RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 01/08/2020

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 1R01AI148264-01
FAIN: R01AI148264

Principal Investigator(s):
CARLOS A SARIOL, MD

Project Title: Dengue-Zika: Correlates of Cross-Protection in Non-Human Primates

Mrs. Roman-Garcia, Irma E
Assistant Director - Pre-Award, AOR
PO Box 365067
SAn Juan, PR 009365067

Award e-mailed to: enga.rcm@upr.edu

Period Of Performance:
Budget Period: 01/09/2020 – 12/31/2020
Project Period: 01/09/2020 – 12/31/2024

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $714,650 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF PUERTO RICO MED SCIENCES in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI148264. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Tamia Y. Powell
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 1R01AI148264-01

Award Calculation (U.S. Dollars)

Salaries and Wages	$94,964
Fringe Benefits	$38,486
Personnel Costs (Subtotal)	$133,450
Materials & Supplies	$131,226
Travel	$2,500
Other	$137,490
Subawards/Consortium/Contractual Costs	$95,151
Federal Direct Costs	$499,817
Federal F&A Costs	$214,833
Approved Budget	$714,650
Total Amount of Federal Funds Obligated (Federal Share)	$714,650
TOTAL FEDERAL AWARD AMOUNT	$714,650
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$714,650

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$714,650	$714,650
2	$695,400	$695,400
3	$685,020	$685,020
4	$677,445	$677,445
5	$667,095	$667,095

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1660433762A6
Document Number:	RAI148264A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2020

IC	CAN	2020	2021	2022	2023	2024
AI	8472364	$714,650	$695,400	$685,020	$677,445	$667,095

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M32C B / **OC**: 41021 / **Released**: PII 01/02/2020
Award Processed: 01/08/2020 12:03:09 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01AI148264-01

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R01AI148264-01

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.

c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI148264. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 1R01AI148264-01

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

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The budget period anniversary start date for future year(s) will be **January** 1.

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This Notice of Award (NoA) includes funds for activity with **Saint Louis University** in the amount of **$95,151** (**$62,806** direct costs + **$32,345** F&A costs).

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In accordance with the NIAID Financial Management Plan, NIAID does not provide funds for inflationary increases. Committed future year (s) funding was adjusted accordingly. See: https://www.niaid.nih.gov/grants-contracts/financial-management-plan.

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Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL 3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL 3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- o A list of the new and/or additional Agent(s) that will be studied;
- o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Shan Liang
Email: shan.liang@nih.gov **Phone**: 301-761-7415 **Fax**: 301-493-0597

Program Official: Sara Elaine Woodson
Email: sara.woodson@nih.gov **Phone**: 301-761-6478

SPREADSHEET SUMMARY
GRANT NUMBER: 1R01AI148264-01

INSTITUTION: UNIVERSITY OF PUERTO RICO MED SCIENCES

Budget	Year 1	Year 2	Year 3	Year 4	Year 5
Salaries and Wages	$94,964	$94,964	$94,964	$94,964	$94,964
Fringe Benefits	$38,486	$38,486	$38,486	$38,486	$38,486
Personnel Costs (Subtotal)	$133,450	$133,450	$133,450	$133,450	$133,450
Materials & Supplies	$131,226	$123,726	$121,726	$114,726	$107,726
Travel	$2,500	$2,500	$2,550	$2,600	$2,700
Other	$137,490	$137,490	$135,520	$134,420	$134,420
Subawards/Consortium/Contractual Costs	$95,151	$95,151	$95,151	$95,151	$95,151
Publication Costs		$3,000		$3,000	$3,000
TOTAL FEDERAL DC	$499,817	$495,317	$488,397	$483,347	$476,447
TOTAL FEDERAL F&A	$214,833	$200,083	$196,623	$194,098	$190,648
TOTAL COST	$714,650	$695,400	$685,020	$677,445	$667,095

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	50%	50%	50%	50%	50%
F&A Cost Base 1	$429,666	$400,166	$393,246	$388,196	$381,296
F&A Costs 1	$214,833	$200,083	$196,623	$194,098	$190,648

PI: **SARIOL, CARLOS A**	Title: Dengue-Zika: Correlates of Cross-Protection in Non-Human Primates	
Received: 02/04/2019	FOA: PA19-056 Clinical Trial:Not Allowed	Council: 10/2019
Competition ID: FORMS-E	FOA Title: Research Project Grant (Parent R01 Clinical Trial Not Allowed)	
1 R01 AI148264-01	Dual:	Accession Number: 4261351
IPF: 578705	Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES	
Former Number:	Department:	
IRG/SRG: ZRG1 IMM-C (02)	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 467,472 Year 2: 466,945 Year 3: 464,010 Year 4: 462,975 Year 5: 460,095	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
CARLOS SARIOL M.D.	UNIVERSITY OF PUERTO RICO MED SCIENCES	PD/PI
Idia Rodriguez	University of Puerto Rico	Other Professional-Veterinarian
James Brien Ph.D	Saint Louis University School of Medicine	Co-Investigator
Amelia Pinto Ph.D	Saint Louis University School of Medicine	Co-Investigator
Aravinda DeSilva Ph.D	University of North Carolina at Chapel Hill	Other (Specify)-Collaborator

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

○ Pre-application ● Application ○ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **Organizational DUNS*:** 9481080630000

Legal Name*: UNIVERSITY OF PUERTO RICO MED SCIENCES
Department:
Division:
Street1*: UPR Medical Sciences Campus
Street2: Office of Sponsored Programs, PO Box 365067
City*: SAN JUAN
County: Select a State
State*: PR: Puerto Rico
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 009365067

Person to be contacted on matters involving this application
Prefix: Mrs. First Name*: Irma Middle Name: E Last Name*: Roman-Garcia Suffix:
Position/Title: Assistant Director - Pre-Award, AOR
Street1*: PO Box 365067
Street2:
City*: SAn Juan
County: Select a State
State*: PR: Puerto Rico
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 00936-5067
Phone Number*: 787-758-2525 x-7042 Fax Number: 787-766-6764 Email: enga.rcm@upr.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 660433762

7. TYPE OF APPLICANT* A: State Government

Other (Specify):
Small Business Organization Type ○ Women Owned ○ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ○ Resubmission
○ Renewal ○ Continuation ○ Revision

If Revision, mark appropriate box(es).
○ A. Increase Award ○ B. Decrease Award ○ C. Increase Duration
○ D. Decrease Duration ○ E. Other *(specify)* :

Is this application being submitted to other agencies?* ○Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Dengue-Zika: Correlates of Cross-Protection in Non-Human Primates

12. PROPOSED PROJECT
Start Date* Ending Date*
10/01/2019 09/30/2024

13. CONGRESSIONAL DISTRICTS OF APPLICANT
00-000

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: CARLOS Middle Name: A Last Name*: SARIOL Suffix: M.D.
Position/Title: Associate Professor
Organization Name*: UNIVERSITY OF PUERTO RICO MED SCIENCES
Department:
Division:
Street1*: Medical Sciences Campus
Street2:
City*: San Juan
County:
State*: PR: Puerto Rico
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 00936-5067
Phone Number*: 7877582525- 5112 Fax Number: Email*: carlos.sariol1@upr.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$3,499,527.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$3,499,527.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Segundo Middle Name: Last Name*: Rodriguez-Quilinchini Suffix: M.D.
Position/Title*: Interim Chancellor
Organization Name*: University of Puerto Rico Medical Sciences Campus
Department: Chancellor;s Office
Division:
Street1*: PO Box 365067
Street2:
City*: SAn Juan
County:
State*: PR: Puerto Rico
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 00936-5067
Phone Number*: 787-758-2525 X-7042 Fax Number: 787-766-6764 Email*: enga.rcm@upr.edu

Signature of Authorized Representative*
Irma Roman-Garcia

Date Signed*
02/04/2019

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of Puerto Rico, Medical Sciences Campus
Duns Number:	9481080630000
Street1*:	PO Box 365067
Street2:	
City*:	SAn Juan
County:	Select a State
State*:	PR: Puerto Rico
Province:	
Country*:	USA: UNITED STATES
Zip/ Postal Code*:	009365067
Project/Performance Site Congressional District*:	00-000

Additional Location(s) File Name:

Funding Opportunity Number: PA-19-056. Received Date: 2019-02-04T14:29:01.000-05:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number A3421-01

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract.pdf
8. Project Narrative*	Narrative.pdf
9. Bibliography & References Cited	REFERENCES.pdf
10.Facilities & Other Resources	FacilitiesResources-Sariol-R01-ZIKV.pdf
11.Equipment	

Abstract

Zika virus (ZIKV) is a re-emerging mosquito-borne *Flavivirus* that recently caused an outbreak in the Americas. The establishment of ZIKV transmission cycle in tropical/sub-tropical regions that are endemic to other close-related flaviviruses such as Dengue virus (DENV) has raised concerns, mainly by their cross-immunological interactions and the implications of this for development of severe clinical manifestations. Several groups have demonstrated that DENV-immune serum from humans can enhance ZIKV infection *in vitro* and *in vivo* in an immunodeficient mice model. This phenomenon known as Antibody Dependent-Enhancement (ADE) has been linked to severe dengue clinical manifestations. Little is known about the effect of a previous immunity to ZIKV on a subsequent DENV infection. It is highly necessary to characterize correlates of protection in the control of a heterologous secondary DENV or ZIKV infection in the presence of previous DENV or ZIKV immunity in an immunological competent animal model that resemble the human immune system like the Non-Human Primates. Our group have preliminary data showing a potential protective role of the cellular immune response in dengue- immune or ZIKV-immune subjects during a heterologous secondary infection with ZIKV or dengue. The overall hypothesis behind this work is that the cross-primed cellular immune response may be critical controlling the DENV and ZIKV infection and provides heterologous protection against each other. To test this hypothesis, we propose a series of straightforward experiments by depleting the CD4+ or CD8+ or CD20+ cells at different time points before a primary or a secondary infection with dengue or ZIKV. This type of experiment has not been performed before in NHP. For these experiments we will use rhesus macaques bred and housed at the Caribbean Primate Research Center that has proven to be the purer Indian-origin macaque population of all populations in the USA or imported animals, without having a significant level of inbreeding. For first time in any study in the flavivirus field, we will use a large data on the MHC typing of this population to characterize specific CD4 and/or CD8 T cells epitopes playing a role in the T cells immune response against dengue and ZIKV. Understanding correlates of protection between ZIKV and DENV is essential to anticipate the outcome of the secondary infection, the design of diagnostics methods and more relevant, to support the design of highly effective ZIKV and DENV vaccines in the scenario of previous DENV or ZIKV immunity, respectively. Undoubtedly, NHP provide us with a unique immunological tool very close to the human system to provide the answers to the questions we are outlying on this application.

Narrative

Zika virus (ZIKV) is a re-emerging mosquito-borne *Flavivirus* that recently caused an outbreak in the Americas. The establishment of ZIKV transmission cycle in tropical/sub-tropical regions that are endemic to other close-related flaviviruses such as Dengue virus (DENV) has raised concerns, mainly by their cross-immunological interactions and the implications of this for development of severe clinical manifestations like Guillain-Barre Syndrome and the Congenital Zika Syndrome. Few is known about consequences of the immunological interaction between those viruses. This project aims to identify correlates of protection or pathogenesis in the complex immunological interactions between DENV and ZIKV using non-human primates as a model. Results from the work proposed here will have a huge impact in the ZIKV and DENV vaccines design and in the way we approach the diagnosis of these viral diseases and anticipate the epidemiological behavior during DENV or ZIKV epidemics in population with previous heterologous immunity.

RESOURCES

Facilities

Laboratory: For the implementation of this grant application Dr. Sariol has a well-equipped laboratory of approximately 400 square feet. In addition, under his direction there are a Biosafety Level-3 Laboratories (about 36 x 30 sq. feet) and one Biosafety Level-2 virology/Molecular biology laboratory (800 sq. feet). Also Dr. Sariol is in charge of The Immunology and Flow Cytometry Laboratory of the CPRC adjacent to the BL 3 Laboratory. Together all those laboratories support procedures as ELISA, tissue culture, virus purification, Flow Cytometry, nucleic acid purification, qReal-Time PCR, Neutralization assay, cytokines determinations, etc.

Clinical: N/A

Animal: The Animal Resources Center (ARC) and the Caribbean Primate Research Center are animal research facilities of the Medical Sciences Campus. Both are under the Unit of Comparative Medicine. The Surgery Research Laboratory of the Medicine Deanship is a satellite of the ARC.

The Animal Resources Center (ARC) is a unit committed to the development and support of animal – based research and resources, which will ultimately contribute to improve human health and animal welfare. It provides researchers, faculty and students with an AAALAC accredited facility, suitable equipped and staffed, for the short and long term maintenance of laboratory animals used in research and/or education. The ARC , located on the 10th and 11th floors of the main building of the Medical Sciences Campus, in metropolitan San Juan, Puerto Rico, is around 26,000-sq. ft in gross size, and is dedicated primarily to research, testing and teaching. It includes ABSL-2 and ABSL-3 areas equipped for SIV/HIV/SHIV/WNV, Dengue and ZIKV work in nonhuman primates. The facility can accommodate up to 90 NHP at once. The ARC has been accredited by AAALAC continuously since 1984 and operates in strict compliance with USDA Animal Welfare Act, the "Guide to the Care and Use of Laboratory Animals", and the NIH Public Health Service Policy/OLAW.

Computer: Three iMac, one 27 inch and two 21 inches with 4GB Ram memory and 150 GB hard disk are available for this project. Also one state of the art MacBook Pro is available. In addition, there is a Pentium III computer at the Virology unit. All the computers have Microsoft application programs (Excel, Microsoft Word, Powert Point, Acces, Prism, etc.) and two laser printers.

Office: One office (15 x 13 sq. feet) and space to accommodate the Post Doc and Technician are available (20 x 12 sq. feet).

Scientific Environment: The University of Puerto Rico, Medical Sciences Campus and the CPRC have been running collaborative projects and others NIH's supported research in the last twelve years. The success for those projects confirmed the UPR administration willingness to do their best to support this project. For this project we will use the unique resources provided by the Caribbean Primate Research center. This facility and its Virology laboratory will provide high quality animals and research support for this project.

Special Facilities: We have a BSL3 facility to support research with Select Agent. However while Dengue and ZIKV are not considered a Select Agent, BSL3 facilities are not required to work with these virus.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

The BL2/3 Virology/Molecular Biology Facilities are well equipped with: Three class II biological safety cabinets; three double-door CO2 incubators, Four REVCO freezers, two LN2 storage tanks; two tissue culture, one Beckman L8-M and one Beckman TJ6 centrifuge, two shakers temperature adjustable water baths, electrophoresis equipment, blotting apparatus. A Luminex100 system (Bio-Rad, Hercules, CA) for multiplex-cytokines array is located in the virology/molecular biology laboratory. Also we have a iQ5 PCR machine to conduct the qReal-Time PCR experiments. An ELISA reader is also available for experiments to be conducted on this protocol. In addition, for the cytometry studies we have a MACSQuant Analyzer 10 with the latest version of the software. All these equipment has been used and in use for the conduction of one federally- funded research projects.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: CARLOS Middle Name A Last Name*: SARIOL Suffix: M.D.

Position/Title*: Associate Professor
Organization Name*: UNIVERSITY OF PUERTO RICO MED SCIENCES
Department:
Division:
Street1*: Medical Sciences Campus
Street2:
City*: San Juan
County:
State*: PR: Puerto Rico
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 00936-5067

Phone Number*: 7877582525- 5112 Fax Number:

E-Mail*: carlos.sariol1@upr.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: PD/PI Other Project Role Category:

Degree Type: MD,MS Degree Year: 1987

Attach Biographical Sketch*: File Name: CARLOS_A._SARIOL.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Idia Middle Name V. Last Name*: Rodriguez Suffix:

Position/Title*: Clinical Vet ARC/ Attending Vet MSC
Organization Name*: University of Puerto Rico
Department:
Division: Unit of Comparative Medicine
Street1*: Office A1067, 10th Floor
Street2: Main Building, Medical Center Area
City*: Sanjuan
County:
State*: PR: Puerto Rico
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 00936-8344

Phone Number*: 787-756-6540 Fax Number:

E-Mail*: idia.rodriguez1@upr.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Other Professional Other Project Role Category: Veterinarian

Degree Type: DVM,BS Degree Year: 2001,1996

Attach Biographical Sketch*: File Name: IDIA_VANESSA_RODRIGUEZ.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: James Middle Name D Last Name*: Brien Suffix: Ph.D

Position/Title*: Associate Professor
Organization Name*: Saint Louis University School of Medicine
Department:
Division:
Street1*: 1100 South Grand Blvd., Room 711
Street2:
City*: St. Louis
County:
State*: MO: Missouri
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 631041015

Phone Number*: 3149778895 Fax Number:

E-Mail*: brienj@slu.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD,BS Degree Year: 2008,1997

Attach Biographical Sketch*: File Name: Brien_JD_biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Amelia Middle Name Kahler Last Name*: Pinto Suffix: Ph.D

Position/Title*: Assistant Professor
Organization Name*: Saint Louis University School of Medicine
Department:
Division:
Street1*: 1100 South Grand
Street2: Doisy Research Center
City*: Saint Louis
County:
State*: MO: Missouri
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 631041015

Phone Number*: 3149778897 Fax Number:

E-Mail*: pintoak@slu.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD,BS Degree Year: 2007,1997

Attach Biographical Sketch*: File Name: PintoAK_biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Aravinda Middle Name M Last Name*: DeSilva Suffix: Ph.D

Position/Title*: Professor
Organization Name*: University of North Carolina at Chapel Hill
Department: Department of Microbiology
Division:
Street1*: 160 Dental Circle
Street2: UNC-Chapel Hill CB # 7292
City*: Chapel Hill
County:
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27599-7290

Phone Number*: 919 962 4891 Fax Number:

E-Mail*: desilva@med.unc.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: PHD,MPH,BA Degree Year: 1992,1997,1986

Attach Biographical Sketch*: File Name: SF424R-R_biosketch-desilva_CS.pdf

Attach Current & Pending Support: File Name:

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: Carlos A. Sariol Curbelo

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

POSITION TITLE: Associate Investigator. Director, Unit of Comparative Medicine.

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
Institute of Medical Sciences, Havana, Cuba.	Medical Doctor	1987	Medicine
Institute of Tropical Medicine Pedro Kouri, Havana, Cuba.	Microbiologist, Major	1992	Microbiology/ Virology
Institute of Tropical Medicine Pedro Kouri, Havana, Cuba.	Master Degree	1994	Virology
University of Tübingen, Medicine Clinic I, Molecular Gastroenterology, Tübingen Germany	Post Doc	1999-2000	Cancer and Apoptosis

A. Personal Statement

I consider being well qualified to serve as PI on this application. My expertise working with NHP began when in 2001 when I joined to the Caribbean Primate Research Center's Virology and Genetics Laboratory (VGL), which focused on the diagnosis of viral infections of CPRC's rhesus population. From 2002 as Laboratory Director, I developed the virology algorithm to test the animals to be included in the incipient SPF Programs at CPRC. Since them I coordinated and supervised the implementation of this algorithm and the management of the SPF program based in the virology and genetic results. From 2002 to present as CPRC's Virology and Genetics Laboratory Director, I coordinated and supervised different projects on HIV/SIV pathogenesis, therapeutics and vaccine protocols using rhesus macaques as a model. Starting in 2007, with the support of North East Biodefense Center I built up a program to study dengue and other flavivirus pathogenesis and vaccine development in rhesus macaques. After that I developed a complete system, including the animal model and the logistic to support complex studies on dengue pathogenesis and vaccine in NHPs. Recently we also have implemented a Zika program at CPRC with relevant results on Zika pathogenesis. We are focused in the interactions of dengue and Zika and other flavivirus in NHP. Results of my works in collaboration with researchers from different Institutions around the world have been published in high impact journals. As PI I have been able to put together great groups of researchers to get the best from our NHPO model. The collaborators and their labs participating on this application, Drs. Aravinda de Silva (Humoral immune response) and Amelia Pinto (T cells immune response) are part of a great team that continuous working together with active ongoing collaboration. Since 2012 I have been serving as PI of two major Specific Pathogen Free animals grants. That responsibility allowed me to acquire vast experience in the handle and use for NHP for biomedical research and in the administration of large NIH grants. As PI of those grants, and relevant to this application, I lead the implementation of the MHC typing of the CPRC's colony.
I will bring all my experience and staff to execute this research project.

