Sample R01: Reviewers' Comments

SUMMARY STATEMENT
(Privileged Communication)

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Release Date: **06/15/2010**

Application Number: **1 R01 AI092571-01**

Principal Investigator

PARRISH, COLIN R. PHD

Applicant Organization: CORNELL UNIVERSITY ITHACA

Review Group: **ZRG1 IDM-R (03)**
Center for Scientific Review Special Emphasis Panel
Member Conflict: Viruses

Meeting Date: **05/25/2010**
Council: **OCT 2010**
Requested Start: **12/01/2010**

RFA/PA: **PA10-067**
PCC: **M34A**

Project Title: **Structural controls of functional receptor and antibody binding to viral capsids**

SRG Action: **Impact/Priority Score: 20 Percentile: 7 #**
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	388,910
2	250,000	388,910
3	250,000	388,910
4	250,000	388,910
5	250,000	388,910
TOTAL	1,250,000	1,944,550

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.

1R01AI092571-01 Parrish, Colin

RESUME AND SUMMARY OF DISCUSSION: This outstanding application focuses on capsid structure and function in parvovirus family and seeks to examine (1) the structural heterogeneity that exists in wild type virus populations; (2) binding of the virus to its receptor (transferrin receptor, TfR); and (3) virus neutralization due to binding of antibody to the capsid surface. Its significance relate to its promise to further our understanding of how altered receptor binding can lead to the emergence of new epidemic or pandemic viruses, and to identify new ways to make more effective vaccines or therapeutics. A major strength of this application is the highly accomplished investigator with an excellent record of productivity. This application generates enthusiasm for well supported preliminary studies, publications record and strong collaboration which ensures feasibility and the success of cryoEM studies. Despite these strengths it is noted that the discussion of the extent to which the findings may be applicable beyond parvoviruses is limited. It is also noted that the interpretation of examination of virion heterogeneity by approaches that could change the virion may be difficult. These limitations, however are considered minor and only slightly diminish the enthusiasm for this high impact application.

DESCRIPTION (provided by applicant): Here we seek to understand how structural flexibility and variation in parvoviral capsids control their ability to bind receptors leading to cell infection and also to variation in host range, and also how capsid structures control antibody binding and neutralization. Those areas of study are significant because they are features of all animal and human viruses. While parvovirus capsids appear structurally simple, they are clearly sophisticated biomolecular machines that carry out many functions using variants of a single capsid protein, and the features controlling many functions have now been mapped to specific mutations and capsid structures, presenting an opportunity to gain a complete understanding of how virus-host interactions occur in fine detail. Parvoviruses include the B19 virus, human bocavirus, and Parv4, all of which cause disease in humans. Here we use feline and canine parvoviruses as models to build on our previous studies showing that cell infection and animal host ranges are controlled by specific interactions of the capsids with the transferrin receptors type-1 (TfR) of different hosts. There are also distinct outcomes for viral infection of antibody binding, depending on the binding site and angle of attachment. The three overlapping areas to be investigated in this project are: 1. Define the functional effects of variant and flexible structures of the parvovirus capsids. There is both flexibility and structural variation in the viral capsids which we will further characterize in detail. Some of that variation is asymmetric, only occurring in a small proportion of the capsid subunits, while other variation occurs in most viral sites. Many of those sites are known to affect viral functions. 2. Characterize how different interactions between parvovirus capsids and TfR or other receptors control infection. Capsid binding to different TfRs is controlled by the protein structure, and flexibility, and potentially by protease cleavages. To define the functional TfR binding to the capsids we will use a variety of approaches, including cryoEM analysis and analysis of capsid mutants affecting binding. To define the TfR interaction we will use receptors containing amino acid changes or added glycans within the interaction sites. 3. Use antibody binding to capsids to define their structures, and also to explain the mechanisms of antibody neutralization. We have many antibodies that bind the capsid structures. Some neutralize as Fabs, and others do not. We will use those antibodies as probes for structural variation in the capsids. The antibodies and their domains will be used in competition assays to further define the binding of different TfRs to the capsids, and to detect cleaved subunits or other capsid modifications. Antibodies with altered binding affinities, different attachment sites, or different angles of attachment would be tested for effects on the virus functions involved in cell infection.

