray analysis proposed in Aim 2 may generate an overwhelming amount of data for analysis. This might be an unrealistic approach to study this response by undergraduate students. The investigator indicates that several publications will be generated with data from this application. While a great optimistic thought, it might not be that easy to publish several manuscripts based on results from this work.
- Other assays such as flow cytometry for apoptosis or intracellular staining might be more feasible by undergraduate students.

5. Environment:

Strengths

- Colgate University is a great environment for an R15 project, since the availability of undergraduates justifies the need for this type of resource. Interestingly, all the preliminary data for this application have been generated by undergraduate students. There seems to be a strong institutional support for the Principal Investigator and he has collaborations with reo-virologists outside of Colgate University.

Weaknesses

- There are no other virologists or immunologists in the investigator's department.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2:

Significance: 3
Investigator(s): 2
Innovation: 3
Approach: 5
Environment: 2

Overall Impact: The fact that the experiments proposed are based upon convincing correlates of pathogenesis in the mouse model is an attractive aspect of the application. The relevance of orthoreovirus to human disease is somewhat limited and could be better justified. Comparison of closely related viruses with different pathogenesis is innovative and important to understanding of viral disease. However, a single phenotype should be chosen and the two viruses compared and cell types relevant to *in vivo* pathogenesis should be used. Aim 2 experiments will be difficult to interpret and assumptions about the mechanisms of apoptosis induction are too narrow.

1. Significance:

Strengths

- Orthoreoviruses have long been used to model the interaction of RNA viruses with host cells, in particular with the innate immune system.
- The results of the studies, especially those involving apoptosis induction, could have direct impact upon use of reoviruses as oncolytic vectors or other reovirus-based therapeutic strategies.
- Exposure of undergraduate students to biomedical research, an emphasized component of the parental announcement, will clearly be increased.

Weaknesses

- Othoreoviruses have limited potential for impact upon human health so findings related to their pathogenesis are diminished in significance somewhat. Antagonism of IRF-3 activation is being studied with many other viruses.

2. Investigator(s):

Strengths

- Dr. Holm's postdoctoral studies were productive and directly related to the subject matter of this application.
- Dr. Parker will be an asset with respect to reovirus expertise, increasing the quality of research facilities and increasing the exposure of undergraduates to biomedical research.

Weaknesses

-

3. Innovation:

Strengths

- Studying the differential activation of IRF-3 and NF-κB pathways is innovative.
- The idea to compare closely related viruses with different disease pathogenesis is innovative.

Weaknesses

- Standard techniques for the field are proposed.

4. Approach:

Strengths

- The approach is based upon phenotypes (IRF-3 activation and apoptosis induction) with direct relevance to the manifestations of orthoreovirus disease in the mouse model.
- Using ISVPs/cores and uncoating defective mutants is an interesting strategy to dissect the role of uncoating in IRF-3 activation.
- Microarray analyses should identify pro-apoptotic genes upregulated in HeLa cells after T3D infection.

Weaknesses

- The comparison of the T1 and T3 viruses lacks full justification as the effects upon IRF-3 activation and apoptosis are only single factors identified in the complex pathogenic process of each virus. Comparison between the two may not be fruitful as many unique aspects of each virus could combine to determine encephalitic versus myocarditic outcome. Choice of one phenotype for a detailed molecular analysis would be more appropriate.
- Cell types relevant to *in vivo* disease are not being used.
- The approach is highly ambitious considering the percentage effort devoted to the project by Dr. Holmes and, especially, the technician (6 calendar months). There appears to be a fair amount of reassortant analysis required, particularly for alternative strategies, which is time consuming.
- The multiplicities of infection used (100 pfu/cell), while perhaps standard, could lead to artificial responses to the DS RNA genome not typical of a single infecting virus *in vivo*.
- In Aim 2, only the induction of pro-apoptotic genes is considered to distinguish the viruses. In addition, it is assumed that all of these processes are regulated at the transcriptional level. Use of the reassortant viruses may be premature in these analyses.
- In Aim 2, the microarray data analysis, combined with virus gene mapping, will be technically difficult and may not yield interpretable results. Table 1 could have assisted the reviewer with understanding how these analyses would be hypothesis-driven. However, the description was a bit confusing.
- Aim 3 appears to outline specific undergraduate training plans and does not add to the scientific aspects of the application.

5. Environment:

Strengths

- Undergraduate students at Colgate should be prepared for this type of effort.

Weaknesses

- Equipment costs requested suggest that some basic research support may be lacking.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3:

Significance: 2
Investigator(s): 2
Innovation: 3
Approach: 2
Environment: 1

Overall Impact: The goals of the proposed project are to evaluate how different reovirus variants interact with the innate immune system and activate transcriptional networks. The proposed studies should produce significant new information and these studies build nicely on the research experience of the investigator. The studies, especially in Aim 2, are rather descriptive, but the information can be used to design more mechanistic investigations once the microarray data have been generated. The techniques will provide a good experience for undergraduates. Aim 3 is not really a relevant scientific aim, but it does confirm the investigator's commitment to undergraduate research experience. The Colgate NIH Study Group, which allows 12-16 undergraduate students to study in NIH laboratories for a semester, is excellent. There appears to be a strong commitment by Colgate to research. Four of the investigator's students have had NIH experience and three have received departmental honors. The research environment is very rich for students and this is viewed enthusiastically. The investigator is an assistant professor of biology and has apparently been at Colgate since 2008 but has already had 17 students work in his laboratory. Also, much of the preliminary data was generated by students in the laboratory. While the investigator has a good publication record and has recent papers, none of these are independent from his postdoctoral training. This is a concern.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

Resource Sharing Plans:

Acceptable

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:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-10-080 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-10-080.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. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

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