B. Positions and Honors

1981-1983 School of Medicine, Havana, Cuba.
1983-198 Navy Hospital, Havana City, Cuba
1986-1987 **Internship,** Central Hospital of Luanda, Luanda, Angola.
1987-1989 **Social service** at Carlos J. Finlay, Havana City, Cuba.
1989-1992 **Medical Residence, Infectious Diseases,** Institute of Tropical Medicine, Havana City, Cuba.
1993-1998 **Physician-Researcher** Institute of Tropical Medicine, Havana City, Cuba.
1992-1998 **Researcher,** Institute of Tropical Medicine "Pedro Kouri", Havana, Cuba
1992-1998 **Associated Researcher,** Center of Genetic Engineering and Biotechnology, Havana, Cuba.
1998-1999 **Guest Researcher,** Hygiene Institute, Department of Virology, Epidemiology and virus diseases, Eberhard Karls University, Tübingen, Germany
1999-2000 **Post-Doc,** University of Tübingen, Medicine Clinic I, Molecular Gastroenterology, Germany
2001-2002 **Molecular Virologist,** Primate Research Center, School of Medicine, University of Puerto Rico.
2002 to present: **Director,** Virology Laboratory, Caribbean Primate Research Center, School of Medicine, University of Puerto Rico, P.R.
2012 to present: **Director**, Unit of Comparative Medicine, UPR-MSC.

Honors

2008 Nomination to Charles C. Shepard Science Award, 2008, CDC.

Other Experience and Professional Memberships

2002 to present: American society of Microbiology
2002 to present American Society for Tropical Medicine and Hygiene
2013 to present Fellow American College of Physicians

C. Contribution to Science

1. The most relevant contribution related to this application is related to the dengue-Zika interactions in non-human primates

Zika virus pathogenesis in rhesus macaques is unaffected by pre-existing immunity to dengue virus. **Pantoja P, Pérez-Guzmán EX, Rodriguez IV, White LJ, González O, Serrano C, Giavedoni L, Hodara V, Cruz L, Arana T, Martinez MI, Hassert MA, Brien JD, Pinto AK, de Silva A, Sariol CA.** Nat Commun. 2017 Jun 23;8:15674. doi: 10.1038/ncomms15674.

A Tale of Two Viruses: Does Heterologous Flavivirus Immunity Enhance Zika Disease? Sariol CA, Nogueira ML, Vasilakis N. Trends in microbiology. 2017; PMID: 29122447

2. My contribution to science started in the Institute of Tropical Medicine and Hygiene in Havana, Cuba. I had the opportunity to be part of the team that identified and characterized the dengue virus strain that caused the major Dengue Hemorragic Fever in the Americas Region (more than 300 deaths in one and half year). That epidemic took place in 1981 and when I joined the Institute Pedro Kouri in 1992 I started to work in the molecular characterization of that dengue strain. In 1999 we published our results in the Journal of the American Society for Tropical Medicine and Hygiene:

Detection and genetic relationships of Flavivirus sequences in seventeen-years old paraffin-embedded samples from DHF/DSS epidemic in Cuba in 1981. **Sariol** CA, Pelegrino JL, Martínez A, Arteaga E, Kourí G and Guzmán MG. *Am J Trop Med Hyg.* Dec;61(6):994-1000,1999. **PMID: 10674684.**

During those years we were able to produce the recombinant Envelope protein from dengue in the yeast host *Pichia pastoris* to be used as immunogen in a dengue vaccine formulation. That work resulted in an international and European patent application:

Process for the Expression of Genes of the Dengue Viruses. Patent number WO98/23754. Sariol, Carlos Augusto, et al. June 4, 1998.

In 2005 I was able to regain my dengue research supported by the North East Biodefense Center. As a major achievement, combining my expertise in dengue and in the use of NHP as model, in 2007 we published the first molecular evidences showing why rhesus macaques do not develop dengue hemorrhagic manifestation in comparison with humans.

Transcriptional activation of interferon stimulated genes but not of cytokine genes after primary infection of rhesus macaques with dengue virus type 1. C. A. **Sariol,** Jorge L. Muñoz-Jordán, Kristina Abel, Lymarie C. Rosado, Petraleigh Pantoja, Luis Giavedoni, Idia Vanessa Rodriguez, Laura J. White, Melween Martínez, Teresa Arana and Edmundo N. Kraiselburd. C V Immunology, June 14 (6): 756-766, 2007. PMID: 17428947

Years later we were able to provide the first in vivo evidence that Dengue virus was capable to counteract the activation of dendritic cells even in the presence of Toll Like Receptors Agonists.

Dengue Decreased Dengue Replication and an Increased Anti-viral Humoral Response with the use of Combined Toll-Like Receptor 3 and 7/8 Agonists in Macaques. Carlos A. **Sariol,** Melween I. Martínez, Francheska Rivera, Idia Vanessa Rodríguez, Petraleigh Pantoja, Kristina Abel, Teresa Arana, Luis Giavedoni, Vida Hodara, Laura J. White, Yesseinia I. Angleró, Luis J. Montaner, Edmundo N. Kraiselburd. 2011, April. PloS ONE 6(4)e19323. **PMID: 21559444**

3. After that, I continue my collaboration with great teams of researchers. These collaborations have let to at least two major contributions in the dengue field. In 2013, in collaboration with Drs. Robert Johnston and Laura White at the former Global Vaccine and with Dr. Aravinda de Silva, we published an article showing that the NHP response to dengue recombinant antigens and to dengue infection is qualitatively different. Even though, the epitopes recognized by the NHP immune system are also different in those two cases. But most important is that we showed that the NHP immune response is very similar to the one observed in humans naturally exposed to dengue. This result was published in Journal of Virology.

An alphavirus vector based tetravalent dengue vaccine induces a rapid and protective immune response in macaques that differs qualitatively from immunity induced by live virus infection. White, L., **Sariol,** C.A, Mattocks, M., Wahala, W., Yingsiwaphat, V., Collier, M., Whitley, J., Mikkelsen, R., Rodriguez, I., Martinez, M., de Silva, A., and Johnston, R. 2013. Journal of Virology 87(6). **PMID: 23302884**

4. Recently in 2015 in collaboration with Aravinda de Silva and Ralph Baric and their teams, we published the characterization of a quaternary structure DENV2 as a responsible of type-specific neutralizing antibodies.

Gallichotte EN, Widman DG, Yount BL, Wahala WM, Durbin A, Whitehead S, Sariol CA, Crowe JE Jr, de Silva AM, Baric RS. A New Quaternary Structure Epitope on Dengue Virus Serotype 2 Is the Target of Durable Type-Specific Neutralizing Antibodies. MBio. 2015 Oct 13;6(5). pii: e01461-15. doi: 10.1128/mBio.01461-15. PubMed PMID: 26463165.

Finally, as a summary of my experience working with dengue and NHP, in collaboration with Dr. Laura White, we recently published a broad review on the use of NHP for dengue research program. The most important contribution is that we proposed the first guidelines to standardize the use of this model.

Utility, limitations, and future of non-human primates for dengue research and vaccine development. Sariol CA, White LJ. Front Immunol. 2014 Sep 24;5:452. 2014. **PMCID**: PMC4174039

5. On the other hand, since I started to work as Virologist at the Caribbean Primate Research Center in 2001, my main contribution has been the establishment and development of the virology algorithm to support the Specific Pathogen Free program of rhesus macaques (free of B virus, STLV, SIV and SRVD) at CPRC. Since 2002 I became Director of the Virology Laboratory and in 2012, PI of two major SPF grants. This work allowed me to be acquired vast experience in the handle and use for these animals for biomedical research and published the first virological and MHC typing characterizations of the Cayo Santiago and CPRC rhesus macaques populations.

Differential Distribution of Antibodies to Different Viruses in Young Animals in the Free-Ranging Rhesus Macaques of Cayo Santiago. C. A. Sariol, Janis González-Martínez, Teresa Arana, Sandra Gascot, Erick Suárez, Elizabeth Maldonado, Melissa S. Gerald, Maria Rodríguez and Edmundo N. Kraiselburd. Journal of Med Primatol, 35 (2006) 369–375, 2006. PMID: 17214665.

Herpes-B virus seroreactivity in a colony of Macaca mulatta: Data from the Sabana Seca Field Station, a new Specific-Pathogen-Free Program. Sariol, CA, Arana T, Maldonado E, Gerald M, Gonzalez-Martinez j, Rodriguez M and. Kraiselburd EN. J Med Primatol; 34:13–19, 2005. PMID: 15667339.

I also served as PI for a trial of the Takeda's dengue vaccine being responsible for the subjects recruitment, implementation of the vaccine schedule, vaccine administration and patients follow up.

Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation. For publicly available citations, URLs or PMC submission identification numbers may accompany the full reference; copies of publicly available publications are not accepted as appendix material.

https://www.ncbi.nlm.nih.gov/sites/myncbi/carlos.sariol.1/bibliography/44213425/public/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

2P40 OD012217 -29 Martinez (PI) 01/15/16-11/30/20
Caribbean Primate Research Center Program
The major goal of this project is to maintain and enhance the Caribbean Primate Research Center basic infrastructure, which is needed for the support of several biomedical research initiatives of national interest.
Role:Co-Investigator

5U24OD010421– Sariol (PI) 07/01/16-06/30/20
Maintenance of a Closed CPRC-SPF Colony
The major goal of this project is to maintain a colony of specific pathogen free rhesus macaques to support research using this model.
Role:PI

Clinical Consortium For Clinical Investigation (PRCCI). This initiative will support the development of R&D capabilities and streamline clinical trial conduct in PR.
Role: Co-PI

Completed Research Support

RO1 AI110792- Sariol (PI) 04/015/15-03/31/17
Tailoring Virulence dengue mammals and mosquitoes
The major goal of this project is to explore changes of the specific sequence space of genomes of arboviruses that have evolved to replicate efficiently in cells of two taxa (mammals and insects) at two different temperatures (27 and 37 C) and with a delicately balanced codon pair bias (CPB) accommodating CPB differences in mammals and insects.
Role: Project Leader

2016-00017 Rodriguez Orengo (PI) 9/11/15-3/31/17
Development of clinical trials Project

The goal of the project is to encourage and promote innovation, transfer and commercialization of technology and creation of jobs in the technology sector. As part of this strategy the PRST recently launched an initiative to establish the PR consortium for Clinical Investigation.

OD010421 – Sariol (PI) 09/01/12-06/30/16
Enhancement of the CPRC-SPF Rhesus Monkey Program
The major goal of this project is to establish a colony of specific pathogen free rhesus macaques to support research using this model.
Role: PI

U42OD011128 – Sariol (PI) 07/01/12-04/30/16
Establishment & Maintenance of a closed CPRC SPF colony
This grant supports the development of a Specific Pathogen-Free colony of rhesus macaques. This colony has been established and we follow-up the animals for the viral status to prevent the introduction or an outbreak of the specific target pathogens.
Role: PI

1R01AI094603 Edmundo Kraiselburd (PI) 03/01/11-02/28/16
Luis J. Montaner (PI)
Early Innate/IgA Anti-HIV/SIV Response in Exposed Uninfected
This grant support to determine the presence of local cellular cervical tissue infiltrate, IFN-mediated gene expression, and mucosal anti-HIV IgA antibody responses in 3 well-defined groups of women with differential exposure risk based on sexual activity/partners. 2. Determine if repeated cervico-vaginal exposures to non-infectious SIV E660 exposures induce a persistent innate cellular infiltrate (plasmacytoid DCs, NK, macrophages) that in combination with mucosal SIV-specific IgA antibody levels decreases mucosal infectivity SIV mac251. This proposal represents a collaborative effort between The University of Puerto Rico, Nebraska University, University of Minnesota, Duke University, University of Massachusetts, Tulane University, National Cancer Institute, and The Wistar Institute.
Role: Research Associated

2P40 OD012217 – Martinez, (PI) 12/01/10-11/30/15
Caribbean Primate Research Center Program
The major goal of this project is to maintain and enhance the Caribbean Primate Research Center basic infrastructure, which is needed for the support of several biomedical research initiatives of national interest
Role: Co-PI

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: **FIS** Rodriguez Idia Vanessa

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

POSITION TITLE: Clinical Veterinarian/Associate Director- Animal Resources Center, Medical Sciences Campus (MSC) / Attending Vet (MSC)–University of Puerto Rico

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
Kansas State University, Manhattan ,KS	BS	1996	Animal Science and Industry
Kansas State University, Manhattan, KS	DVM	2001	Veterinary Medicine

A. Personal Statement

As the Attending Veterinarian for the AAALAC accredited Medical Sciences Campus (MSC) Facilities for the past 10 years, I monitor and evaluate operations, processes and/or practices involving all animal handling and veterinary care insuring that all procedures comply with the recommendations set forth in the "Guide for the Care and Use of Laboratory Animals" as well as in all laws, regulations and policies governing the use of laboratory animals. This includes compliance with the Institutional Regulatory Committees like IACUC and Biosafety. As the Clinical Veterinarian of the Animal Resources Center (ARC) and Alternate Clinical Veterinarian at the Institute of Neurobiology, MSC I have had 15 years of experience within the Lab animal field within biosafety level conditions 1-3, including barrier facilities, transgenic and regular rodent models, rabbits, aquatic species, swine and non-human primate care within several research projects. Over the past 4 years I have also serve as Associate Director of the ARC and have help develop, coordinate and supervised different projects on disease pathogenesis, therapeutics and vaccine protocols using rhesus macaques as a model. I am a part of the animal care team that provides veterinary care of the animals from arrival to the facility to the completion of the procedures, including the procurement of the materials and preparation and organization of the areas, administration of physical exams and treatments as required, induction and maintenance of anesthesia throughout the various procedures and keeping detailed records. I have the experience and expertise, leadership and motivation necessary to successfully carry out the proposed work and meet the aims of this application.

B. Positions and Honors

Positions and Employment

2001-present Clinical Veterinarian, Animal Resources Center, Medical Science Campus, University of Puerto Rico, San Juan, PR.
2001-2002 Veterinary Clinician, Avian and Small Animal Hospital, San Juan, Puerto Rico,

Other Experience and Professional Memberships

2001- present	IACUC Continuing Education Speaker – Medical Sciences Campus UPR
2003–present	Instructor - Animal Health Technology Program, Medical Sciences Campus, UPR
2001-present	Member of American Veterinary Medical Association, May 2001 to present.
2001-present	Member of Colegio de Medicos Veterinarios, de Puerto Rico.
2001-present	Member of American Association of Laboratory Animal Science
2001-present	Member of the Association of Primate Veterinarians
2001-present	Member of the American Society of Lab. Animal Practitioners
2001- 2006	Alternate Member of the Institutional Animal Care and Use Committee, Medical Sciences Campus, University of Puerto Rico.
2001-present	Alternate Member of the Biosafety, Medical Sciences Campus, University of Puerto Rico
2002-present	Member of the Thesis Advisory Committee for the Degree of Master of Science in Pharmacy Candidates, Department of Pharmacy, MSC, UPR
May 2006 - Present	Attending Veterinarian, Medical Sciences Campus, UPR
May 2006 - Present	Member of the Institutional Animal Care and Use Committee, Medical Sciences Campus, University of Puerto Rico.

Honors

2001	Award of Recognition for Dedication and Compromised to the Program of Animal Care and Use - Medical Sciences Campus, UPR, PR
2008	Charles C. Shepard Science Award ceremony nominee under Laboratory Methods Category - CDC, Atlanta, Georgia

C. Contribution to Science

My contribution to science started in the Medical Sciences Campus (MSC), University of Puerto Rico (UPR). As the Clinical Veterinarian at the Animal Resources Center, MSC, UPR, I had the opportunity to be part of several studies advancing the knowledge on pathogenesis and Vaccine development and treatment for infectious diseases such as dengue and SIV/SHIV.:

R. Kumar, C. Torres, Y Yamamura, **I Rodriguez**, M. Martinez, S. Staprans, R. Donahoe, E. Kraiselburd, EB Stephens, A. Kumar. Modulation by Morphine of Viral Set Point in Rhesus Macaques Infected with SIV and SHIV. Journal of Virology 2004 Oct; 78 (20): 11425-8

Kumar, R., Perez-Casanova, A.E., Tirado, G., Noel, R.J., Torres, C., **Rodriguez, I.**, Martinez, M., Staprans, S., Kraiselburd, E., Yamamura, Y., Higley, J.D., and Kumar, A. Increased viral replication in Simian Immunodeficiency Virus/Simian-HIV-Infected macaques with self-administering model of chronic alcohol consumption. J Acquir Immune Defic Syndr. Volume 39, Number 4, August 1, 2005.

Ellenberger D., Wyatt L. , Li B., Buge L., Lanier N., **Rodriguez IV**, Sariol C., Martinez M., Monsour M., Dowd J., Smith J., Otten R., Montefiori D., Kraiselburd E., Moss B., Robinson H., Mc Nicholl,J., Butera S. Comparative immunogenicity in rhesus monkeys of multi-protein 3 HIV-1 (CRF02_AG) DNA/MVA vaccines expressing 4 mature and immature VLPs.Virology, 340(1)21:32, 2005

Ellenberger, D., Otten RA, Li b, Aidoo M, **Rodriguez IV**, Sariol C., Martinez M, Monsour M, Wyatt L, Hudgens MG, Kraiselburd E, Moss B, Robinson H, Folks T, Butera S. HIV-1 DNA/MVA vaccination reduces the per exposure probability of infection during repeat mucosal SHIV challenges. Virology 15; 353 (1): 216-25, 2006.

Sariol CA, Munoz-Jordan JL, Abel K, Rosado LC, Pantoja P, Giavedoni L, **Rodriguez IV**, White LJ, Martinez M, Arana T, Kraiselburd E. Transcriptional Activation of Interferon Stimulates Genes but not of Cytokine genes after primary infection in Rhesus Macaques with Dengue Virus Type 1. Clin Vaccine Immunol. 2007; Jun; 14(6):756-66.

M. Aidoo, R. Otten, **V. Rodriguez**, CA Sariol, M. Martinez, E. Kraiselburd, H. Robinson, T. Folks, S. Butera, D. Ellenberger : Absence of SHIV infection in gut and lymph node tissues in rhesus monkeys after repeated rectal challenges following HIV-1 DNA/MVA immunizations. Vaccine 25 (2007) 6474-6481.

Rivera-Amill V, Kumar R, Noel RJ, Garcia Y, **Rodriguez IV**, Martinez M, Sariol CA, Kraiselburd E, Iszard M,Mukherji M, Kumar S, Giavedoni LD, Kumar A. Short Communication: Lack of Immune Response in Rapid Progressor Morphine-Dependent and SIV/SHIV-Infected Rhesus Macaques Is Correlated with Downregulation of T(H)1 Cytokines. AIDS Res Hum Retroviruses. 2010 Jul 30. [Epub ahead of print] **PMID: 20672973**

Carlos A. Sariol, Melween I. Martínez, Francheska Rivera, **Idia Vanessa Rodriguez**, Petraleigh Pantoja, Kristina Abel, Teresa Arana, Luis Giavedoni, Vida Hodara, Laura J. White, Yesseinia I. Angleró, Luis J. Montaner, Edmundo N. Kraiselburd. Decreased Dengue Replication and an Increased Anti-viral Humoral Response with the use of Combined Toll-Like Receptor 3 and 7/8 Agonists in Macaques. 2011, April. PloS ONE 6(4)e19323. **PMID: 21559444**

Rafael Rodríguez-Mercado, Gregory Ford, Shefeng Xu, Edmundo Kraiselburd, Melween I Martinez, Vesna A Eterovic, Edgar Colon, **Idia V Rodriguez**, Peter Portilla, Pedro A Ferchmin, Lynette Gierbolini, Maria Rodriguez-Carrasquillo, Michael D Powell, John VK Pulliam, Casey O McCraw, Alicia Gates, and Byron D Ford: Acute Neuronal Injury and Blood Genomic Profiles in a Nonhuman Primate Model for Ischemic Stroke. Comparative Medicine 2012; Oct; 62 (5): 427-438.

White, L., Sariol, C.A, Mattocks, M., Wahala, W., Yingsiwaphat, V., Collier, M., Whitley, J., Mikkelsen, R., **Rodriguez, I.**, Martinez, M., de Silva, A., and Johnston, R. An alphavirus vector based tetravalent dengue vaccine induces a rapid and protective immune response in macaques that differs qualitatively from immunity induced by live virus Infection. March 2013. Journal of Virology 87(6): Pages 3409-24 **PMID: 23302884**

Abdulhaqq SA, Martinez MI, Kang G, Foulkes AS, **Rodriguez IV**, Nichols SM, Hunter M, Sariol CA, Ruiz LA, Ross BN, Yin X, Speicher DW, Haase AT, Marx PA, Li Q, Kraiselburd EN, Montaner LJ. Serial Cervicovaginal exposures with Replication-deficient SIVsm induce higher Dendritic Cell (pDC) and CD4+ T-Cell Infiltrates not associated with prevention but a More Severe SIVmac251 Infection of Rhesus Macaques. April 2014. J Acquir Immune Defic Syndr. 65(4): Pages 405-13 **PMID: 24226059**

Zika virus pathogenesis in rhesus macaques is unaffected by pre-existing immunity to dengue virus.