PUBLIC HEALTH RELEVANCE: This project addresses several issues of central importance to the success of all viruses infecting humans and other animals. Those include: how viral proteins vary in structure over their life cycles, the mechanisms of binding to different receptors, how differences in viral structural proteins can control their host ranges, the processes of cell infection, and how antibody binding can neutralize the virus in some cases but not others. These studies will show how altered

receptor binding can lead to the emergence of new epidemic or pandemic viruses, and will also reveal new ways to make more effective vaccines or therapeutics. The specific model viruses being examined infect animals, but they are similar to a variety of important viruses of humans. The small sizes and simple genetic and capsid structures of the parvoviruses make them excellent models for defining the most basic aspects of the viral-host interaction, and the results and broad conclusions will be directly relevant to the better understanding of many different viruses of humans.

CRITIQUE 1:

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

Overall Impact:

Strengths

- The proposed project is a study of capsid structure and function in canine parvovirus and feline panleukopenia virus. The structures of these simple (T=1) capsids is now well known with an X-ray crystallographic structure available. The capsids are now suitable for analysis of features not revealed by crystallography, and that is the goal of the proposed project. Studies will be performed to characterize sub-populations of virions (aim 1), capsid receptor binding (aim 2) and virus neutralization by antibodies (aim 3). All three aims are strong with considerable research momentum developed in each. The project is enhanced by appropriate collaborators for cryo-EM analysis (Hafenstein) and genetic selection (Jin).

Weaknesses

- The proposal would be strengthened by information detailing how results would be interpreted and how the project relates to structural analyses of other parvoviruses.

1. Significance:

Strengths

- The most obvious function of a virus capsid is to contain and protect the nucleic acid. Protection is particularly important during the period when the virus is separated from a host cell and exposed to the external environment. Its role in genome protection accounts for the structural rigidity of the capsid and its relative inertness to chemical reaction. The capsid has numerous other functions, however, and many are of central importance to the ability of a virus to replicate and cause disease. For instance, the capsid must be assembled in the host cell, packaged there with the virus nucleic acid and released to find a new host cell. Thereafter the capsid is involved in recognition of the host cell receptor (in non-enveloped viruses), entry of the virus into the host cell and delivery of the virus nucleic acid to a compartment where it can be replicated. In many cases the capsid is recognized by host antibodies that can neutralize the virus infectivity.
- The capsids to be studied here are those of a model system, canine parvovirus (CPV) and feline panleukopenia virus (FPV; also a parvovirus). CPV arose in the late 1970's as a variant of FPV with altered receptor recognition. The capsids of both viruses are simple ones (T=1 icosahedra) with extensive structural analysis, including X-ray crystallography, already completed. The capsids are therefore most attractive for the proposed studies of capsid structural variability, receptor recognition and antibody neutralization. B19 is the best-known parvovirus able to cause disease in humans. It causes a xanthem (fifth disease) in children and an aplastic anemia in adults. Adeno-associated viruses are also able to infect humans and are now being actively studied as delivery vehicles in gene therapy protocols.

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- Dr. Colin R. Parrish is a Professor in the Baker Institute for Animal Health at Cornell University College of Veterinary Medicine where he has served since 1984. He is well known for his studies of parvoviruses including CPV and FPV. Preliminary studies he has done on all three aims are strengths of the proposal. X-ray crystallography and cryo-EM studies carried out with Dr. Michael Rossmann (Purdue Univ.) form an important part of the background for the current project. Dr. Susan L Hafenstein is a consortium collaborator. She is an Assistant Professor in the Department of Medicine (Infectious Disease Division) at Pennsylvania State University School of Medicine where she has served since 2009. Prior to her current appointment, Dr. Hafenstein was a post-doctoral fellow with Michael Rossmann at Purdue where she worked with Dr. Parrish on structural analysis of CPV/FPV. Her role will be to carry out the cryo-EM studies on receptor binding as described in aim 2, an effort for which she is very well qualified.

Weaknesses

- None.

3. Innovation:

Strengths

- The proposed project abounds with methodological innovations that enhance the chances for success of the overall effort. I was particularly attracted to the two screens described in aim 2 to identify amino acids involved in TfR-capsid contacts. Both seem well suited for their goal and full of ways they could be successful. I was also attracted to the effort to engineer an integrin-binding site (RGD) into an exposed site on the CPV capsid and test whether the mutant virus would be able to use integrin as a receptor.