Pantoja P, Pérez-Guzmán EX, Rodríguez IV, White LJ, González O, Serrano C, Giavedoni L, Hodara V, Cruz L, Arana T, Martínez MI, Hassert MA, Brien JD, Pinto AK, de Silva A, **Sariol CA**.
Nat Commun. 2017 Jun 23;8:15674. doi: 10.1038/ncomms15674.

D. Research Support

Active

2P40 OD012217 -29 Martinez (PI) 01/15/16-11/30/20
Zika initiative at the Caribbean Primate Research Center. Translational Science Initiative.
Role: Collaborator
Project Leader: C. A. Sariol

Utilizing Laboratory Animals as a Tissue for Testing Monoclonal Antibody Products 10/23/06-Ongoing
(Quality Assurance Department BD Biosciences)
Role: Collaborator

Fresh tissue from recently euthanized animals will be used to test, as a quality control measure, the binding specificities and staining characteristics for all monoclonal and polyclonal antibodies and recombinant and native cytokines.

Neuroprotective Role of Neuregulin-1 in Ischemic Stroke (NIH/NINDS B. Ford) 4/15/07-Ongoing

Role: Collaborator

The objective of this study is to determine the efficacy of neuregulin-1 (NRG-1) as a neuroprotectant in stroke using a non-hp model

NIAID RFA-AI-06-033 (U01) **07/30/08- Ongoing**

Role: Collaborator

Recently Completed

1R01AI094603 Edmundo Kraiselburd, PI 03/01/11-02/28/16
Luis J. Montaner,PI
NIH/NIAID
Early Innate/IgA Anti-HIV/SIV Response in Exposed Uninfected

Role: Collaborator

This grant support to determine the presence of local cellular cervical tissue infiltrate, IFN-mediated gene expression, and mucosal anti-HIV IgA antibody responses in 3 well-defined groups of women with differential exposure risk based on sexual activity/partners. 2. Determine if repeated cervico-vaginal exposures to non-infectious SIV E660 exposures induce a persistent innate cellular infiltrate (plasmacytoid DCs, NK, macrophages) that in combination with mucosal SIV-specific IgA antibody levels decreases mucosal infectivity SIV mac251. This proposal represents a collaborative effort between The University of Puerto Rico, Nebraska University, University of Minnessota, Duke University, University of Massachusetts, Tulane University, National Cancer Institute, and The Wistar Institute.

NIAID RFA-AI-06-033 (U01) 07/30/08-June 2013
PI: Laura White

Immunization of rhesus macaques with a dengue vaccine candidate based on a Venezuelan equine encephalitis (VEE) replicon vector.
(Collaborative project with Global Vaccine, Inc. PI: Laura White

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: James Drew Brien

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

POSITION TITLE: Assistant Professor

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
University of Rochester, Rochester, NY	BA, BS	05/1997	Biology, Environmental Science
University of Massachusetts Medical School, Worcester, MA			Immunology
Oregon Health & Science University, Portland, OR	PhD	06/2008	Microbiology and Immunology
Washington University School of Medicine, Saint Louis, MO	Postdoctoral Scholar	07/2013	Virology and Immunology

A. Personal Statement

My laboratory has extensive experience in identifying immunological correlates of protection and mechanisms of viral pathogenesis, with a long-term focus on flaviviruses. To understand protection we study the mechanisms of B cell and antibody responses using both animal models (mice and non-human primates); then apply our discoveries to investigations using samples from human vaccine trial subjects through the Vaccine and Therapeutic Evaluation Units (VTEU).

One factor that contributes to the productivity of my laboratory is our long-term collaboration with Dr. Carlos Sariol. This collaboration stems from our shared interests in immunological correlates of protection and mechanisms of disease. The collaboration with Carlos has produced a study investigating cross-reactive immune responses in *Nature Communications* with a second publication currently under review at *Nature Communications* and a third manuscript in preparation.

My laboratories expertise in identifying T cell and B cell viral antigens and the role of these responses during infection actively contributes to the goals of this application. My previous and current collaborations with Carlos, our previous publications, as well as my laboratories expertise demonstrate our ability to develop a deeper understanding of "Dengue-Zika: correlates of protection in non-human Primates".

1. Pantoja P, Perez-Guzman EX, Rodriguez IV, White LJ, Gonzalez O, Serrano C, Giavedoni L, Hodara V, Cruz L, Arana T, Martinez MI, Hassert MA, **Brien JD**, Pinto AK, de Silva A, Sariol CA. Zika virus pathogenesis in rhesus macaques is unaffected by pre-existing immunity to dengue virus. Nat Commun. 2017;8:15674. doi: 10.1038/ncomms15674. PubMed PMID: 28643775; PMCID: PMC5490051.

2. Hassert MA, Wolf KJ, Schwetye KE, DiPaolo RJ, **Brien JD**, Pinto AK. CD4+T cells mediate protection against Zika associated severe disease in a mouse model of infection. PLoS Pathog. 2018 Sep 13;14(9):e1007237. doi: 10.1371/journal.ppat.1007237. eCollection 2018 Sep. PubMed PMID: 30212537

3. Hassert MA, **Brien JD**, & Pinto AK (2018). The Temporal Role of Cytokines in Flavivirus Protection and Pathogenesis. Current Clinical Microbiology Reports. https://doi.org/10.1007/s40588-018-0106-x

B. Positions and Honors

Positions and Employment

- 1997-1999 Guest Student, Biology Department, Woods Hole Oceanographic Institute, Woods Hole, MA.
- 1999-2000 Research Assistant, Pathology Department, University of Massachusetts Medical Center, Worcester, MA.
- 2000-2008 Graduate Student, Molecular Microbiology and Immunology Department, Oregon Health & Science University, Portland, OR.
- 2008-2013 Postdoctoral Research Scholar, Infectious Disease Department, Washington University Medical School, St. Louis, MO.
- 2013-2015 Research Instructor, Infectious Disease Department, Washington University Medical School, St. Louis, MO.
- 2015- Assistant Professor, Saint Louis University School of Medicine, Department of Molecular Microbiology & Immunology

Professional Society memberships

- Member American Society of Virology
- Member American Association of Immunologists
- Member American Society of Tropical Medicine & Hygiene

Honorary Societies, Honors and Awards

- Saint Louis University Presidential Research Fund Award, 2017
- Travel grant: Pan American Dengue Virus Conference, Panama City, Panama, 2016
- National Institutes of Health NIAID Career Transition Award (K22) 2014
- Travel grant: Workshop on Molecular Evolution: Cesky Krumlov, Czechoslovakia, 2013
- Travel grant: WHO/IVI Next Generation Dengue Vaccines and Diagnostics, Atlanta, GA , 2010
- Washington University School of Medicine, Immunology Training grant recipient, 2007-2010
- OHSU Molecular Microbiology and Immunology Training grant recipient, 2006-2008
- AAAS award: Science Program for Excellence in Science, 2006-2008
- Travel grant: Aging Research in Immunology: Paris, France, 2006
- Tartar Trust Fellowship 2003, 2005
- Travel grant: ASM conference: Immunity to Bacterial, Viral, and Protozoal Pathogens. Savannah, GA , 2002

Professional Services

Ad Hoc Reviewer:

- mBio
- Journal of Virology
- Virology
- PLoS NTDs
- PLoS One
- Applied Microbiology and Biotechnology
- Journal of Interferon & Cytokine Research
- Viruses
- Frontiers in Immunology
- Frontiers in Microbiology

Editorial Responsibilities:

- Frontiers in Microbiology: Associate Editor "Dengue Virus Research"

C. Contributions to Science

1. Correlates of protection against flaviviruses. As a graduate student I began to use small animal models to systematically defined components essential for the protection against a lethal encephalitic flavivirus, WNV. I was first to identify and define the antigen specific CD4 T cell population and the WNV epitopes that they recognize in C57BL/6 mice. I used this previous knowledge of CD4 T cells epitope identification to aid in the study of CD4 T cell epitopes to ZIKV, which was recently published with Dr. Pinto. In back to back publication with Dr. Michael Diamond I identified the WNV specific CD8 T cell population and the majority of CD8 T cell epitopes, as well as the generation of the first antigen specific tetramer for WNV specific CD8 T cells. The identification of the immunodominant CD8 T cell epitope in C57BL/6 mice has facilitated WNV research in multiple laboratories. As a postdoctoral research scholar in Dr. Michael Diamond's laboratory, I identified key defects in the immune response that both directly and indirectly led to an increase in susceptibility to severe WNV disease. These studies have provided fundamental insights into the host-pathogen interface in animals and has helped to understand the correlates of protection for flaviviruses.

1. **Brien JD**, Uhrlaub JL, Nikolich-Zugich J. Protective capacity and epitope specificity of CD8(+) T cells responding to lethal West Nile virus infection. European journal of immunology. 2007;37(7):1855-63. doi: 10.1002/eji.200737196. PubMed PMID: 17559175.

2. **Brien JD**, Uhrlaub JL, Nikolich-Zugich J. West Nile virus-specific CD4 T cells exhibit direct antiviral cytokine secretion and cytotoxicity and are sufficient for antiviral protection. Journal of immunology. 2008;181(12):8568-75. PubMed PMID: 19050276; PubMed Central PMCID: PMC3504655.

3. Pinto AK, Daffis S, **Brien JD**, Gainey MD, Yokoyama WM, Sheehan KC, et al. A temporal role of type I interferon signaling in CD8+ T cell maturation during acute West Nile virus infection. PLoS pathogens. 2011;7(12):e1002407. doi: 10.1371/journal.ppat.1002407. PubMed PMID: 22144897; PubMed Central PMCID: PMC3228803.

2. Protective viral vaccines in vulnerable populations. The elderly, pregnant women and neonates are all vulnerable populations when it comes to designing safe and effective vaccines. Immune senescence, aging of the immune system, affects the elderly and is the diminished ability to mount a protective immune response. Previous to this work many defects associated with the aging immune system were documented using in vitro systems or transgenic mouse models, contributing to the large number of conflicting reports. Very few studies have used live pathogens or have been able to correct specific defects in vivo to show the relative contribution of the immune defects to immune senescence. Utilizing small animal models of Herpes simplex virus type I, Murine Cytomegalovirus and West Nile virus I have identified key defects in the antiviral immune response that leads to a functional deficit in immunity and increased susceptibility to severe disease and poor responses to vaccination.

1. **Brien JD**, Uhrlaub JL, Hirsch A, Wiley CA, Nikolich-Zugich J. Key role of T cell defects in age-related vulnerability to West Nile virus. The Journal of experimental medicine. 2009;206(12):2735-45. doi: 10.1084/jem.20090222. PubMed PMID: 19901080; PubMed Central PMCID: PMC2806630.

2. Cicin-Sain L, **Brien JD**, Uhrlaub JL, Drabig A, Marandu TF, Nikolich-Zugich J. Cytomegalovirus infection impairs immune responses and accentuates T-cell pool changes observed in mice with aging. PLoS pathogens. 2012;8(8):e1002849. doi: 10.1371/journal.ppat.1002849. PubMed PMID: 22916012; PubMed Central PMCID: PMC3420928.

3. Uhrlaub JL, **Brien JD**, Widman DG, Mason PW, Nikolich-Zugich J. Repeated in vivo stimulation of T and B cell responses in old mice generates protective immunity against lethal West Nile virus encephalitis. Journal of immunology. 2011;186(7):3882-91. doi: 10.4049/jimmunol.1002799. PubMed PMID: 21339368; PubMed Central PMCID: PMC3501996.

4. Lang A, **Brien JD**, Messaoudi I, Nikolich-Zugich J. Age-related dysregulation of CD8+ T cell memory specific for a persistent virus is independent of viral replication. Journal of immunology. 2008;180(7):4848-57. PubMed PMID: 18354208; PubMed Central PMCID: PMC4161215.

3. Immune and antiviral control of flaviviruses. My development of small animal models of viral diseases has facilitated multiple collaborations that have allowed us to evaluate innate regulator of infection as well as small molecule therapeutics as well antibody based therapeutics. Recently, in collaboration with Dr. Amelia Pinto we developed a new mouse model of DENV disease that develops both a dengue fever and dengue hemorrhagic fever like disease, utilizing that model we have made significant strides in evaluating therapeutics. The advantage of this new model is that the mice are highly immunocompetent allowing us to evaluate therapeutics that target the innate immune system within the context of DENV. In addition, within this model, we have adapted clinical veterinary hematology and chemistry analyzers to improve the quantification of disease allowing us to make direct comparisons to the clinical parameters of human disease.

1. **Brien JD**, Sukupolvi-Petty S, Williams KL, Lam CY, Schmid MA, Johnson S, et al. Protection by immunoglobulin dual-affinity retargeting antibodies against dengue virus. Journal of virology. 2013;87(13):7747-53. doi: 10.1128/JVI.00327-13. PubMed PMID: 23658441; PubMed Central PMCID: PMC3700316.

2. Pinto AK*, **Brien JD***, Lam CY, Johnson S, Chiang C, Hiscott J, et al. Defining New Therapeutics Using a More Immunocompetent Mouse Model of Antibody-Enhanced Dengue Virus Infection. mBio. 2015;6(5):e01316-15. doi: 10.1128/mBio.01316-15. PubMed PMID: 26374123; PubMed Central PMCID: PMC4600115.(* denotes co-first author)

3. Barklis E, Still A, Sabri MI, Hirsch AJ, Nikolich-Zugich J, **Brien J**, Dhenub TC, Scholz I, Alfadhli A. Sultam thiourea inhibition of West Nile virus. Antimicrob Agents Chemother. 2007;51(7):2642-5. doi: 10.1128/AAC.00007-07. PubMed PMID: 17452483; PMCID: PMC1913232.

4. **Brien JD**, Daffis S, Lazear HM, Cho H, Suthar MS, Gale M, Jr., et al. Interferon regulatory factor-1 (IRF-1) shapes both innate and CD8(+) T cell immune responses against West Nile virus infection. PLoS pathogens. 2011;7(9):e1002230. doi: 10.1371/journal.ppat.1002230. PubMed PMID: 21909274; PubMed Central PMCID: PMC3164650.

Full publication list: http://www.ncbi.nlm.nih.gov/pubmed/?term=brien+jd

D. Additional Information: Research Support and/or Scholastic Performance

NIH/NIAID HHSN 272201300021I (Patel P.I.) 04/13/16-03/09/19
Rapid Research Response to Zika Virus Infection in US Residents: Humoral and Cellular Immune Responses
The goal is to evaluate the associations of ZIKV-specific humoral and cellular immune responses with viral load and viral persistence in blood and other body fluids.
Role: Co-Investigator

Private Source **(Pinto P.I.)** 10/18/18 - 12/31/19
In Vivo Evaluation of Human Zika IVIG
To evaluate Antibody Dependent Enhancement (ADE) of Zika polyclonal antibodies by in-vivo testing against Zika, Dengue 2 and Dengue 3 in susceptible animal models.
Role: P.I.

NIH/NIAID HHSN 272201300014-16 (George P.I.) 05/11/16 - 02/28/20
Phase 1 Zika Virus Vaccine Trials in US and Puerto Rico

The goal is to compare of safety, reactogenicity, and immunogenicity of a formalin-inactivated, alum-adjuvanted, whole virus Zika vaccine with placebo.
Role: Co-Investigator

NIH/NIAID HHSN272201300021I (George P.I.) 09/25/17 – 02/28/20
HHSN27200021
Vaccine and Treatment Evaluations Units Task 16-0033.D1.0086
The purpose of this Task Order is to provide analysis of immune responses developed following Zika virus vaccination
Role: Co-investigator

Federal Bureau of Investigation (DiPaolo P.I.) 05/12/15 – 05/11/19
DJF-15-1200-P-0001007
Immunological Profiling to Distinguish Virus (Monkeypox) Infection from (Smallpox) Vaccination
The goal of this proposal is to identify TCR sequences that can be used to detect previous exposure to vaccines, infectious agents.
Role: Co-investigator

Completed Research Support

NIH/NIAID HHSN 272201500008C (Painter P.I.); Buller Co-PI) 06/30/15 - 06/29/18
Targeting Therapeutics Development to Relieve Bottlenecks; Optimizing Lead Therapeutic Compounds against Infectious Pathogens
The goals of the project are to evaluate the antiviral efficacy of therapeutics against emerging and re-emerging RNA viral pathogens.
Role: Co-Investigator

K22AI104794 (Brien P.I.) 02/01/16 – 01/31/18
NIH/NIAID
Immune Control Of West Nile Virus Quasispecies Dynamics
The goal of this project is to understand the interaction between immune restriction and quasispecies development.
Role: Principal Investigator

Private Source **(Brien P.I.)** 07/01/17 - 12/31/17
In Vitro Safety Evaluation of Human Zika IVIG
To evaluate Antibody Dependent Enhancement (ADE) of Zika polyclonal antibodies by in-vitro testing against Zika, Dengue 2 and Dengue 3 with K562 cell line.
Role: Principal Investigator

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: Amelia Kahler Pinto

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

POSITION TITLE: Assistant Professor

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
Portsmouth University, Portsmouth England	B.Sc.	06/1997	Molecular Biology
University of Massachusetts Medical School, Worcester, MA		06/2000	Immunology
Oregon Health & Science University, Portland, OR	Ph.D.	07/2007	Microbiology and Immunology
Washington University School of Medicine, Saint Louis, MO	Postdoctoral	06/2015	Virology and Immunology

A. Personal Statement

I am an Assistant Professor of Microbiology and Immunology, in the Department of Molecular Microbiology & Immunology at Saint Louis University School of Medicine. Over the past 18 years I have studied the host immune response to viral infection investigating both viral and host factors that influence the development of an effective immune response using multiple viral families. I have a history of successful collaborations stemming from a shared interest in immune control of viral infections. I began my research career aiding in the establishment of models to study the impact of multiple sequential infections on immune memory. I have established a strong long lasting collaboration that has resulted in multiple publications including a recent study published with Dr. Carlos Sariol, in *Nature Communications* looking at cross-reactive immune response. Throughout my career, to address what constitutes an effective anti-viral immune response I have designed research projects to study the role of T cell recognition, and the protective capacity of virus specific and cross-reactive T cells to infection. The identification of novel Zika virus epitopes and the function of CD4 virus specific T cells in a murine model of Zika virus infection were recently published in *PLOS Pathogens*. I have continued to be involved in the productive collaborative effort with Dr. Sariol evaluating the immune response to ZIKV in non-human primates that have led to a second publication submitted to *Nature Communications* and a third manuscript in process. Based upon my training in virology and immunology, previous experimental work, and close highly successful collaboration with Dr. Sariol's group I am confident that we will successfully complete the studies proposed.

B. Positions and Honors

Positions and Employment

1997	Research Student, University of Portsmouth, England,
1999-2001	Research Assistant, University of Massachusetts Medical Center, Supervisors: Liisa Selin MD, PhD, and Raymond Welsh PhD
2001-2007	Graduate Student, Oregon Health & Science University, Advisor: Ann Hill PhD

2008-2010	Postdoctoral Research Associate, Washington University School of Medicine, Advisor: Herbert Virgin IV MD, PhD
2010-2013	Postdoctoral Research Associate, Washington University School of Medicine, Advisor: Michael Diamond MD, PhD
2013-2015	Research Instructor, Washington University School of Medicine
2015-	Assistant Professor, Saint Louis University School of Medicine, Department of Molecular Microbiology & Immunology

Other Experience and Professional Memberships

2011-	Member, American Association for the Advancement of Science
2012-	Member, American Association of Immunologists
2013-	Ad Hoc reviewer: Microbiology and Biotechnology
2014-	Ad Hoc reviewer: Emerging Infectious Diseases
2014-	Ad Hoc reviewer: PLoS One
2016-19	Member, American Society for Virology, Membership committee member
2016	Arbovirus Expert: GENeS
2016	Reviewer: DoD, Emerging Infectious Diseases Panel
2016	Reviewer: NIH Special Emphasis Panel PAR-16-106 Rapid Assessment of Zika Virus Complications (R21)
2017	Reviewer: DoD, Emerging Infectious Diseases Panel
2017-	Ad Hoc reviewer: Ebio Medicine
2018	Reviewer: Peer Review Medical Research Program (PRMRP) for CDMRP

Honors and Awards

2002, 2005	Tartar Fellowship
2002-2004	American Heart Association Fellowship 0215188Z
2004-2007	National Eye Institute Training Grant ACAEI0071
2004	Travel grant: Keystone Symposia Taos New Mexico
2005	30th Annual International Herpesvirus Workshop Honoraria
2007-2008	W.M. Keck Postdoctoral Fellowship
2013	Global Health World Health Interest Group Honoraria
2014	Travel grant: IV Pan American Dengue Network Meeting
2016	Travel grant: V Pan American Dengue Network Meeting
2017	Saint Louis University Presidential Research Fund Award

C. Contributions to Science

Below are summaries of my contributions to science. As of January 2019, I have 35 publications in peer reviewed journals that have been cited over 1,000 times.

1. Immune response to viral infections

I have sought to understand virus disease progression and mechanisms of immune control. For these studies I have used mice infected with multiple pathogens and monitor both the virus specific and cross-reactive T cell responses. For many virus infections it has been difficult to understand the biology of infection, as there are very few models that that can accurately mimic human disease progression. As a graduate student and postdoc I established models for the study of arbovirus diseases including Zika (ZIKV), West Nile virus (WNV), Oropouche virus (OROV), and Dengue virus (DENV). Using ZIKV my laboratory demonstrated a role for CD4 T cells in controlling neuroinvasive disease. Using WNV infection, I demonstrated that the selective loss of type I interferon receptor signaling on myeloid cells leads to an increase in susceptibility of myeloid cells to infection and induces the production of cytokines leading to cytokine storm and organ damage, mimicking the symptoms of viral sepsis. Starting with the model I established with WNV, I have applied this approach to both Oropouche and DENV to evaluate the influence of immune recognition, and cytokine regulation on the severity of disease and test therapeutics. My studies directly lead to a novel DENV model system that uses primary DENV isolate to

recapitulate the clinical parameters of dengue disease allowing for the investigation of mechanisms of viral sepsis. These studies have been widely referenced in establishing newer models for ZIKV. I have also shown the importance of a functional immune response for the study of antivirals, as some highly effective antivirals do not function in the absence of type I interferon signaling.

- Brehm MA, **Pinto AK**, Daniels KA, Schneck JP, Welsh RM, Selin LK. T cell immunodominance and maintenance of memory regulated by unexpectedly cross-reactive pathogens. Nature Immunolology. 2002;3(7):627-34. doi: 10.1038/ni806. PubMed PMID: 12055626.

- **Pinto AK**, Ramos HJ, Wu X, Aggarwal S, Shrestha B, Gorman M, et al. Deficient IFN signaling by myeloid cells leads to MAVS-dependent virus-induced sepsis. PLoS pathogens. 2014;10(4):e1004086. doi: 10.1371/journal.ppat.1004086. PubMed PMID: 24743949; PMCID: PMC3990718.

- Hassert M, Wolf KJ, Schwetye KE, DiPaolo RJ, Brien JD, **Pinto AK**. CD4+T cells mediate protection against Zika associated severe disease in a mouse model of infection. PLoS Pathog. 2018 Sep 13;14(9):e1007237. doi: 10.1371/journal.ppat.1007237. eCollection 2018 Sep. PubMed PMID: 30212537

- **Pinto AK,** Brien JD, Lam CY, Johnson S, Chiang C, Hiscott J, et al. Defining New Therapeutics Using a More Immunocompetent Mouse Model of Antibody-Enhanced Dengue Virus Infection. mBio. 2015;6(5):e01316-15. doi: 10.1128/mBio.01316-15. PubMed PMID: 26374123; PubMed Central PMCID: PMC4600115

2. T cell driven immune restriction and immune recognition and control.

The proteins encoded by herpesvirus immune evasion genes are well known for their effect on major histocompatibility complex (MHC) class I molecules yet they are dispensable for viral replication within their hosts. Murine cytomegalovirus (MCMV) encodes three immune evasion genes, *m04, m06* and *m152*, that all function to affect MHC class I antigen presentation to CD8 T cells. In this work I identified a large panel of CD8 T cell epitopes encoded by MCMV that are presented and recognized by CD8 T cells. Using these tools I demonstrated that immune evasion genes inhibit CD8 T cell killing not by downregulating epitope-specific MHC class I but rather by reducing the total MHC class I levels weakening the T cell response. As I transitioned into my postdoctoral career I further developed my understanding of T cell driven immunity by identifying critical mechanisms by which T cells are programed to recognize virally encoded antigen and mechanisms of viral clearance. Using this model system and a IFN-α receptor blocking antibody I uncoupled the influence of type I IFNs on the innate immune response from its role in the adaptive immune response allowing the identification of a novel role for type I IFN signaling in the development of a functional adaptive immune response. These studies have provided fundamental insights into the protective capacity of CD8 T cells *in vivo*.

- **Pinto AK**, Munks MW, Koszinowski UH, Hill AB. Coordinated function of murine cytomegalovirus genes completely inhibits CTL lysis. Journal of Immunology. 2006;177(5):3225-34. Epub 2006/08/22. PubMed PMID: 16920962.

- Hensley SE, **Pinto AK**, Hickman HD, Kastenmayer RJ, Bennink JR, Virgin HW, Yewdell JW. (2009) Murine norovirus infection has no significant effect on adaptive immunity to vaccinia virus or influenza A virus. Journal of Virology, 83(14):7357-60. PubMed PMID: 19403665

- Chou C, **Pinto AK**, Curtis JD, Persaud SP, Cella M, Lin CC, Edelson BT, Allen PM, Colonna M, Pearce EL, Diamond MS, Egawa T. c-Myc-induced transcription factor AP4 is required for host protection mediated by CD8+T cells. Nature Immunology. 2014;15(9):884-93. doi: 10.1038/ni.2943. PubMed PMID: 25029552; PMCID: PMC4139462.

- **Pinto AK**, Daffis S, Brien JD, Gainey MD, Yokoyama WM, Sheehan KC, et al. A temporal role of type I interferon signaling in CD8+ T cell maturation during acute West Nile virus infection. PLoS Pathogens. 2011;7(12):e1002407. doi: 10.1371/journal.ppat.1002407. PubMed PMID: 22144897.

3. Flavivirus immune response development.

I have used animal models to elucidate cell-mediated mechanisms of control of virus infection, and to evaluate the generation of innate and a protective adaptive immune response to a novel flavivirus vaccine. Through this work I identified critical mechanisms by which T cells are programed to recognize virally encoded antigen and mechanisms of viral clearance. Using this model system and a IFN-α receptor blocking antibody I uncoupled the influence of type I IFNs on the innate immune response from its role in the adaptive immune response allowing the identification of a novel role for type I IFN signaling in the development of a functional adaptive immune response. I have also demonstrated a unique role of gamma delta T cells in controlling infection. These studies have provided fundamental insights into the protective capacity of T cells in vivo, and how this knowledge can be used to generate a protective response in a vulnerable population such as the elderly.

- Kim S*, **Pinto AK***, Myers NB, Hawkins O, Doll K, Kaabinejadian S, et al. A novel T-cell receptor mimic defines dendritic cells that present an immunodominant West Nile virus epitope in mice. European Journal of Immunology. 2014;44(7):1936-46. doi: 10.1002/eji.201444450. PubMed PMID: 24723377. (*denotes co-first author)

- **Pinto AK**, Richner JM, Poore EA, Patil PP, Amanna IJ, Slifka MK, et al. A hydrogen peroxide-inactivated virus vaccine elicits humoral and cellular immunity and protects against lethal West Nile virus infection in aged mice. Journal of Virology. 2013;87(4):1926-36. doi: 10.1128/JVI.02903-12. PubMed PMID: 23221549.

- Pantoja P, Perez-Guzman, E. X., Rodriguez, I. V., White, L. J., Gonzalez, O., Serrano, C., Giavedoni, L., Hodara, V., Cruz, L., Arana, T., Martinez, M. I., Hassert, M. A., Brien, J. D., **Pinto, A. K.,** de Silva, A., Sariol, C. Zika virus pathogenesis in rhesus macaques is unaffected by pre-existing immunity to dengue virus. Nature Communications. 2017 Jun 23;8:15674. doi: 10.1038/ncomms15674. PubMed PMID: 28643775

Full publication list: http://www.ncbi.nlm.nih.gov/pubmed/?term=pinto+ak

D. Additional Information: Research Support and/or Scholastic Performance

NIH/NIAID HHSN 272201300021I (Patel, P.I.) 04/13/16 - 03/09/19

Rapid Research Response to Zika Virus Infection in US Residents: Humoral and Cellular Immune Responses

The goal is to evaluate the associations of ZIKV-specific humoral and cellular immune responses with viral load and viral persistence in blood and other body fluids.

Role: Co-Investigator

NIH/NIAID HHSN 272201300014-16 (George, P.I.) 05/11/16 - 02/28/20

Phase 1 Zika Virus Vaccine Trials in US and Puerto Rico

The goal is to compare of safety, reactogenicity, and immunogenicity of a formalin-inactivated, alum-adjuvanted, whole virus Zika vaccine with placebo.

Role: Co-Investigator

NIH/NIAID HHSN272201300021I (George, P.I.) 09/25/17 – 02/28/20

HHSN27200021

Vaccine and Treatment Evaluations Units Task 16-0033.D1.0086

The purpose of this Task Order is to provide analysis of immune responses developed following Zika virus vaccination

Role: Co-investigator

Private Source **(Pinto, P.I.)** 10/01/18 - 12/31/19
Master Service Agreement
In vivo Evaluation of Human Zika IVIG to cause ADE
To evaluate Antibody Dependent Enhancement (ADE) of Zika polyclonal antibodies by in-vivo testing against Zika
Role: Principal Investigator

Completed Research Support

Private Source **(Brien, P.I.)** 07/01/17 - 12/31/17
Master Service Agreement
In Vitro Safety Evaluation of Human Zika IVIG
To evaluate Antibody Dependent Enhancement (ADE) of Zika polyclonal antibodies by in-vitro testing against Zika, Dengue 2 and Dengue 3 with K562 cell line.
Role: Co-Investigator

NIH/NIAID HHSN 272201500008C (Painter P.I.);Buller Co-PI) 06/30/15 - 06/29/18
Targeting Therapeutics Development to Relieve Bottlenecks; Optimizing Lead Therapeutic Compounds against Infectious Pathogens
The goals of the project are to evaluate the antiviral efficacy of therapeutics against emerging and re-emerging RNA viral pathogens.
Role: Co-Investigator

Private Source **(Pinto, P.I.)** 10/01/17 - 12/31/18
Master Service Agreement
In vivo Safety Evaluation of Human Zika IVIG
To evaluate Antibody Dependent Enhancement (ADE) of Zika polyclonal antibodies by in-vivo testing against Zika, Dengue 2 and Dengue 3
Role: Principal Investigator

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: **ARAVINDA M. DE SILVA, PH.D.**

POSITION TITLE: **PROFESSOR**

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

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
Vassar College, New York	BA	1986	Biology
Yale Univ, Connecticut	PhD	1992	Cell Biology/Virology
Yale Univ, Sch of Public Hlth, Connecticut	MPH	1997	Epid & Public Hlth
Univ of Rochester, New York	Postdoc	1994	Biology
Yale Univ, Sch of Med, Connecticut	Postdoc	1997	Infectious Dis

A. Personal Statement.

I have worked on flaviviruses for the past 22 years and currently lead a group of 18 scientists studying viral pathogenesis and immunology. I am well versed in the biology and immunology of flaviviruses, the epidemiology and public health burden of these viruses and the challenges facing clinicians, public health officials and vaccine developers. My group has developed new methods and reagents for studying the specificity and functional properties of the antibody response to dengue, Zika and related viruses. Our work has uncovered novel human neutralizing antibodies that recognized complex quaternary epitopes displayed on assembled virions. Our discoveries about the importance "quality" (as opposed to "quantity") of flavivirus neutralizing antibodies have challenged dogma and stimulated new avenues of research. Several dengue vaccine developers are actively collaborating with us to characterize the human immune response to their vaccines. For the past 20 years I have also collaborated with colleagues in Sri Lanka, Nicaragua, Colombia and Mozambique to support public health efforts to control dengue and to conduct research on dengue epidemiology and pathogeneisis. Dr. Carlos Sariol and I have collaborated for the past 10 years to study dengue and Zika pathogenesis and host immunity in non-human primates. I look forward to continuing this rewarding and productive partnership with Dr. Sariol and his team.

- **de Silva AM**, Harris E. The Path to a Dengue Vaccine: Learning from Human Natural Infection and Vaccine Studies. ***Cold Spring Harb Perspect Biol.*** 2017 Jul 17. pii: a029371. PMID: 28716891.
- Emily N. Gallichotte, Thomas Baric, Boyd L. Yount Jr, Douglas Widman, Anna Durbin, Steve Whitehead, Ralph S. Baric, **A. M. de Silva** Human dengue virus serotype 2 neutralizing antibodies target two distinct quaternary epitopes. **Plos Pathogens.** 2018 Feb 26;14(2):e1006934. doi: 10.1371/journal.ppat.1006934. eCollection 2018 Feb. PMID: 29481552
- Collins MH, McGowan E, Jadi R, Young E, Lopez CA, Baric RS, Lazear HM, **de Silva AM.** *Lack of Durable Cross-Neutralizing Antibodies Against Zika Virus from Dengue Virus Infection.* **Emerg Infect Dis.** 2017 May;23(5):773-781. PubMed PMID: 28418292.
- Lazear HM, Stringer EM, **de Silva AM**. The Emerging Zika Virus Epidemic in the Americas: Research Priorities. JAMA. 2016 May 10;315(18):1945-6. doi: 10.1001/jama.2016.2899. PubMed PMID: 26963564

B. Positions and Honors.

1992-1994 Postdoctoral Fellow, Department of Biology, University of Rochester, NY
1994-1997 Postdoctoral Fellow, Department of Internal Medicine, Yale University Sch of Medicine, CT
1998-2005 Assistant Professor, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC

2005-2006 Visiting Professor, Department of Microbiology, Faculty of Medicine, University of Sri Jayawardhanapura, Sri Lanka. (Sabbatical partly supported by a competitive W. R. Kenan research and scholarly grant from the University of North Carolina at Chapel Hill)
2005-2012 Associate Professor, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC
2012-present Professor, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC
2016- Outstanding Postdoctoral Mentoring Award from the University of North Carolina at Chapel Hill.

Other Experience and Professional Memberships (2013-2018)
Editorial Board, Journal of Virology (2016-); **Associate Editor**, PloS Neglected Tropical Diseases (2012-). **Ad Hoc Reviewer**: Science, Nature Medicine, Journal of Virology, Infection and Immunity, Archives of Virology, Journal of Clinical Microbiology, Emerging Infectious Diseases and Proceedings of the National Academy of Sciences (USA). **Study Sections:** NIH Small Business Vaccine Grants (ZRG1_IMM) (2011-2014). NIH Immunity and Host Defense Study Section Permanent Member (2016-2022); **Other:** Member US Gov. delegation to discuss collaborative research with Cuba (Havana, Nov 2016); **Scientific Advisory Group** of Partnership for Dengue Control (PDC) meetings on immune correlates of protection (2016) and Zika/arbovirus diagnostics (2017). **Executive committee** of the Pan American Dengue Research Network (2012-16); **Co-Chair of the Scientific Committee** of the Pan American Dengue Research Network (2015-2016); **Program Committee** American dengue network meeting to be held in Brazil (September 2014); **Program Committee** of DVI/ NIAID co-sponsored meeting on dengue vaccines (June 2013); **Program Committee** American dengue network meeting held in Colombia (September 2012).

C. Contribution to Science. (Total publications 101; Citations 6945; h-index 46; i10-index 78)

Human Antibodies in Protective and Pathogenic Immunity to Dengue and Zika *(2009-2018):* The potential for antibodies to be beneficial or harmful in the face of a dengue infection has complicated the development of vaccines. In collaboration with Ralph Baric at UNC and James Crowe at Vanderbilt, my group has helped to develop a variety of methods that range from removal of specific antibody populations from human immune sera, production of human monoclonal antibodies from dengue-exposed people and recombinant virus with specific mutations or chimeric envelop proteins to dissect the human antibody response to dengue. Our studies with human B-cells and antibodies challenge dogma in the dengue field based on studies with mouse antibodies. We have discovered that people exposed to natural DENV infections have distinct populations of antibodies responsible for protective versus potentially disease enhancing immunity. The antibodies linked to protection bind to complex quaternary epitopes on the virion. The location of epitopes differ between serotypes but the mechanism of DENV neutralization appears to be similar and involve inhibition of steps after viral attachment to cells. More, recently we have extended our studies to Zika virus.
Selected Publications

- Patel B, Longo P, Miley MJ, Montoya M, Harris E, de Silva AM. Dissecting the human serum antibody response to secondary dengue virus infections. PLoS Negl Trop Dis. 2017 May 15;11(5):e0005554. doi: 10.1371/journal.pntd.0005554. eCollection 2017 May. PubMed PMID: 28505154.
- Henein S, Swanstrom J, Byers AM, Moser JM, Shaik SF, Bonaparte M, Jackson N, Guy B, Baric R, **de Silva AM**. Dissecting antibodies induced by a chimeric yellow fever-dengue, live-attenuated, tetravalent dengue vaccine (CYD-TDV) in naïve and dengue exposed individuals. J Infect Dis. 2016 Dec 8. pii: jiw576. PubMed PMID: 27932620.
- Fibriansah G, Ibarra KD, Ng TS, Smith SA, Tan JL, Lim XN, et al. Dengue Virus. Cryo-EM structure of an antibody that neutralizes dengue virus type 2 by locking E protein dimers. Science. 2015 Jul 3;349(6243):88-91. PMC4672004.
- de Alwis R, Williams KL, Schmid MA, Lai CY, Patel B, Smith SA, Crowe JE, Wang WK, Harris E*, **de Silva AM*.** Dengue viruses are enhanced by distinct populations of serotype cross-reactive antibodies in human immune sera.. PLoS Pathog. 2014 Oct 2;10(10):e1004386. PMID: 25275316. (*co-senior authors).

Molecular epidemiology of dengue viruses in South Asia (1998-2017): I have collaborated with colleagues in Sri Lanka for the past 15 years to understand the changing epidemiology of dengue on the Island. I support the regular exchange of students and other researchers between UNC and Colombo to promote collaborative research and training activities. My colleagues and I have established a research laboratory and

clinical sites in Sri Lanka with support from the NIH, Gates Foundation and The European Union. Our studies have demonstrated that some dengue strains are more invasive and pathogenic compared to other strains. Our studies also led to the first population based estimates of the incidence of dengue infection and disease in the Indian subcontinent. I am currently continuing these collaborative studies to understand dengue pathogenesis and also to build capacity for infectious disease research in Sri Lanka.

Selected Publications

- Corbett KS, Katzelnick L, Tissera H, Amerasinghe A, de Silva AD, **de Silva AM**. 2015. Preexisting Neutralizing Antibody Responses Distinguish Clinically Inapparent and Apparent Dengue Virus Infections in a Sri Lankan Pediatric Cohort. Journal of Infectious Diseases 211: 590-599. PMID: 25336728.
- Tissera H, Amarasinghe A, De Silva AD, Kariyawasam P, Corbett KS, Katzelnick L, Tam C, Letson GW, Margolis HS, **de Silva AM**. Burden of dengue infection and disease in a pediatric cohort in urban Sri Lanka. Am J Trop Med Hyg. 2014 Jul 2;91(1):132-7. PMID: 24865684.
- Kanakaratne N, Wahala WM, Messer WB, Tissera HA, Shahani A, Abeysinghe N, **de-Silva AM**, Gunasekera M. Severe dengue epidemics in Sri Lanka, 2003-2006. Emerg Infect Dis. 2009 Feb;15(2):192-9. PMC2662655.
- Messer WB, Vitarana UT, Sivananthan K, Elvtigala J, Preethimala LD, Ramesh R, Withana N, Gubler DJ, **De Silva AM**. Epidemiology of dengue in Sri Lanka before and after the emergence of epidemic dengue hemorrhagic fever. Am J Trop Med Hyg. 2002 Jun;66(6):765-73. PMID: 12224589

Molecular interactions between Lyme Disease spirochetes and ticks (1994-2010): As a postdoctoral fellow with Erol Fikrig at Yale and subsequently at UNC, I studied how Lyme disease spirochetes were transmitted by ticks. These were among the first studies to use molecular approaches to understand interactions between ticks and pathogens. My postdoctoral work demonstrated that the outer surface proteins on the spirochete were differentially expressed in the mammalian host versus the tick vector. Even within the tick, proteins expressed and required for establishing an infection in the tick were different from the proteins expressed and required for transmission out of the tick. I demonstrated changes in surface protein expression were coincident with ability of spirochetes to escape from the tick gut and invade the salivary gland for subsequent transmission to the host. Others have followed up on these studies and established the molecular mechanisms involved in differential gene expression as well as the specific functions of proteins expressed at different stages.

Selected Publications

- **de Silva AM**, Tyson KR, Pal U. Molecular characterization of the tick-Borrelia interface. Front Biosci (Landmark Ed). 2009 Jan 1;14:3051-63. PMID: 19273256.
- Strother KO, Hodzic E, Barthold SW, **de Silva AM**. Infection of mice with lyme disease spirochetes constitutively producing outer surface proteins A and B. Infect Immun. 2007 Jun;75(6):2786-94. PMC1932870.
- Ohnishi J, Piesman J, **de Silva AM**. Antigenic and genetic heterogeneity of Borrelia burgdorferi populations transmitted by ticks. Proc Natl Acad Sci U S A. 2001 Jan 16;98(2):670-5. PMC14646.
- **de Silva AM**, Telford SR 3rd, Brunet LR, Barthold SW, Fikrig E. Borrelia burgdorferi OspA is an arthropod-specific transmission-blocking Lyme disease vaccine. J Exp Med. 1996 Jan 1;183(1):271-5. PMC2192397.