Weaknesses

- None

4. Approach:

Strengths

- The proposed project begins with the considerable amount we already know about the CPV/FPV capsid and extends it in three areas: variability and flexibility in the capsid not detected by X-ray crystallography (aim 1), interaction of the capsid with the host cell receptor for the virus (transferrin receptor; TfR; aim 2), and neutralization of virus infectivity by binding of antibodies specific for the capsid (aim 3). In aim 1 Dr. Parrish and his colleagues will emphasize studies of ion binding to the major capsid protein, cleavage of the N-terminal ~20 amino acids of VP2 and residues (297-301) that affect proteolytic cleavage of VP2, loop 3 during infection. Mutagenic changes will be made in all three regions and the effects tested on variables including virus infectivity and capsid stability.
- Aim 2 is focused on interaction of the host cell receptor (TfR) with the capsid. The goal is to identify capsid amino acids involved in contacting the receptor and receptor residues involved in direct contacts with the capsid. Clever selection methods are described for each part of the project, and the results will be complemented with cryo-EM studies to compare receptor binding to the capsid as the contact amino acids are changed (see Fig. 5, pg. 38).

- Aim 3 is a study of neutralization of CPV infectivity by specific antibodies. The goal is to distinguish between two models, one in which neutralization results from interference with receptor binding and the other in which neutralization is related to the strength of antibody binding. The project is designed to extend a prior study in which cryo-EM was used to define the binding sites of eight antibodies Fab's on the capsid surface (ref. 21). A clever selection method is described for preparing Mab's that differ in their affinity for the capsid.
- I am enthusiastic about all three proposed aims. Dr. Parrish begins with the considerable amount we already know about parvovirus capsid structure and would extend it by making use of preliminary results from his lab. Divalent ion binding is an example. From crystallographic analysis of the capsid we know that two or three divalent ions (probably Ca2+) are bound to VP2 (the major capsid protein) and we know where they are bound. In the proposed project, the amino acids holding the ions in place will be mutated and the effects of the mutations tested. Similar studies will be carried out with sites in loop 3 affecting proteolytic digestion and sites at which VP2 is cleaved near the N-terminus. Relevant tests will be carried out on the mutated viruses; these will include infectivity, capsid stability, DNA exposure, receptor binding and other variables.
- Identification of the amino acids involved in capsid-receptor contact is a challenging goal for which the CPV system is most attractive (aim 2). Clever selection methods are proposed for both aspects of the study (i.e. identification capsid AA's and receptor aa's). Enhanced resolution of the current cryo-EM images of the receptor-capsid complex, as described in the proposal, should complement the biochemical studies of receptor-capsid contact. For example, amino acids found in biochemical analyses should be included in the contact region identified by cryo-EM. Appropriate collaborators will be involved in the yeast selection (Jin) and cryo-EM (Hafenstein) portions of the proposed analysis.
- As a result of previous studies, analyses of antibody Fab binding to the CPV capsid are now at an advanced state. Cryo-EM has been used to map eight Mab's and the eight are found to bind to one of two antigenic sites (A and B) on the capsid (ref. 21). Strong evidence has been presented to show that interference with receptor binding can result in virus neutralization by a MAb. Studies are proposed to perform a similar test for the strength of antibody binding. Using a yeast selection system, antibodies with different binding affinities will be selected and tested for their ability to neutralize the virus. The proposed study will advance what we already know by analyzing binding of Fab's with different specificities at once and by testing the effects of Fab binding on receptor (TfR) attachment.

Weaknesses

- The proposal would be improved by an overall hypothesis integrating the various capsid functions into an overall model of infection. Ion binding, or loop 3 cleavage, for example. What stage of infection so they affect? Such a model might clarify how results of the proposed studies will be interpreted. Do they result from capsid variation or are they the result of incomplete capsid maturation or perhaps pre-mature launching of the infectious program?
- Structural studies of Adeno-associated viruses are at a comparable stage of development as those described here with CPV/FPV. The application doesn't indicate how the two studies complement each other and areas where the same information is being developed.
- I missed a discussion of the phospholipase activity of CPV. The proposal lacks a discussion of how exposure of the phospholipase may relate to capsid-receptor binding.

5. Environment:

Strengths

- There is an excellent environment for research and for virus research in the Cornell College of Veterinary Medicine (Ithaca).

Weaknesses

- None.

Protections for Human Subjects:

- NA

Vertebrate Animals:

- NA

Biohazards:

- Infectivity studies of CPV will be performed only in cell culture.

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2:

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

Overall Impact:

Strengths

- Studies of parvovirus capsid-receptor interactions and capsid-antibody will be applicable to other non-enveloped viruses with similar structures.
- Parvoviruses are an excellent model system to understand the relationship between viral structure and factors that could influence infectivity.