Protein folding and quality control in the endoplasmic reticulum (1986-1992): With my PhD supervisor, Ari Helenius, I used viral glycoproteins to study membrane protein folding and assembly in the endoplasmic reticulum (ER). Our work demonstrated that the ER has a quality control system in place whereby only properly folded proteins were permitted to exit the compartment. My studies demonstrated that quality control was an active process and involved ER chaperones that recognized misfolded proteins. I also demonstrated that the quality control system was present in the ER but not other compartments in the secretory pathway. Looking back it is gratifying to see current work in this area that have established molecular details of ER quality control and the key role of this compartment in the unfolded protein cellular stress response.

Selected Publications

- **de Silva, A.M.**, Braakman, I. and Helenius, A. (1993) Post-translational folding of VSV G protein in the endoplasmic reticulum: involvement of noncovalent and covalent complexes. J Cell Biol.120:647-655.
- **de Silva, A.M.**, Balch, W.E. and Helenius, A. (1990). Quality control in the endoplasmic reticulum: folding and misfolding of vesicular stomatitis virus G protein in cells and in vitro. J. Cell Biol. 111:857-866.

D. Research Support.

U19 AI109965 (PI. Ting) (Core Leader. de Silva) 3/1/14-2/28/19
NIH/NIAID
Novel Nanoparticles for Vaccine Delivery

1R01 AI125198 (PI. de Silva) 5/04/16-4/30/21
NIH/NIAID
Preclinical Assays To Predict Tetravalent Dengue Vaccine Efficacy

Not Assigned (PI. Sette) (Sub Contract PI. De Silva) 9/1/14-8/31/19
Private Source
The Identification of HLA Class I and Class II T Cell Epitopes from Dengue Virus

00008956 (PI. Harris) (Project 2 PI. de Silva) 7/29/15-6/30/20
Univ. of California-Berkeley/NIH/NIAID
Protective Immunity Following Dengue Virus Natural Infections and Vaccination

Not Assigned (PI: Baric) 1/01/16-07/31/19
Private Source
In Vitro and In Vivo Characterization of Bivalent DENV Live Virus Vaccines
To provide expertise in molecular virology required for creating recombinant dengue viruses for in vitro and in vivo testing.

Not assigned (PI: de Silva) 10/1/16-9/30/19
Private Source
UNC-Takeda study to characterize human antibody response to DENVax-Phase 2

00HVCLJB-2017-04191 (PI: de Silva) 4/17/17-3/31/21
CDC
Diagnosis of recent and remote Zika infections

R01 AI107731-04 (de Silva) 8/5/13-8/31/23
NIH/NIAID
Molecular Basis of Dengue Virus Neutralization by Human Antibodies

R21 AI134073 (MPI: deSilva/Lakshmanane) 09/05/17-08/31/19
NIH/NIAID
Structure Based design of Recombinant Zika virus antigens for Serodiagnosis

W81XWH1820034 (PI: deSilva) 9/1/18-8/31/21
DOD/USA Med Research ACQ Activity
Structure Guided Design of Dengue and Zika Virus Protein Subunit Vaccines

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 9481080630000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2019 **End Date*:** 09-30-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	CARLOS	A	SARIOL	M.D.	PD/PI	Institutional Base Salary	EFFORT			21,048.00	6,583.00	27,631.00
2.	Dr.	Idia	V	Rodriguez		Veterinarian					16,886.00	5,598.00	22,484.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **50,115.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			45,000.00	18,655.00	63,655.00
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	1.2			3,600.00	1,653.00	5,253.00
1	Animal Health Technologist	6.0			8,430.00	5,997.00	14,427.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**83,335.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**133,450.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-02-04T14:29:01.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 9481080630000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2019 **End Date*:** 09-30-2020 **Budget Period: 1**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,500.00
2. Foreign Travel Costs	
Total Travel Cost	**2,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 9481080630000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2019 **End Date*:** 09-30-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	131,226.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	95,151.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal procedures and Perdiem	137,490.00
Total Other Direct Costs	**363,867.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**499,817.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost Base	50.0	429,666.00	214,833.00
		Total Indirect Costs	**214,833.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Region II, Darryl W. Mayes, 212-264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**714,650.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	714,650.00

L. Budget Justification* File Name: BudgetJustificationFinal-2-04-19.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12781495 Funding Opportunity Number: PA-19-056 . Received Date: 2019-02-04T14:29:01.000-05:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 9481080630000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2020 **End Date*:** 09-30-2021 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	CARLOS	A	SARIOL	M.D.	PD/PI	Institutional Base Salary	EFFORT			21,679.00	6,732.00	28,411.00
2.	Dr.	Idia	V	Rodriguez		Veterinarian					17,393.00	5,718.00	23,111.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **51,522.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			46,350.00	18,975.00	65,325.00
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	1.2			3,708.00	1,678.00	5,386.00
1	Animal Health Technologist	6.0			8,683.00	6,057.00	14,740.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**85,451.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**136,973.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-02-04T14:29:01.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 9481080630000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2020 **End Date*:** 09-30-2021 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,500.00
2. Foreign Travel Costs	
Total Travel Cost	**2,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 9481080630000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2020 **End Date*:** 09-30-2021 **Budget Period:** 2

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	123,726.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	95,833.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . ANIMAL PERDIEM & PROCEDURES	137,490.00
Total Other Direct Costs	**360,049.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**499,522.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost Base	50.0	403,689.00	201,845.00
		Total Indirect Costs	**201,845.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Region II, Darryl W. Mayes, 212-264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**701,367.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	701,367.00

L. Budget Justification* File Name: BudgetJustificationFinal-2-04-19.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 9481080630000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2021 **End Date*:** 09-30-2022 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	CARLOS	A	SARIOL	M.D.	PD/PI	Institutional Base Salary	EFFORT			22,311.00	6,882.00	29,193.00
2.	Dr.	Idia	V	Rodriguez		Veterinarian					17,900.00	5,837.00	23,737.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **52,930.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			47,700.00	19,294.00	66,994.00
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	1.2			3,816.00	1,704.00	5,520.00
1	Animal Health Technologist	6.0			8,936.00	6,116.00	15,052.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**87,566.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**140,496.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-02-04T14:29:01.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 9481080630000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2021 **End Date*:** 09-30-2022 **Budget Period:** 3

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel		Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)		2,550.00
2. Foreign Travel Costs		
	Total Travel Cost	**2,550.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 9481080630000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2021 **End Date*:** 09-30-2022 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	121,726.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	96,533.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . ANIMAL PERDIEM & PROCEDURES	135,520.00
Total Other Direct Costs	**353,779.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**496,825.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost Base	50.0	400,292.00	200,146.00
		Total Indirect Costs	**200,146.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Region II, Darryl W. Mayes, 212-264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**696,971.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	696,971.00

L. Budget Justification* File Name: BudgetJustificationFinal-2-04-19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 9481080630000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2022 **End Date*:** 09-30-2023 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	CARLOS	A	SARIOL	M.D.	PD/PI	Institutional Base Salary	EFFORT			22,942.00	7,031.00	29,973.00
2.	Dr.	Idia	V	Rodriguez		Veterinarian					18,406.00	5,958.00	24,364.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **54,337.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			49,050.00	19,614.00	68,664.00
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	1.2			3,924.00	1,729.00	5,653.00
1	Animal Health Technologist	6.0			9,189.00	6,176.00	15,365.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**89,682.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**144,019.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-02-04T14:29:01.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 9481080630000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2022 **End Date*:** 09-30-2023 **Budget Period:** 4

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel		Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)		2,600.00
2. Foreign Travel Costs		
	Total Travel Cost	**2,600.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 9481080630000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2022 **End Date*:** 09-30-2023 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	114,726.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	97,278.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . ANIMAL PERDIEM & PROCEDURES	134,420.00
Total Other Direct Costs	**349,424.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**496,043.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost Base	50.0	398,766.00	199,383.00
		Total Indirect Costs	**199,383.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Region II, Darryl W. Mayes, 212-264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**695,426.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	695,426.00

L. Budget Justification* File Name: BudgetJustificationFinal-2-04-19.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 9481080630000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2023 **End Date*:** 09-30-2024 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	CARLOS	A	SARIOL	M.D.	PD/PI	Institutional Base Salary	EFFORT			23,574.00	7,180.00	30,754.00
2.	Dr.	Idia	V	Rodriguez		Veterinarian					18,913.00	6,077.00	24,990.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **55,744.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			50,400.00	19,933.00	70,333.00
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	1.2			4,032.00	1,755.00	5,787.00
1	Animal Health Technologist	6.0			9,442.00	6,237.00	15,679.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**91,799.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**147,543.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-02-04T14:29:01.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 9481080630000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2023 **End Date*:** 09-30-2024 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,700.00
2. Foreign Travel Costs	
Total Travel Cost	**2,700.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 9481080630000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: UNIVERSITY OF PUERTO RICO MED SCIENCES

Start Date*: 10-01-2023 **End Date*:** 09-30-2024 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	107,726.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	98,030.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . ANIMAL PERDIEM & PROCEDURES	134,420.00
Total Other Direct Costs	**343,176.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**493,419.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost Base	50.0	395,389.00	197,694.00
		Total Indirect Costs	**197,694.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Region II, Darryl W. Mayes, 212-264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**691,113.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	691,113.00

L. Budget Justification* File Name: BudgetJustificationFinal-2-04-19.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. Senior/Key personnel:

"I certify that I have first-hand knowledge of (or have used suitable means of verifying) work performed by this individual and salary distribution prior to the effective date of this change is reasonable in relation to the work performed."

Carlos A. Sariol, MD, MS, (PI, EFFORT Cal. Months) is a molecular virologist with extensive experience using non-human primates (NHP) as a model for dengue vaccine and dengue and Zika pathogenesis. As PI he will coordinate and supervise all the complex activities at the Animal Resources Center and at the Virology Laboratory, University of Puerto Rico related to the animal's infection. Dr. Sariol will coordinate with the collaborators Drs. Amelia Pinto and Aravinda de Silva the timing and the details of the NHP experiments. Dr. Sariol has current collaboration with both Drs Pinto and De Silva Labs. In this way the execution of the experiments proposed on this application will be a continuation of work and do not need new arrangements. His lab will responsible for testing the animals to identify the proper ones to run the experiments included on this proposal. Also, under his supervision the Virology Laboratories will be in charge for samples reception, processing storage, experiments execution. Dr. Sariol will also responsible to find a solution to any unforeseen problem that may arise. He will be also led the results analysis, discussion and manuscripts preparation.

Idia V. Rodriguez, DVM, (Clinical Veterinarian, EFFORT Cal. Months), is Board Certified in Laboratory Animal Medicine. In the last 15 years, Dr. Rodriguez has been involved in SIV/HIV and Dengue and Zika vaccine protocols with rhesus macaques. She has been working with the PI for many years and still continue working together in the execution of current protocols on Zika and dengue. She will be in charge of all animal procedures and health. They will conduct and oversee animal immunizations, bleeding and all procedures described on this application.

Aravinda de Silva, PhD (Collaborator, EFFORT Cal Month in kind) is a renowned scientist in the immunology field. Dr. Drs. Sariol and de Silva have an ongoing collaboration for more than 10 years and the experiments proposed on this application will be a continuation of that collaboration. Dr. de Silva will continue making the resources, expertise and knowledge at his lab in support of the work proposed on this application (See letter of support). He will have periodical meeting with the PI to follow up the execution of the experiments and to discuss the results. Dr. de Silva will also be involved in the results analysis and manuscript preparation.

B. Other personnel

Erick Perez-Guzman, Post Doc, (EFFORT Cal Month), was a graduate student under Dr. Sariol mentorship. During his PhD career he become familiar with all the experiments and procedures currently implemented at Dr. Sariol Lab. He is first author in a Nature Communication publication and currently a second manuscript is being submitted. Perez-Guzman will support the PI in the coordination and execution of the experiments proposed on this application. He will also execute some of the experiments. Also, he will be involved in the manuscripts writing process.

TBA, (Animal Health Technologist ,50% effort,6 Cal Month), will support Dr. Rodriguez in all procedures and animals care.

TBA, (Administrator, 10% effort, 1.20 Cal Month) The Administrator will devote 10 % of his/her time effort on this application.

Total Cost Personnel for year 1:	**$133,450.00**
Total Cost Personnel for year 2:	**$136,973.00**
Total Cost Personnel for year 3:	**$140,496.00**
Total Cost Personnel for year 4:	**$144,020.00**
Total Cost Personnel for year 5:	**$147,543.00**

C. Equipment
No budget requested

D. Travel
Travel support for year 1 ($2,500) to attend a national or international meeting to present the work at peer reviewed venues, such as the American Association of Tropical Medicine to discuss the work with the PI and to get rigorous scientific feedback on the work and methods. Funds are requested to cover air ticket ($560), lodging (three nights x $120/n = $360), meals ($120), ground transportation ($100), and incidentals ($60).

Total Cost Travel for year 1:	**$2,500.00**
Total Cost Travel for year 2:	**$2,500.00**
Total Cost Travel for year 3:	**$2,550.00**
Total Cost Travel for year 4:	**$2,600.00**
Total Cost Travel for year 5:	**$2,700.00**

E. Participant/Trainee Support Costs
Not Applicable

F. Other Direct Costs

Year 1

F1. Materials and Supplies:
In order to ask for a well balance budget animals' cost and lab cost will be divided in the 5 years period.
This proposal will use 108 rhesus macaques in the 5 years.
The market price of these animals is Proprietary Info
for this protocol for the price Proprietary For 108 animals Proprietary Info
For year 1 we are requesting Proprietary Info
In addition, the following costs are requested.

General Lab Supplies	$	30,000
Elisa Ig/Igm. Neutralization Tests, Antibodies for CD4, CD8/CD20; RT-PCR, Immunophenotyping, Cytokines	$	27,100
MHC TYPING	$	2,376
Reagents for Antibodies depletion	$	8,000
Isotyping & ADE	$	2,500
Dry Ice for sample shipping	$	250
Fed Ex Shipping	$	2,500
Liquid Nitrogen	$	2,000
Laptop	$	1,500
Computer Mac	$	2,500

Total Supplies Costs: **$131,226**

Other Direct Costs:
In order to provide a balance budget, the animals maintenance costs (per diem) will be split across the five years period. Animals will be available for 450 days. Animal per diem Proprietary Info In year one we are requesting Proprietary Info
For animals' procedures including immunization, bleedings, antibodies administration for depletion, CBC, Liver profile and euthanasia costs we are requesting Proprietary Info
Total Other Direct Costs Y1 **$137,490**

Total Direct Costs Year 1 **$404,666**

Year 2

F1. Materials and Supplies:
For year 2 we are requesting Proprietary Info to cover the cost of the animals as described under Y1.
In addition, the following costs are requested.

General Lab Supplies	$	27,000
Elisa Ig/Igm. Neutralization Tests, Antibodies for CD4, CD8/CD20; RT-PCR, Immunophenotyping, Cytokines	$	27,100
MHC TYPING	$	2,376
Reagents for Antibodies depletion	$	7,500
Isotyping & ADE	$	2,500
Dry Ice for sample shipping	$	250
Fed Ex Shipping	$	2,500
Liquid Nitrogen	$	2,000

Total Supplies Costs: **$123,726**

Other Direct Costs:
As described under Y1, to cover the animal's per diem cost in Y2 we are requesting Proprietary Info
For animals' procedures including immunization, bleedings, antibodies administration for depletion, CBC, Liver profile and euthanasia costs we are requesting Proprietary Info

Publication Costs:
At this time, we anticipate submitting a publication with the results obtained in years 1 and 2. To cover the cost of the publications we are requesting $3,000

Total Other Direct Costs Y2 **$140,490**

Total Direct Costs Year 2 **$403,689**

Year 3

F1. Materials and Supplies:
For year 3 we are requesting Proprietary Info to cover the cost of the animals as described under Y1.
In addition, the following costs are requested.

General Lab Supplies	$	32,000
Elisa Ig/Igm. Neutralization Tests, Antibodies for CD4, CD8/CD20; RT-PCR, Immunophenotyping, Cytokines	$	22,600
MHC TYPING	$	2,376
Reagents for Antibodies depletion	$	5,000
Isotyping & ADE	$	2,500
Dry Ice for sample shipping	$	250
Fed Ex Shipping	$	2,500
Liquid Nitrogen	$	2,000

Total Supplies Costs: **$121,726**

Other Direct Costs:
As described under Y1, to cover the animal's per diem cost in Y3 we are requesting Proprietary Info
For animals' procedures including immunization, bleedings, antibodies administration for depletion, CBC, Liver profile and euthanasia costs we are requesting Proprietary Info

Total Other Direct Costs Y3 **$135,520**

Total Direct Costs Year 3 **$400,292**

Year 4

F1. Materials and Supplies:
For year 4 we are requesting Proprietary to cover the cost of the animals as described under Y1.
In addition, the following costs are requested.

General Lab Supplies	$	25,000
Elisa Ig/Igm. Neutralization Tests, Antibodies for CD4, CD8/CD20; R T-PCR, Immunophenotyping, Cytokines	$	22,600
MHC TYPING	$	2,376
Reagents for Antibodies depletion	$	5,000
Isotyping & ADE	$	2,500
Dry Ice for sample shipping	$	250
Fed Ex Shipping	$	2,500
Liquid Nitrogen	$	2,000

Total Supplies Costs: **$114,726**

Other Direct Costs:
As described under Y1, to cover the animal's per diem cost in Y4 we are requesting Proprietary Info
For animals' procedures including immunization, bleedings, antibodies administration for depletion, CBC, Liver profile and euthanasia costs we are requesting Proprietary Info

Publication Costs:
At this time, we anticipate submitting a publication with the results obtained in years 3 and 4. To cover the cost of the publications we are requesting $3,000

Total Other Direct Costs Y4 **$137,420**

Total Direct Costs Year 4 **$398,766**

Year 5

F1. Materials and Supplies:
For year 5 we are requesting Proprietary to cover the cost of the animals as described under Y1.
In addition, the following costs are requested.

General Lab Supplies	$	18,000
Elisa Ig/Igm. Neutralization Tests, Antibodies for CD4, CD8/CD20; RT-PCR, Immunophenotyping, Cytokines	$	22,600
MHC TYPING	$	2,376
Reagents for Antibodies depletion	$	5,000

Isotyping & ADE	$	2,500
Dry Ice for sample shipping	$	250
Fed Ex Shipping	$	2,500
Liquid Nitrogen	$	2,000

Total Supplies Costs: **$107,726**

Other Direct Costs:
As described under Y1, to cover the animal's per diem cost in Y5 we are requesting $118,000.
For animals' procedures including immunization, bleedings, antibodies administration for depletion, CBC, Liver profile and euthanasia costs we are requesting $16,420

Publication Costs:
At the end of the application, we anticipate having enough data to submit additional work for publication. To cover the cost of the publications we are requesting $3,000

Total Other Direct Costs Y5 **$137,420**

Total Direct Costs Year 5 **$395,389**

Total Direct Costs Year 1-5 **$2,002,802**

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		264,648.00
Section B, Other Personnel		437,833.00
Total Number Other Personnel	15	
Total Salary, Wages and Fringe Benefits (A+B)		702,481.00
Section C, Equipment		0.00
Section D, Travel		12,850.00
1. Domestic	12,850.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		1,770,295.00
1. Materials and Supplies	599,130.00	
2. Publication Costs	9,000.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	482,825.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	679,340.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		2,485,626.00
Section H, Indirect Costs		1,013,901.00
Section I, Total Direct and Indirect Costs (G + H)		3,499,527.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		3,499,527.00

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 0502207220000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Saint Louis University

Start Date*: 10-01-2019 **End Date*:** 09-30-2020 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Amelia	K	Pinto		PD/PI	Institutional Base Salary	EFFORT			4,690.00	1,421.00	6,111.00
2.	Dr.	James	D	Brien		CO-INVESTIGATOR					1,876.00	568.00	2,444.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **8,555.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician	1.2			4,030.00	1,221.00	5,251.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**5,251.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**13,806.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 0502207220000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Saint Louis University

Start Date*: 10-01-2019 **End Date*:** 09-30-2020 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 0502207220000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Saint Louis University

Start Date*: 10-01-2019 **End Date*:** 09-30-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	49,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**49,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**62,806.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	51.5	62,806.00	32,345.00
		Total Indirect Costs	**32,345.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): US DHHS, MR. ARIF KARIM, 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**95,151.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	95,151.00

L. Budget Justification* File Name: BudgetJustification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 0502207220000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Saint Louis University

Start Date*: 10-01-2020 **End Date*:** 09-30-2021 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Amelia	K	Pinto		PD/PI	Institutional Base Salary	EFFORT			4,805.00	1,504.00	6,309.00
2.	Dr.	James	D	Brien		CO-INVESTIGATOR					1,922.00	602.00	2,524.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **8,833.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician	1.2			4,130.00	1,293.00	5,423.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**5,423.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**14,256.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 0502207220000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Saint Louis University

Start Date*: 10-01-2020 **End Date*:** 09-30-2021 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 0502207220000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Saint Louis University

Start Date*: 10-01-2020 **End Date*:** 09-30-2021 **Budget Period:** 2

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	49,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**49,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**63,256.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	51.5	63,256.00	32,577.00
		Total Indirect Costs	**32,577.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): US DHHS, MR. ARIF KARIM, 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**95,833.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	95,833.00

L. Budget Justification* File Name: BudgetJustification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 0502207220000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Saint Louis University

Start Date*: 10-01-2021 **End Date*:** 09-30-2022 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Amelia	K	Pinto		PD/PI	Institutional Base Salary	EFFORT			4,925.00	1,591.00	6,516.00
2.	Dr.	James	D	Brien		CO-INVESTIGATOR					1,970.00	636.00	2,606.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **9,122.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician	1.2			4,230.00	1,366.00	5,596.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**5,596.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**14,718.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-02-04T14:29:01.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 0502207220000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Saint Louis University

Start Date*: 10-01-2021 **End Date*:** 09-30-2022 **Budget Period:** 3

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 0502207220000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Saint Louis University

Start Date*: 10-01-2021 **End Date*:** 09-30-2022 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	49,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**49,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**63,718.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	51.5	63,718.00	32,815.00
		Total Indirect Costs	**32,815.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): US DHHS, MR. ARIF KARIM, 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**96,533.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	96,533.00

L. Budget Justification* File Name: BudgetJustification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 0502207220000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Saint Louis University

Start Date*: 10-01-2022 **End Date*:** 09-30-2023 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Amelia	K	Pinto		PD/PI	Institutional Base Salary	EFFORT			5,050.00	1,682.00	6,732.00
2.	Dr.	James	D	Brien		CO-INVESTIGATOR					2,020.00	673.00	2,693.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **9,425.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician	1.2			4,340.00	1,445.00	5,785.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**5,785.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**15,210.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-02-04T14:29:01.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 0502207220000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Saint Louis University

Start Date*: 10-01-2022 **End Date*:** 09-30-2023 **Budget Period:** 4

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 0502207220000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Saint Louis University

Start Date*: 10-01-2022 **End Date*:** 09-30-2023 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	49,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**49,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**64,210.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	51.5	64,210.00	33,068.00
		Total Indirect Costs	**33,068.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): US DHHS, MR. ARIF KARIM, 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**97,278.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	97,278.00

L. Budget Justification* File Name: BudgetJustification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 0502207220000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Saint Louis University

Start Date*: 10-01-2023 **End Date*:** 09-30-2024 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Amelia	K	Pinto		PD/PI	Institutional Base Salary	EFFORT			5,175.00	1,775.00	6,950.00
2.	Dr.	James	D	Brien		CO-INVESTIGATOR					2,070.00	710.00	2,780.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **9,730.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician	1.2			4,450.00	1,526.00	5,976.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**5,976.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**15,706.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 0502207220000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Saint Louis University

Start Date*: 10-01-2023 **End Date*:** 09-30-2024 **Budget Period:** 5

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 0502207220000
Budget Type*: ○ Project ● Subaward/Consortium
Organization: Saint Louis University

Start Date*: 10-01-2023 **End Date*:** 09-30-2024 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	49,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**49,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**64,706.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	51.5	64,706.00	33,324.00
		Total Indirect Costs	**33,324.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): US DHHS, MR. ARIF KARIM, 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**98,030.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	98,030.00

L. Budget Justification* File Name: BudgetJustification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

EFFORT

BUDGET JUSTIFICATION: SLU

A. Senior/Key Personnel:

Amelia K Pinto, PhD, Collaborator, calendar months EFFORT. Dr. Pinto is an Assistant Professor of Molecular Microbiology & Immunology at Saint Louis University School of Medicine. Dr. Pinto is a leading expert in viral immunology. Her laboratory seeks to improve our understanding of how the immune system recognizes and restricts re-emerging infectious diseases with the goal of designing treatments to reduce morbidity and mortality. We use immunological techniques to investigate fundamental biological processes that control virus infection and identify the correlates of protection required for the development of vaccines and therapeutics. For this project: she will design and oversee flow cytometric analysis of T cell responses following Zika and dengue.

EFFORT

B. Other Personnel:

James D. Brien, PhD, Assistant Professor, calendar months EFFORT Dr. Brien is a highly regarded flavivirologist with a unique expertise in understanding the immune response to arboviruses and has successfully identified immunological correlates of protection and mechanisms of viral pathogenesis. For this project Dr. Brien will aid in flow cytometric analysis and participate in discussions of the results.

TBA, Research Assistant, 1.2 calendar months 10% effort. This project requires that flow cytometric assays be run on the non-human primate samples sent from Dr. Sariol's group to Dr. Pinto's laboratory. For this project: They will perform the flowcytometric assays on all samples received for this proposal.

C. Supplies:

Disposables, etc. Disposable products will be needed for processing samples. Lab supplies include (pipette tips, chemwipes, gloves, tubes, bleach, and plasticware), are estimated at **$3,000** per year.

Cytometric calibration reagents. With this project there will be a considerable amount of flow cytometric assays. The funds requested will be used for daily and monthly calibrating of the screening equipment to ensure rigor and reproducibility of the data and are estimated at **$1,000** per year.

Immunochemical Reagents. Antibodies and secondary reagents will be used for the flow cytometric analysis at **$45,000** per year.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		45,665.00
Section B, Other Personnel		28,031.00
Total Number Other Personnel	5	
Total Salary, Wages and Fringe Benefits (A+B)		73,696.00
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		245,000.00
1. Materials and Supplies	245,000.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		318,696.00
Section H, Indirect Costs		164,129.00
Section I, Total Direct and Indirect Costs (G + H)		482,825.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		482,825.00

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	467,472	466,945	464,010	462,975	460,095	**2,321,497**

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount ($) *Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	SpecificAims020319.pdf
3. Research Strategy*	ResearchStrategy020419.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	VAR01-Zika-Dengue.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	LOSfinal.pdf
10. Resource Sharing Plan(s)	RESOURCE_SHARING_PLAN.pdf
11. Authentication of Key Biological and/or Chemical Resources	
Appendix	
12. Appendix	

SPECIFIC AIMS

Zika virus (ZIKV) is a re-emerging mosquito-borne *Flavivirus* that recently caused an outbreak in the Americas. The establishment of ZIKV transmission cycle in tropical/sub-tropical regions that are endemic to other close-related flaviviruses such as Dengue virus (DENV) has raised concerns, mainly by their cross-immunological interactions and the implications of this for development of severe clinical manifestations. Several groups have demonstrated that DENV-immune serum from humans can enhance ZIKV infection *in vitro* (1, 2) and *in vivo* in an immunodeficient mice model (3). This phenomenon known as Antibody Dependent-Enhancement (ADE) has been linked to severe dengue clinical manifestations (4-7). However, our group provides evidence that a pre-existing DENV immunity may confer some degree of protection against ZIKV infection (8). Our results were recently confirmed in human population (9). Furthermore we found that the cross-protection is associated to the interval of time between DENV and ZIKV infections and may be mediated by the cellular immune response, particularly CD4+ T cells (10). This protective effect against ZIKV by previous DENV immunity has also been documented in humans (44). On the other hand, little is known about the effect of a previous immunity to ZIKV on a subsequent DENV infection. During the recent ZIKV epidemic, part of the population naïve to DENV such as newborns, children, adults and travelers from non-flavivirus endemic areas could be exposed to a ZIKV infection before to DENV. Eventually, herd immunity will reduce ZIKV transmission allowing DENV to re-emerge and potentially infect the ZIKV-immune population. It has being documented that ZIKV-immune serum can enhance DENV infection *in vitro*, however little evidence is available about this phenomenon occurring *in vivo*. Recently George *et al.*, showed that a short-term immunity to ZIKV can enhance DENV infection in rhesus macaques inducing a pro-inflammatory cytokine profile and higher peak of DENV viremia (11). However, we have result showing that previous ZIKV immunity can modulate the immune response against DENV but does not result in enhancement (12). We also showed that the T cells immune response plays a relevant role in the modulation of the immune response, but it is unclear if the role is protective or not. Currently there are not known correlates of protection from previous DENV immunity against ZIKV or vice versa and this is a key information in order to design effective ZIKV vaccines to prevent the devastating effect associated with this virus like Guillain-Barré Syndrome (13) and Congenital Zika Syndrome (14). In addition to our findings in Non-Human Primates (NHP), recent publications using small animals (immunodeficient mice), showed that CD8+ T cells are essential for the control of ZIKV viremia and pathogenesis including control of ZIKV viremia during pregnancy (15-17). Also the protective role of CD4+ T cells against neuroinvasive ZIKV disease have been documented by one of our collaborators, Dr. A.K. Pinto's group (18). In addition, the link between the CD4+ T and the B cells during the immune response to these pathogens and their role in the cross-protection need to be clarified. The role of the CD4+ T cells in the B cells immune response against dengue have been previously documented (19). However, all above mentioned works have been performed in immunodeficient animals lacking essential antiviral mechanisms that otherwise, will effectively contribute to the control of the viral replication. Because of that, it is highly necessary to characterize the cellular immune response, and its impact in the humoral immune response, in the control of a heterologous secondary DENV or ZIKV infection in the presence of previous DENV or ZIKV immunity in an immunological competent animal model that resemble the human immune system like NHP. The overall hypothesis behind this work is that the cross-primed cellular immune response may be critical controlling the DENV and ZIKV infection and provides heterologous protection against each other. To test this hypothesis we propose a series of straightforward experiments with the following aims:

Aim 1: Characterize the contribution of DENV-crossreactive CD4+ or CD8+ T cells in the heterologous protection against ZIKV (Years 1 - 3). We will assess ZIKV replication, the clinical presentation, the serological and cytokine profiles and the cellular immune response in the presence of an intact or a blunted cellular immune response to a previous DENV infection. In **Task 1.1** we will determine the contribution of either CD4+ or CD8+ T cells in the outcome of ZIKV infection by depleting those cell types in groups of 6 animals each prior to ZIKV challenge. One extra group, with intact T cells will serve as control. **Task 1.2** will complement the results from Task 1.1 by challenging DENV-immune animals with a blunted CD4+ T cells response, with ZIKV. For this experiment animals will be depleted of CD4+ cells before they are primarily infected with DENV. **Task 1.3** will be similar to Task 1.2 but animals will have a blunted CD8+ T cell. In total 42 macaques will be used on this aim.

Aim 2: Characterize the contribution of ZIKV-crossreactive CD4+ or CD8+ T cells in the heterologous protection against DENV (Years 2-4). We will assess DENV replication, the clinical presentation, the serological and cytokine profiles and the cellular immune response in the presence of an intact or a blunted cellular immune response to a previous DENV infection. For **Tasks 2.1, 2.2** and **2.3** will use new groups of animals (total 42) to reproduce the experimental design under Aim 1 but where the initial infecting agent will be ZIKV followed by DENV as secondary heterologous infection.

AIM 3: To determine the net contribution of a previous cross-reactive cellular immunity, in the presence of a blunted humoral response, to the heterologous protection against ZIKV in DENV-immune subjects and vice versa (Years 3.5-5). For this aim we will proceed to deplete the B cells in the experimental groups before the primary infection with DENV or ZIKV. We will assess DENV or ZIKV replication, the clinical presentation, the serological and cytokine profiles and the cellular immune response in the presence of an intact or a blunted humoral immune response to a previous ZIKV or DENV infections. In **Task 3.1** two groups of 6 new animals each (total 12) will be used.

A. SIGNIFICANCE

This study is designed to identify novel correlates of protection based on the synergistic contribution of the T cell and the antibodies response during a secondary heterologous flavivirus challenge, particularly DENV and ZIKV.DENV is a global public health threat, causing ~390 million infections annually worldwide, and with the highest level of mortality within arboviruses (18-22). Exposure to a primary DENV infection confers long-lived immunity against a homotypic secondary infection. However, a heterotypic secondary infection is the major risk factor to induce severe DENV disease, characterized by dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (25-27). The ADE mechanism suggests that antibodies (Abs), generated in response to the primary infection with DENV, are not of sufficient concentration or avidity to neutralize a secondary infection with DENV of a different serotype, and may even facilitate its replication. Some works *in vitro* and *in vivo* using mice showed that previous DENV-immunity can results in ADE of ZIKV (1, 3, 20-22). However, our group (8) and other (23) showed that in NHP, previous flavivirus immunity does not result in ZIKV enhancement. On the other scenario (ZIKV-DENV), there are works in vitro, in mice (20) and one work in NHP (11) showing that ZIKV may induce ADE of DENV. It has also been suggested that a ZIKV vaccine may induce ADE of DENV infection (24). We have preliminary results showing that that may not be the case (12). This situation is highly significant for the consequences this may have when DENV epidemic arise in areas with ZIKV-immune population. Understanding the mechanism of cross-protection between those two viruses is crucial to guarantee safe vaccines designs and epidemiological interventions.

A.2 T cells: pathogenesis vs. protection: Another mechanism proposed for the development of DHF/DSS is the production of cross-reactive T-cell clones during the primary infection, a theory known as the original antigen sin (25, 26). According to this hypothesis, T cells induced by a primary infection dominate the secondary heterologous infection but are of lower efficacy in clearing the infection (27, 28) and inducing a response that is qualitatively different from the response induced by the original antigen, such as inducing a different pattern of cytokine production, and thus contribute to immunopathogenic of severe disease (29, 30). However, there are another works in mice (31-35) and from humans (36-38) showing that both CD4 and C D8 may play a resolving DENV infection. Currently, by January 2019, there is not published data in human or NHP, on the protective or pathogenic role of the T cells during heterologous infections with DENV and ZIKV vice versa. Our preliminary results and the experiments proposed on this application will help to clarify the discrepancies in the literature.

A.3 The ZIKV issue: ZIKV re-emergence and arrival to the Americas with association to severe clinical manifestations, promotes an explosive research response from the scientific community. The introduction of this virus in the Americas have been linked to unique severe adverse outcomes such as fetal loss (39), congenital Zika syndrome (CZS) (14), Guillain-Barré syndrome (GBS) (13), and rare cases of encephalopathy (40), meningoencephalitis (41), myelitis (42), uveitis (43), and severe thrombocytopenia (44). Little is known about the cause of the high ZIKV-associated microcephaly incidence in South America. One of the main factors pointed out is the complex immunological background of the population, mainly based on long-term pre-existing immunity to DENV (endemicity), Yellow Fever (vaccination), among other viruses (45-47). There is currently no treatment or vaccine available to prevent ZIKV, although in the last two years there have been numerous efforts developing ZIKV vaccine candidates in proof-of-concept pre-clinical and clinical studies (48).

A.4 Role of DENV-ZIKV cross-reactive cellular immune response: Less is known about the impact of previous exposition to ZIKV on a subsequent flavivirus infection such DENV. It is estimated that in the recent epidemic of ZIKV approximately 750,000 individuals (suspected and laboratory- confirmed cases) were infected in the Americas, where most countries are endemic to DENV (46). There is not data about how many of them were naive to DENV and other locally transmitted flaviviruses during the epidemic of ZIKV. In spite of disperse evidences in mice (31-35) and from humans (36-38) showing the role of the cellular immune response in the protection against DENV, the only commercial DENV vaccine available in different countries (no FDA approved yet, but filed for approval) Dengvaxia, was designed using the structural proteins from DENV but in a genetic background of the attenuated yellow fever vaccine strain. Because of that, the vaccine lacks the main T cells epitopes to induce a competent cellular immune response. This drawback is one of the reasons that may explain the low effectiveness of the vaccine and the significant higher number of severe dengue in vaccine recipients compared to the control groups after a natural exposition to DENV (49, 50). Recent works performed in mice suggest that prior DENV immunity can protect against ZIKV infection during pregnancy and CD8+ T cells are sufficient for this cross-protection (15). Our collaborators also showed that protective role of CD4+ T cells against neuroinvasive ZIKV disease (18). However as far as we know, the depletion experiments proposed on this application, to understand the contribution of the DENV or ZIKV heterologous cellular immune response in the protection against ZIKV or DENV respectively, have not been performed so far. Understanding correlates of protection between ZIKV and DENV is essential to anticipate the outcome of the secondary infection, the design of diagnostics methods and more relevant, to support the design of highly effective ZIKV and DENV vaccines in the scenario of previous DENV or ZIKV immunity, respectively. Undoubtedly, NHP provide us with a unique immunological tool very close to the human system to provide the answers to the questions we are outlying on this application.

B. INNOVATION

Recent results confirmed that NHPs, particularly rhesus macaques (RM) are a good model to study ZIKV infection and pathogenesis (51-56). However, current evidence in the role of flaviviral pre-existing immunity in the outcome of ZIKV infection is scarce. So far only three works addressing the role of previous exposition to DENV in the outcome of ZIKV

infection have been published in NHP models and two of them have been produced by our group (8, 10, 23) (Serrano-Collazo et al. is under consideration but we do expect that at the time you review this application it should be accepted/published). The works from our group are the only one addressing the potential mechanisms of protection conferred by a previous DENV or ZIKV immunity against a subsequent infection with ZIKV or DENV. The depletion experiments in NHPs we are proposing here are not novel as they have been extensively used in the HIV/SIV-AIDS-related field. However, they are novel in the sense that these experiments have not been performed in NHP to understand the correlates of protection against ZIKA with or without previous DENV-immunity or viceversa or in the flavivirus research field at all. Surprisingly, in more than 75 years of DENV research, this experiment, to determine the net contribution of the cellular immune response to DENV has not been performed in NHP yet. Another point that can be considered novel is the timing of one year between DENV and ZIKV infection or vice versa proposed on this application. The rational is supported in our recent work (10) (and preliminary results) showing for first time, that the interval of time of one year but not three months or almost three years (2.8y), between DENV and ZIKV infections can confer a level of protection against ZIKV, most likely mediated by cross-reactive T cells. Similarly relevant is the genetic quality of the animal we will use for this application in comparison with other works using NHP from different and dissimilar stocks. The population of rhesus macaques bred and housed at the Caribbean Primate Research Center has proven to be the purer Indian-origin macaque population of all populations in the USA or imported animals, without having a significant level of inbreeding, (57-60). As we reported before, this is highly significant as it allows for consistency and reproducibility in all the flavivirus-related experiments we have conducted before with these animals (8, 61-65). In addition, since year 2016 with the support of the Wisconsin National Research Primate Center (WNPRC), we implemented the MHC typing via deep sequencing using Illumina MiSeq genotyping. The standard assay includes both class I and class II DRB sequencing and allows for an increased efficiency in genotyping nonhuman primate populations and to generate essentially a complete allele libraries (see table 1 below). For first time, in any study in the flavivirus field, we will use this information to characterize specific CD4 and CD8 T cells epitopes playing a role in the T cells immune response against dengue and ZIKV. This is a unique and critical contribution of this application.

C. PRELIMINARY RESULTS

C.1 Relevant results supporting Aim 1 and 3: Previously we challenged with ZIKV a cohort of 4 animals previously exposed to DENV 2.8 years earlier (8). We found that previous DENV immunity of almost three years was related to a trend to control ZIKV viremia. After ZIKV infection, animals developed ZIKV-specific and DENV-cross reactive antibodies confirming the activation of naïve B cells and the expansion of B Memory Cells (MBC). However, the DENV-cross reacting antibodies showed a very limited neutralization potential against DENV suggesting that those cross-antibodies generated after ZIKV infection target non-neutralizing epitopes. Immune profiling of the memory CD4+ and CD8+ T-cell responses in the naive and DENV-immune macaques revealed no defects in the ability of the DENV-immune cohort to respond to ZIKV infection. Notably we noted that the DENV-immune macaques appeared to have higher percentage of CD107a+ CD8+ T cells in response to stimuli as compared to the naive macaques, suggesting that rather than hampering the immune response, a prior DENV infection enhanced the ability of the CD8+ T cells to release lytic granules in response to their cognate antigen (8). In the work proposed on this application we will add the CD27 marker (66) to characterize the contribution of the MBC in the initial infection with ZIKV or DENV and then during the secondary heterologous challenge. Also, continuing with our collaboration with Dr. Aravinda de Silva's lab, we will better characterize the composition of the antibodies population quantifying the presence of virus-specific vs. cross-reacting antibodies.