Weaknesses

- None noted

1. Significance:

Strengths

- An integrated plan to probe the requirements for parvovirus structure and its interaction with ligands in solution. The anticipated results will link various biochemical properties for capsid with infectivity.
- Defining the requirements for interactions between parvovirus capsid and different versions of the receptors will contribute to better understanding of the interactions needed for parvovirus entry.
- Defining the interactions between neutralizing and nonneutralizing antibodies and parvovirus capsid (Aim 3) will be a significant contribution to treatment of parvovirus infection.

Weaknesses

- Some of the approaches are of low resolution and will provide more correlative rather than mechanistic insights.

2. Investigator(s):

Strengths

- Dr. Parish is a highly productive and accomplished virologist who is exceptionally well qualified to perform the proposed work.
- Dr. Hafenstein, an assistant professor at U. of Penn has been collaborating with Dr. Parrish, parvoviruses and the Transferrin receptor for several years and will provide structural biology expertise for the project.
- Dr. Jin at Cornell University will assist in the selection of the yeasts that be altered for affinities for the TfR.

Weaknesses

- none

3. Innovation:

Strengths

- Innovative selection of altered TfR using yeast expression library.
- The use of the combination of approaches is unusual and will help ensure progress.

Weaknesses

4. Approach:

Strengths

- Defined assays have already been developed in the Parrish laboratory to analyze population of viruses and for virus-TfR interactions.
- Understanding how the manipulated viruses in Aim 2 will attach to and infect cells will provide valuable information.
- Thoughtful aims that together, will contribute to both understanding of parvovirus capsids as well as potential therapies.

Weaknesses

- Aim 1. Since the goal of this aim is to understand variations within parvovirus, the analysis of populations of particles lead to emsemble averaging of the results and obscure the differences between particles. The use of proteases and antibodies could cause conformational changes in virions that can result in changes. Methods to overcome the limitations of sampling populations will be necessary for more clear-cut answers. Dr. Parrish should consider methods for the analysis of single vial particles.
- Aim 1 could benefit from analysis of mutant capsid binding and entry into cells.
- Many of the methods require very careful manipulations to obtain quantitative results. These include the differential penetration of dye, cleavage of capsids, determination of the number of receptors bound, and changes in the fluorescence of the capsids.

- The goal for the resolution in cryoEM studies should be better defined since that will significantly impact the amount of analysis. If the resolution is poorer than 8 Å, the amount of detailed information concerning interaction with the transferrin receptor will be limited.

5. Environment:

Strengths

- Cornell has excellent facilities for the proposed biological and biochemical analyses on parvoviruses.

Weaknesses

- The need for cryoelectron microscopy facility at Purdue University and Dr. Hafenstein's location in Hershey PA could make the preparation of samples and the acquisition of images a logistic challenge.

Vertebrate Animals:

Acceptable

Biohazards:

Acceptable

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3:

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

Overall Impact:

Strengths

- The proposed studies aim to integrate current understanding of parvovirus capsid structures and functions involved in virus-cell interactions during infection and host range determinants. The projection is that the studies will have implications for other non-enveloped viruses and also provide parallels to structural changes and interactions that occur during enveloped virus infection as well. State-of-the-art approaches, including structural and biophysical analyses, combined with basic viral studies should provide mechanistic insights.
- A significant amount of published results and preliminary data strongly support the proposed studies.

- The proposed studies will expand existing protocols and develop new approaches that will certainly provide new insight into parvovirus-host interactions. How much the results will extend directly to understanding other viruses remain to be seen. Nonetheless, the study will likely have broader implications as a model and applicability for refinement of experimental approaches that can be used to study other virus-host interactions. The impact on the parvovirus field should be very high and there is potential for high impact more broadly.
- The principal investigator has significant experience and expertise in virology. His lab has been very productive and made contributions to the field. Excellent publication record in high impact journals. Excellent collaborators help form a strong team to carry out the proposed studies.
- The experimental approaches are carefully described and considered. Expected results, potential problems and alternative approaches are covered.
- The scientific environment at Cornell is excellent for the proposed studies. The facilities at Purdue are excellent for the structural studies.
- The likelihood of successful outcomes from the proposed studies is very high.

Weaknesses

- There are no major weaknesses.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Acceptable

Biohazards:

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:

VERTEBRATE ANIMAL (Resume): ACCEPTABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Meeting Roster

The roster for this review meeting is displayed as an aggregate roster that includes reviewers from multiple CSR Special Emphasis Panels of the IDM IRG for the 2010/05 council round. This roster is available at:

http://www.csr.nih.gov/SummaryStatementRoster/IDM201010.pdf