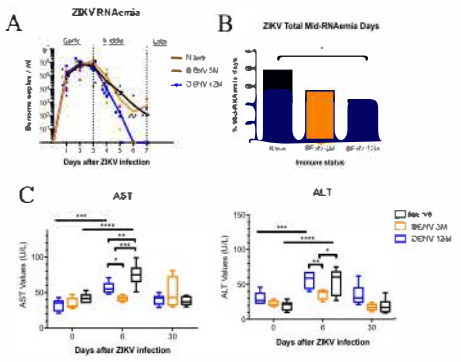


Figure 1: ZIKV RNAemia and viremia days. (A) RNAemia detected in the early (days 1-3) and middle periods (days 4-7) after Zika infection. Late viremia times were not assayed due to the impact of the hurricane Maria on September 20, 2017. On Day 4 DENV 12M group had a viremia peak significantly lower than the naïve ($p<0.05$) and 3M ($p<0.05$) groups. (B) Average viremia days per cohorts is showed. Only the DENV 12M group showed a significant difference with the naïve group ($p<0.05$).

Furthermore, we exposed to ZIKV two cohorts of animals previously exposed to DENV 2, one year (six animals) or three months earlier (four animals)(10). Of note, DENV-middle convalescent animals (one year of previous DENV immunity) had significantly lower peak viremia on day 4 compared with the rest of the animals ($P<0.05$) (fig. 1A). By days 6 and 7 p.i., there was no viral RNA detection in this group, while early convalescent animals (DENV 3M) animals showed a trend towards an intermittent viremia. The animals exposed to DENV 12 months earlier had the least average viremia days in comparison with the naïve group, and the difference was statistically significant ($P<0.05$) (fig. 1B). Also, the liver enzymes (AST and ALT) showed statistically lower values in the DENV-immune animals (fig1C). These results suggest that a previous infection with DENV contributes to a more efficient control of ZIKV viremia in a subsequent infection and with a limited liver damage, but only if at middle convalescence period (at least 12 months) have passed between infections. To assess if the period of DENV convalescence previous a ZIKV infection has an impact on the T cell response to a ZIKV infection we phenotypically characterized the changes in the CD4+ and CD8+ T cells compartments before and after the

ZIKV challenge. We found that the group heterologous challenged 12 months post-DENV infection (the group that was better at controlling ZIKV viremia and had significantly lower levels of liver enzymes) showed a significantly higher frequency of pre-existing T effector memory cells (TEM) (CD4+CD3+CD28-CD95+) cells prior to and 3 days after ZIKV infection. Particularly relevant is the significant preexistent combination of high TEM and lower TCM cell frequencies in that group compared to the 3M convalescent and naïve groups (data not shown). We also aimed to evaluate the effector response of the CD4+ and CD8+ T cells producing IFN-γ, TNF-α, and CD107a in response to various stimuli. The IFN-γ response in the CD4+ T cells from the DENV 12M group before ZIKV infection is remarkable (fig 2A). The frequency of these cells was significantly higher in response to the whole inactivated dengue virus ($P<0.0001$) but also showed a strong trend to have a higher frequency of IFN-γ producing cells in response to peptides derived from the DENV and ZIKV envelopes and ZIKV non-structural proteins as well. DENV 12M animals had also a significantly higher frequency of CD107a+ cells prior to ZIKV infection, while a significant increase in reactivity of CD107a+ CD4+ cells was observed against ZIKV envelope and non-structural antigens 30 days p.i. (fig 2A). These results correlate with the protective effect observed in this group. In contrast, data from CD8+ T cells denote similar responses between DENV immune animals, with no significant variations compared to the naïve animals (data not shown). This suggests that previous DENV immune status preferentially shapes the CD4+ T cells effector responses to a ZIKV infection. The significant role of the T cells in controlling Zika replication and liver damage in the animals with a DENV-middle convalescence period before Zika infection was reinforced by the significant increase of circulating cytolytic protein perforin at day 7 post-infection in that group (data not shown). We hypothesize that likely this represents the acquisition of cytotoxic function of the T cells (67) in that group compared to the other two groups and correlates with the higher expression of CD107a on the CD4+T cells isolated from the middle convalescent animals. In summary, we determined that the magnitude and the breadth of the cellular immune response were dependent on the convalescent status with significant consequences. Our study suggests that TEM cells play a role in controlling ZIKV viremia and liver damage. We noted that this only happens after a convalescent period of about one year. The cytotoxic properties of TEMs present 12 months after DENV infection and during heterologous ZIKV challenge correlate with better performance relative to the TEM cells present early (3 months) or late (2.8 years) (8) after the primary DENV infection. These results suggest that, similar to the heterologous dengue infections, there is a window of optimal cross-protection between ZIKV and DENV with significant consequences and have pivotal implication while interpreting Zika pathogenesis in flavivirus-experimented population, diagnostic results interpretation and vaccine designs among others. The experiments we are proposing on this application are key to complement and expand our preliminary results. On this application we will perform specific T cells depletion to confirm the contribution of the DENV cross-reactive CD4+ T cells or CD8+ T cells controlling ZIKV infection.

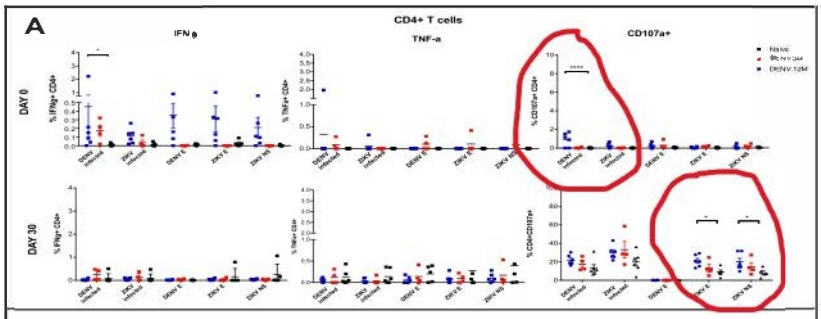


Figure 2: Antigen-specific CD4+ previous and after Zika infection. The frequency of the specific response to dengue and Zika antigens are significantly different among cohorts. In all panels, animals exposed to DENV 12 months before ZIKV infection are in blue, while animals exposed to DENV 3 months before are in orange. Naïve animals are in black. All percentages shown are subtracted from the unstimulated background. (A) Analysis of CD4 T cell response to different stimuli before (upper panel) and 30 days after ZIKV infection (lower panel). Statistically significant differences among groups were calculated by two-way ANOVA using Tukey's multiple comparisons test ($p<0.05$).

C.2 Relevant results supporting Aim 2 and 3: We also assessed the impact of previous ZIKV immunity on DENV outcome and the contribution of the elapse of time between ZIKV and DENV in the immune quality and magnitude of the response. To accomplish this, we challenge with DENV2 two cohorts of animals previously exposed to ZIKV two or ten months earlier. A third cohort of flavivirus naïve animals was also challenge with DENV2 as control group. ZIKV-immune animals were challenged two and ten months earlier with ZIKV strains PRVABC-59 and H/PF/2013 respectively (12). In addition to evaluate the role of the time elapse between DENV and ZIKV, the use of two different ZIKV strains allowed also to identify if there were any differences in the immune response to DENV due to ZIKV strain specificity. RNAemia levels in NHPs serum were quantified by qRT-PCR at baseline, 1 to 10, and 15 dpi to determine if the presence of early- (ZIKVPR-2mo) or mid-convalescence (ZIKVPF-10mo) immunity to ZIKV alters DENV kinetics. In general, no significant differences between groups were observed in detected levels of DENV genome copies per ml of serum over time (fig. 3A). Despite of this, ZIKVPF-10mo keep the RNAemia level below 10^3 genome copies/ml in 75% of the animals at 1 dpi. Moreover, at day 2 and 3 pi at least 50% of ZIKVPF-10mo animals show the lowest levels of DENV genome copies

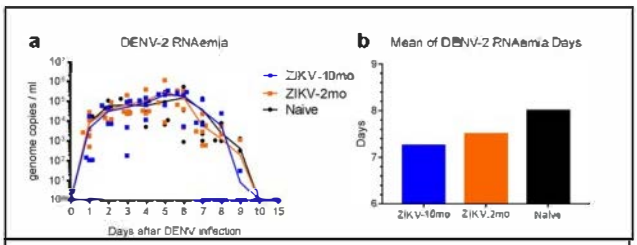


Figure 3: DENV-2 RNA kinetics of ZIKV-immune and naïve rhesus macaques.

compared to other groups. In addition, a strong trend but not significant decrease in the average DENV viremia days was associated with a 10 months period of previous ZIKV immunity (fig 3B). We aimed to evaluate the dynamic of the T cells immune response against DENV in the presence of previous ZIKV immunity with variable periods of time. As showed in figure 4 the frequency of activated CD4+ and CD8+ T cells, compared to its basal levels, was statistically significant higher early after DENV infection only in the ZIKVPF-10mo. This result suggests that the period of time between the two challenge is essential to positively modulate activation of the effector memory cells. We them investigated if different convalescent periods to ZIKV impact the outcome of the effector role of $CD4^+$ and $CD8^+$ T cells following DENV infection (fig. 5). In summary, results of T cell functional response before DENV infection suggest: (i) that a mid-convalescence to ZIKV provoke a higher $CD8^+$ T cell effector response capable to cross-react efficiently with DENV E protein; (ii) that an early-convalescence to ZIKV retained (for at least 2 months) the cytotoxic phenotype of both T cell compartments by the expression of CD107a against ZIKV, but also able to cross-react with DENV supernatant of infection at same magnitude; (iii) and that in absence of ZIKV-immunity naïve animals are unable to generate a significant effector T cell response against DENV or ZIKV as expected. Results after DENV infection suggest that a mid-convalescence to ZIKV: (i) produce a cytotoxic $CD107a^+$ response directed to DENV E protein for both T cell compartments comparable to the DENV-specific *de novo* response of the naïve group, (ii) the developed IFN-γ and TNF-α producing $CD8^+$ T cell effector response that cross-react efficiently with DENV E protein since baseline is maintained but a remarkable boost of IFN-γ and TNF-α producing $CD4^+$ T cell response is obtained capable of react against DENV/ZIKV E and ZIKV-NS proteins. In summary we can conclude that the T cell functional effector response against DENV and ZIKV is shaped by the longevity of ZIKV-immunity. With the work proposed on this application we do expect to dissect the cross-protection mechanism between these two viruses by determining the particular role of each T cells subpopulations.

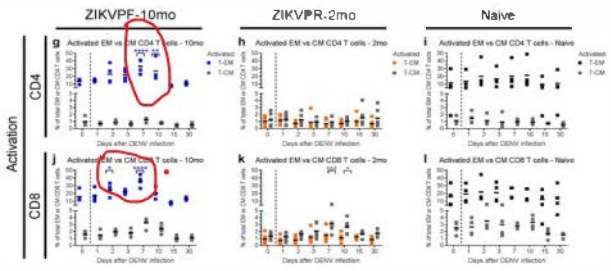


Figure 4: Effector and central memory T cells within $CD4^+$ and $CD8^+$ T cell compartments are modulated by early- and mid-convalescence to ZIKV after DENV infection. (g-l) activation ($CD69^+$) of effector memory (T-EM: $CD3^+CD4^+CD28^-CD95^+$) and central memory (T-CM: $CD3^+CD4^+CD28^+CD95^+$) T cells within $CD4^+$ and $CD8^+$ T cell compartments. Blue, orange and black squares represent T-EM for ZIKVPF-10mo, ZIKVPR-2mo and Naïve, respectively. Gray squares represent T-CM for all groups. Short black lines mark mean value for each group per timepoint. Cutted line divide % of T-EM and T-CM cells quantified before and after DENV infection.

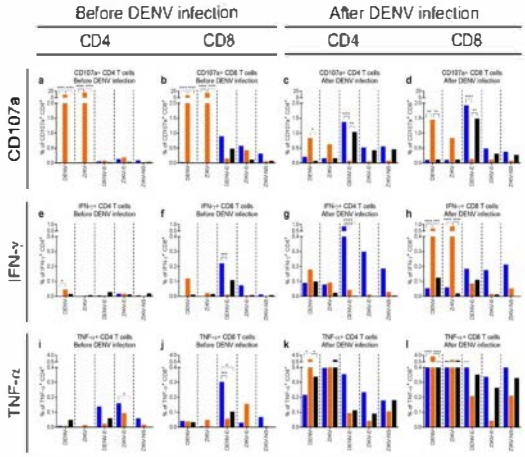


Figure 5: Longevity of ZIKV immunity shapes the functional response of $CD4^+$ and $CD8^+$ T cells. T cell functional effector response was determined by the quantification (%) of **(a-d)** CD107a-expressing and **(e-h)** IFN-γ or **(i-l)** TNF-α producing $CD4^+$ and $CD8^+$ T cells in response to several antigenic stimulation such as DENV and ZIKV supernatants of infection, or peptide pools that encode for DENV and ZIKV envelope (E) proteins or ZIKV non-structural (NS) proteins. Blue, orange and black bars represent the % of T cells quantified for ZIKVPF-10mo, ZIKVPR-2mo and Naïve, respectively. Cutted line divide % of T cells per group quantified for each antigenic stimulation.

C.3 Relevant results supporting Aim 3.

C3.1 Humoral immune response in the DENV-ZIKV sequence: To determine the contribution of the antibodies to the neutralization and the impact of a previous DENV infection in a subsequent ZIKV infection we measure the binding and the neutralizing antibodies in the three groups of animals described in figure 1 (C.1). First, we measure the levels of the binding antibodies. As expected, all DENV immune animals had detectable IgG levels against DENV at baseline. Anti-DENV IgG levels were confirmed in both DENV-pre exposed groups and remained significantly higher compared to the naïve animals slowly decreasing by days 30 and 60 p.i.. All DENV-immune animals had detectable levels of anti-ZIKV IgG at baseline compared to the naïve animals, suggesting a strong cross-reactivity between previously generated anti-DENV IgG to ZIKV (data not shown). When evaluating the neutralizing antibodies, we found that neutralization against ZIKV is kept at a minimum before ZIKV infection, regardless of pre-immunity status. However, and highly relevant, we found that early neutralizing titers are raised after ZIKV infection in all groups, including the naive group in spite of the limited IgG binding antibodies (fig 6A). Neutralizing titers are, however, statistically significant higher in animals with previous immunity to DENV, particularly in the DENV-12M group compared to the naive group. The significant increase in the neutralization titers correlates with an early significant increase of the IgG level in the DENV-12M group (fig 6B). This suggests an early expansion of the MBC population in the DENV-immune animals and that the magnitude of the neutralization is positively affected by the interval of time between DENV and ZIKV

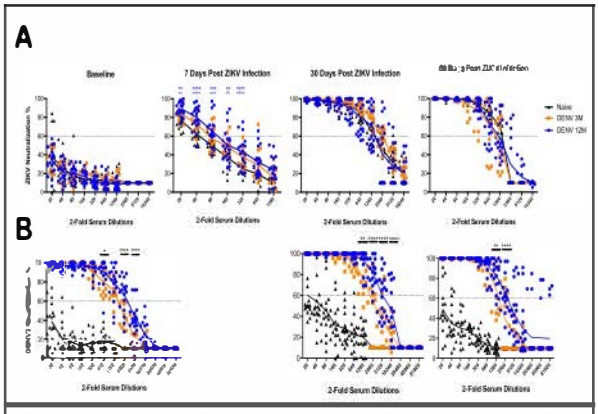


Figure 6. Dynamic of the neutralizing antibodies to Zika and dengue. The magnitude of neutralizing antibodies before and after ZIKV infection is shown. In all panels, animals exposed to DENV 12 months before ZIKV infection are in blue, while animals exposed to DENV 3 months before are in orange. Naïve animals are in black. (A) Neutralization magnitude against ZIKV before, and 30 and 60 days after ZIKV infection was determined using a dilution:neutralization capacity correlation. (B) Neutralization magnitude against DENV 2 before, and 30 and 60 days after ZIKV infection.

infections. By day 60 p.i. DENV-middle convalescent animals continue showing a trend towards the higher magnitude. From our results we can conclude that previous DENV immunity contribute to the induction of early anti-ZIKV neutralizing antibodies that may modify ZIKV replication in vivo and the liver damage (fig 1). This suggests that a subsequent exposure to ZIKV has an impact on neutralization potential in DENV-immune animals, and this effect significantly correlates with the time that has passed between infections.

C3.2 Humoral immune response in the ZIKV-DENV sequence: We assessed the levels of DENV IgM and IgG, and cross-reactivity with ZIKV (IgM, IgG, NS1-IgG and EDIII-IgG) at multiple timepoints. Naïve cohort showed a significant higher IgM peak characteristic of a primary DENV infection at 15 and 30 dpi that confirm the productive and acute DENV infection, while ZIKV immune groups showed lower levels (data not shown). Total DENV IgG levels of both ZIKV immune groups were significantly higher compared to naïve since baseline. Overall, ZIKVPF-10mo developed higher and long-lasting levels of DENV IgG compared to both other groups. After DENV infection, an increase was shown and remain constantly high at 15, 30, 60 and 90 dpi in both ZIKV immune groups suggesting that DENV have the potential to stimulate ZIKV-specific Ab-producing plasmablasts. To elucidate the composition of similar ZIKV IgG levels in ZIKV immune groups, we measured ZIKV-NS1 and ZIKV-EDIII IgG levels. We observed a significantly higher cross-reactive expansion and long-lasting response of ZIKV NS1-specific Abs in ZIKVPF-10mo compared to ZIKVPR-2mo and naïve (data not shown). Also, higher presence of ZIKV-EDIII IgG levels in ZIKV-10mo than ZIKVPR-2mo were observed compared to naïve at baseline (ZIKVPF-10mo only), 15, 30 and 60 suggesting that ZIKV mid convalescence promotes an expansion of ZIKV EDIII IgG Abs from ZIKV-specific memory B cells (data not shown). In summary, a boost of DENV and ZIKV Abs is triggered by the presence of ZIKV immunity and these expansion of specific- and cross-reactive Abs is higher and long lasting when a mid-convalescence to ZIKV is present. These results are consistent with the finding that B cells activation and activation and proliferation were statistically significant higher at early time points after DENV infection only in the ZIKVPF-10mo group. Then we tested the neutralization capacity of NAbs in serum from ZIKV immune and naïve animals before and after DENV infection, in order to determine whether an early- or mid-convalescence to ZIKV affect the neutralization response. Before DENV infection all groups had no detectable levels against all DENV, except ZIKV immune groups that had low cross-NAb titers against DENV-2 (fig. 7a). These cross-reactive levels were higher in ZIKVPF-10mo than ZIKVPR-2mo. The peak of high NAb titers occurred at 30 days after DENV infection for all against DENV. ZIKVPF-10mo neutralized all DENV serotypes with significant higher efficacy than Naïve group and ZIKVPR-2mo, except for DENV-2 that the elicited NAb titers were similar between both ZIKV immune groups (fig 7c). In general, the ZIKVPF-10mo neutralizing response was more long-lasting, maintaining higher NAb titers up to 90 dpi compared to ZIKVPR-2mo and naïve groups. Additionally, we tested the early neutralization capacity of all groups against the infecting serotype at day 7 pi, but no significant differences were found (data no shown). Interestingly, comparing DENV-2 NAb titers and DENV genome copies at early and late phase of RNAemia timeframe (baseline NAb titer vs 1 dpi genome copies: early phase; 7 dpi NAb titers vs 8 dpi genome copies: late phase), an inverse proportion was observed among elevated NAb levels and a subsequent lower (early phase) or no detection (late phase) of DENV genome copies (data not shown). This negative correlation (>NAb titers:<genome copies) was stronger in ZIKVPF-10mo. Therefore, this observation is consistent with the trend of ZIKVPF-10mo to develop a shorter viremic period (fig 3). Collectively, these results demonstrate that a mid-convalescence to ZIKV provoke a boost of the magnitude and durability of the neutralizing response against all DENV serotypes more effectively than an early-convalescence to ZIKV. On this application, by combining results from Aims 1 and 3, we will evaluate the contribution of the T cells, mainly CD4 helper activity, on the quantity and quality of the binding, affinity and neutralizing antibodies against ZIKV in subjects with previous immunity to DENV and combining results from Aims 2 and 3 we will

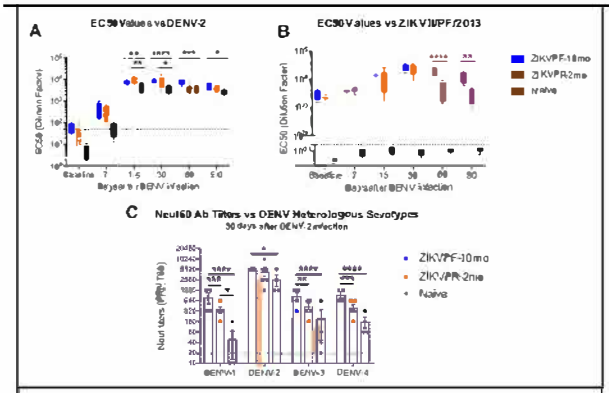


Figure 7: Characterization of the neutralizing antibodies after dengue challenge in ZIKV-immune subjects.

perform similar assessment but of the antibodies against DENV in subjects with previous immunity to ZIKV. We will characterize the quality of these antibodies, previous and after the heterologous challenge with DENV or ZIKV, by determining the predominant isotypes and the specific epitopes they target by implementing antibody depletions experiments as we described before in collaboration with Dr. Aravinda de Silva's lab and collaborator on this application (68). Overall, the experimental design on this application, will allow determining the contribution of the cellular immune response in the humoral immune component in the complex context of heterologous flavivirus infections.

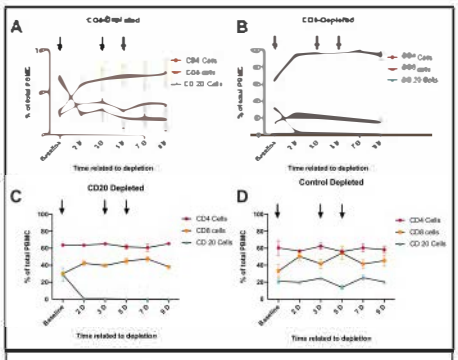


Figure 8: Dynamic of different subset cells depletion in RMs. Two animals per group were depleted of CD4+ (A) or CD8+ (B) T cells or of CD20+ (C) B cells. A control group remains undeleted (D). Depletion was very specific for each type of cells as only the target cell frequency was affected by the three doses of the MAbs. The procedure was also very efficient as in all cases depletion was over 90% compared to the basal levels. Mean of the two animals per group are presented.

C.4 Cells Depletions Experiments: For this application we will perform the depletion of the CD4 (69, 70), CD8 (71, 72) and CD20 cells (73, 74) as previously published and as it have been completed by pour group with some modifications. Figure 8 show the results of CD4 (fig 8A) or CD8 (fig 8B) or CD20 (fig 8C) cells in rhesus monkeys after one s.c administration (day 1) followed by two i.v. administration (days 3 and 7) of the monoclonal anti-CD4 CD4R1, anti-CD8 MT807R1 and anti-CD20 2B8R1 antibodies. All antibodies were obtained from Non-Human Primates Reagent Resources (http://nhpreagents.bidmc.harvard.edu). As it is showed, the depletion of a specific cell subset did not affect the frequency of the other subsets. This confirm the specificity of the depletion procedure. We will use same approach to perform the depletion experiments proposed in Aims 1, 2 and 3 of this application. Control group will receive an unrelated MAbs against Syncytial Respiratory Virus. Following our results, animals will be challenge (primary or secondary challenges) on day 10 after the depletion when the depleted cells reach their cell frequency nadir. In the example provided in figure 8, effective depletion was characterized by a mean nadir for CD4+ T-cell frequency of 18.9% (90.27% depletion from baseline), for CD8+ T-cell frequency of 0.09% (99.88% depletion from baseline) and for CD20+ B-cell frequency of 0.033% (99.86% depletion from baseline). For the experiments proposed here, we will administer higher dose of anti-CD4 T cells mAbs as described in the Vertebrate Animal's section to guarantee a severe CD4+ subset depletion (above 95% from baseline) according to previous reports (70, 75).

C.5 MHC typing of the CPRC RMs population: Since year 2016 with the support of the Wisconsin National Research Primate Center (WNPRC), we implemented the MHC typing via deep sequencing using Illumina MiSeq genotyping. The standard assay includes both class I and class II DRB sequencing and allows for an increased efficiency in genotyping nonhuman primate populations and to generate essentially a complete allele libraries. An average of 53,000 sequence reads per animal are generated. The Genetics Services Unit's custom database of rhesus macaque sequences of major class I and class II alleles is used to manually map the sequence reads, each of which was tagged with a unique barcode that referenced the animal from which it was derived, to these reference sequences and assign them to the allele they most likely represent. Highly relevant is that those "major" alleles that are defined by known haplotypes represent the vast majority of sequence reads and are assumed to initiate most of the immune responses (59). This approach allows the CPRC offering investigators very well characterized animals in support of their research projects including this application. Table 1 shown the MHC class I and II haplotypes frequencies in the CPRC's colony. This is a unique and critical contribution of this application for a better characterization of the CD4 and CD8 DENV/ZIKV specific epitopes in rhesus macaques. In spite of being the most frequent animal model used for dengue (64) and Zika studies (76), as far as we know, this type of study related to any flavivirus, has not been conducted before in macaques.

Table 1: Major histocompatibility complex (MHC) class I and Class II haplotypes frequencies

Mamu-A															
A001	A002a	A224a	A004	A057	A009	A022	A031								
0.131	0.036	0.009	0.579	0.001	0.222	0.001	0.001								
Mamu-B															
B001a	B002	B008	B012a	B012b	B015b	B017a	B024a	B028	B028'	B043a	B047a	B048	B055	B069a	B069b
0.095	0.017	0.024	0.03	0.363	0.082	0.004	0.004	0.128	0.013	0.012	0.129	0.044	0.001	0.04	0.043
DRB															
DR01a	BR01d	DR02	DR02'	DR03a	DR03f	DR04a	DR06	DR08	DR09e	DR14b	BR-U				
0.015	0.001	0.009	0.008	0.212	0.074	0.347	0.111	0.002	0.112	0.001	0.007				
DQA															
01_05_02	01_06	01g1	01g2	02g1	23_01	23_02	23_03	24g1	26g1	26g2					
0.001	0.007	0.067	0.244	0.001	0.112	0.013	0.001	0.004	0.318	0.135					
DQB															
06_01	06_06	06_09	06g2	18_02	18_10	18g1	18g2	18g3							
0.348	0.007	0.001	0.068	0.112	0.004	0.312	0.019	0.134							
DPA															
02_08	02g1	02g2	04g1	06g	07_01										
0.016	0.675	0.127	0.009	0.131	0.042										
DPB															
04g1	03g	04g	06_02	06_04	07_01	08_01	1501	19g							
0.132	0.009	0.001	0.094	0.015	0.124	0.015	0.569	0.042							

D. APPROACH AND EXPERIMENTAL DESIGN

D.1 Proposed NHP cohorts: All RM cohorts will be acquired from the CPRC and housed in the CPRC facilities at the University of Puerto Rico-Medical Sciences Campus (UPR-MSC), San Juan, Puerto Rico. These RMs with Indian genetic background are part of the purest colony of RM used in the United States for comparative medicine and biomedical research

(57). In order to address the aims of this application we intend to use 108 young adult male RM matched in age and weight divided as follow: **Aim1:** Task 1.1: Three groups of six, total 18; Task 1.2: Two groups of six, total 12; Task 1.3: Two groups of six total 12. Total animals: 42. **Aim 2:** Task 2.1: Three groups of six, total 18; Task 2.2: Two groups of six, total 12; Task 2.3: Two groups of six, total 12. Total animals: 42. **Aim 3:** Task 3.1: Four groups of six, total 24. **Total animals Aims 1-3: 108.** Specific cohorts and study design are described below under the specific Aims.

D1.1 Viral stock: Details on the animal testing and challenge are provided under the Vertebrate Animals Section.

For these experiments we will use the DENV-2 New Guinea 44 strain (kindly provided by Steve Whitehead, NIH/NIAID) and the ZIKV PRVABC59 strain, obtained through BEI Resources, NIAID, NIH, NR-50240 (Accession number KU501215.1). known to replicate well in RMs, will be used for challenge in order to obtain comparative results with previous published studies from our group on DENV and ZIKV challenge studies (8, 77, 78). We have standardized the assays to quantify this virus by Plaque assay and Focus Reduction Neutralization Test (FRNT), as described in our previous work (14). In addition, the DENV-1 Western Pacific 74, DENV-3 Sleman 73, and DENV-4 Dominique strains (kindly provided by Steve Whitehead from NIH) will be used for Plaque Reduction Neutralization Test (PRNT) assays.

D1.2 Clinical characteristics after DENV or ZIKV infection and correlates of protection.

On this application we will closely follow the potential development of clinical manifestations or laboratory abnormalities after the T or B cells depletion's experiments and after the viral challenges. Animals will be followed up on daily basis for injury and/or clinical manifestations. Complete Blood Counts (CBC) will be performed for all RMs in several timepoints (tables 2-6) to determine the absolute number (10^6 cells/ml) and percent of LYM, MON, white blood cells (WBC), neutrophils (NEU) and platelets (PLT). Also, liver profile panel will be indicated in several timepoints (tables 2-6) to measure concentration (U/L) of liver enzymes alanine and aspartate aminotransferase (ALT, AST respectively) and CPK.

D1.3 Animals MHC Typing: All animals included in this application will be MHC typed as described by our group (59) and under preliminary results (C.5). This will allow for first time to identify potential DENV and ZIKV-specific CD8 and/or CD4 restricted epitopes in macaques. Undoubtedly, these results will open a door to new approaches while designing DENV and ZIKV to be tested in NHP.

D1.4 T cells approach: Few studies have evaluated the cell-mediated immunity to dengue infection. Those studies have examined the induction of dengue-specific cytokine -producing cells from PBMC stimulated with purified DENV, NS1, NS3 or NS5 peptide pools, using ELISPOT or intracellular cytokine staining or cytotoxicity assays (79-83) (84). In general, these studies suggest that infection of NHP with DENV result in cellular immune responses that are similar to those in humans in kinetics and serotype-specificity (38, 85, 86) and suggest that NHP is an useful model to further understand the cellular response to vaccine candidates or sequential infections and their role in pathogenesis. On this application we are proposing a comprehensive approach to dissect *in vivo* the complex interaction between the cellular and humoral immune compartments and their contribution to the DENV/ZIKV or ZIKV/DENV cross-protection (fig. 9).

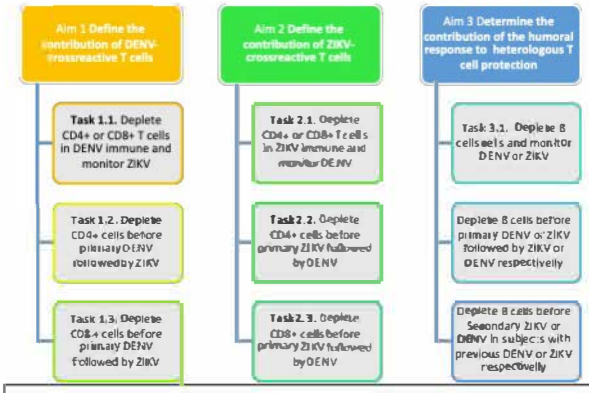


Figure 9: Overview of the Cells depletion experiments per Aims and per Tasks.

D.2 Specific Aims and Proposed Experiments

Aim 1: Characterize the contribution of DENV-crossreactive CD4+ or CD8+ T cells in the heterologous protection against ZIKV (Years 1 - 3). Rational: Recent publications using small animals (immunodeficient mice), showed that CD8+ T cells are essential for the control of ZIKV viremia and pathogenesis including control of ZIKV viremia during pregnancy (15-17). Also the protective role of CD4+ T cells against neuroinvasive ZIKV disease have been documented by our collaborators (18). Ex vivo experiments using human samples nicely showed that DENV exposure prior to ZIKV infection influences the timing, magnitude, and quality of the T cell response (87). In addition, it has been suggested that sequential immunizations for flaviviruses sharing CD4 epitopes (including DENV and ZIKV) should promote protection during a subsequent heterologous infection (88). A significant aspect leading our experimental design is the fact that the contribution of DENV-specific cytotoxic CD4+ T cells are generally detected following secondary DENV infections, these findings further support that DENV-specific cytotoxic CD4+ T cells are induced by repeated T cells Receptors stimulation from conserved DENV antigens (89, 90). If this apply to a combination of DENV followed by Zika sequential infections or vice versa it is currently unknown. With this aim we expect to provided solid evidences on the role a DENV-primed cellular immune response in the cross protection against ZIKV. For this, we will assess ZIKV replication, the clinical presentation, the serological and cytokine profiles and the cellular immune response in the presence of an intact or a blunted cellular immune response to a previous DENV infection. In **Task 1.1** we will determine the contribution of either DENV-cross reactive CD4+ or CD8+ T cells in the outcome of ZIKV infection. For this, three cohorts (cohorts 1,2 and 3) of 6 Indian origin rhesus macaques (total 18) will be infected with DENV-2. One year later all groups will be exposed to ZIKV. Previous to ZIKV infection one group will be CD4+ or CD8+ T cells depleted, and a third group will serve as control group

with intact T cells compartments (Table 2). **Task 1.2** will serve to complement the results from Task 1.1 by infecting DENV-immune animals with a blunted CD4+ T cells response, with ZIKV. Similar to previous Task 1.1, twelve (12) animals will be initially infected with DENV-2 but after being depleted of CD4+ T cells. Animals will be divided in two cohorts (cohorts 4 and 5) of 6 subjects each. After one year will be infected with ZIKV virus. However, one group will be CD4+T cells depleted again before the ZIKV infection while the other group will preserve the DENV naïve CD4+ T cells that replenished the depleted compartment after the initial DENV infection, one year earlier (Table 3). By comparing the groups with double depletion before the secondary heterologous flavivirus infection with the group with the single initial depletion, will allow for testing the contribution, if any, of the naïve CD4+ T cells that were replenish after the initial depletion one year earlier. Also results from this task will serve as a confirmation of the protective role of the CD4+ T cells cross-priming addressed under task 1.1. **Task 1.3** will be similar to Task 1.2 but animals (cohorts 6 and 7) will be CD8+ T cells depleted (Table 2)

Table 2: Schedule of events for tasks 1.1

Aim 1 (Task 1.1)																																												
Procedures	Cohorts																																											
CD4 depletion	1																																							x	x	x		
CD8 depletion	2																																							x	x	x		
Infections	1-3		DENV2																																									ZIKV*
Depletion Follow up																																								x	x	x	x	
Measurements (all cohorts)																																												
Temperature		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x		x		x		x		x		x		x		x	x	x	x	x	x					
Chemistry/Hematology		x							x				x					x															x											
Viremia				x	x	x	x	x	x	x	x	x	x		x		x		x		x		x		x		x		x		x		x											
Antibodies		x						x		x								x															x	x	x	x	x	x	x					
Neutralization		x																x															x	x	x	x	x	x	x					
Cellular Assays		x		x						x								x															x	x	x	x	x	x	x					
B Memory Cells		x		x						x								x															x						x					
Monocytes, NK and DCs		x		x	x	x				x			x					x																					x					
Cytokines		x		x	x	x				x			x					x															x						x					
Days		-30	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	60	90	150	180	270	360	-1	-3	-7	-15	0

* Heterologous challenge will observe same schedule from day 1 till day 60

By comparing the groups with double depletion before the secondary heterologous flavivirus infection with the group with the single initial depletion, will allow for testing the contribution, if any, of the naïve CD8+ T cells that were replenish after the initial depletion one year earlier. Also results from this task will serve as a confirmation of the protective role of the CD8+ T cells cross-priming addressed under task 1.1.

Table 3: Schedule of events for tasks 1.2 and 1.3

Aim 1 (Tasks 1.2 and 1.3)																																																
Procedures	Cohorts																																											Cohorts				
CD4 depletion	4,5		x	x	x																																							4	4	4		
CD8 depletion	6,7		x	x	x																																							6	6	6		
Infections	4-7						DENV2																																									ZIKV*
Depletion Follow up			x	x	x	x																																						x	x	x	x	
Measurements (all cohorts)																																																
Temperature		x						x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x		x		x		x		x		x		x	x	x	x	x	x	x					
Chemistry/Hematology		x											x				x					x															x											
Viremia								x	x	x	x	x	x	x	x	x	x		x		x		x		x		x		x		x		x		x		x											
Antibodies		x										x		x								x															x	x	x	x	x	x	x					
Neutralization		x																				x															x	x	x	x	x	x	x					
Cellular Assays		x						x						x								x															x	x	x	x	x	x	x					
B Memory Cells		x						x						x								x															x						x					
Monocytes, NK and DCs		x						x	x	x				x			x					x																					x					
Cytokines		x						x	x	x				x			x					x															x						x					
Days		-30	-1	-3	-7	-15	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	60	90	150	180	270	360	-1	-3	-7	-15	0

* Heterologous challenge will observe same schedule from day 1 till day 60

Samples collection schedule for this series of experiments are described in tables 2 and 3 above and under Vertebrate Animals section.

Experiment 1.1: DENV-ZIKV IgM, IgG and anti-NS1 IgG ELISAs. Seroreactivity to DENV will be measured at different timepoints before and after DENV challenge using commercial IgM and IgG ELISA kits (Focus Diagnostics, Cypress, CA). Also, levels of anti-DENV NS1 IgG will be tested using an in-house ELISA standardized in our laboratories. Levels of Anti-ZIKV IgM (InBios, Seattle, WA) and Anti-ZIKV NS1 IgG (Alpha Diagnostics, San Antonio, TX) will be examined using commercial kits. This serological characterization will allow us to compare the dynamic of the DENV-specific and ZIKV/DENV cross-reacting antibodies in the setting of an ablated T cell immune versus immunocompetent animals.

Experiment 1.2: ZIKV-binding ELISA. The cross-reactivity of DENV-specific Abs against ZIKV will be determined measuring the binding capacity of Abs in the sera of all cohorts before and after DENV challenge to the ZIKV whole particle using an in-house IgG-binding ELISA as we described before (8). This experiment will provide information on the specificity of the ZIKV cross-binding antibodies induced by DENV infection in the presence and absence of a CD4+ T cells immune response.

Experiment 1.3: Antibodies depletion. Still few is known about the role in the protection, if any, of the dengue and ZIKV cross-reacting antibodies. For this experiment, in collaboration with our collaborator Dr. Aravinda de Silva's group, we will perform antibodies depletion using whole dengue or ZIKV virus. Performing neutralization assays with the virion-depleted vs. control depleted sera we will determine the proportion of virus type-specific vs. cross-reactive antibodies and their contribution to protection. Furthermore, to refine the characterization of epitopes recognition we will complete similar characterization but depleting only the antibodies against the EDIII domains, as it has been demonstrated that antibodies against this domain effectively neutralize ZIKV infection (91-95). Experiments will be conducted as we have described before during previous collaboration and elsewhere (61, 96, 97) (98).

Experiment 1.4: Antibodies Isotyping. There are evidences of the role of particular IgG isotypes (IgG2), in the protection against dengue in humans and macaques (62, 99, 100). Less is known about the contribution to the protection of these

isotypes against ZIKV (94, 101). We will characterize the IgG isotypes switch at different time points after either viral infection to determine the potential role of the isotypes in the protection and cross-protection. Method will be implemented as we described before (62).

Experiment 1.5: ZIKV and DENV serotypes Plaque Reduction Neutralization Test (PRNT). The magnitude and the quality of NAbs against all four DENV serotypes and ZIKV before and after DENV challenge and after ZIKV heterologous challenge will be measured by FRNT (dengue) and PRNT (ZIKV) to dissect the behavior of the acute and convalescent neutralizing response among cohorts. Experiments will be conducted as we described before (8). This experiment will provide for the first-time, evidences on the dynamic interactions between the cellular and the humoral immune response and their impact in the quality of the neutralizing immune response after a primary infection with dengue and after a heterologous challenge with ZIKV and the contribution of the cellular immune response.

Experiment 1.6: *In vitro* DENV Antibody-Dependent Enhancement (ADE). The ability of serum Abs from DENV-immune and naïve RMs to enhance ZIKV *in vitro* and vice versa, using K-562 cells, will be performed based on a protocol that was previously described for ZIKV-ADE (102-104) with some modifications. A monoclonal Ab 4G2 conjugated to Alexa-488 (kindly provided by our collaborator Dr. Aravinda de Silva will be used for intracellular stain of DENV Envelope (E) protein and percent of infection will be determined by flow cytometry (8). As described before, ADE is a critical phenomenon blamed for the worst clinical presentations after a secondary dengue infection and it has been proposed that the same mechanism may result in an increase in the ZIKV pathogenesis. Performing this experiment in the setting of a blunted T cells immune response, will also provide the first evidences of the contribution of the T cells to the enhancement properties of the antibodies after a primary DENV or a secondary heterologous infection with ZIKV.

Experiment 1.7: DENV RNAemia. Levels of DENV and ZIKV RNAemia will be determined as describe by our group before (8) and under preliminary results. With these determinations will evaluate the contribution of the T cell immune responses (CD4+ or CD8+ T cells) controlling the viremia. Furthermore, we will be able to correlate RNAemia levels with DENV or ZIKV pathogenesis and by monitoring the clinical status of the animals as described under section D1.2.

Experiment 1.8: Phenotyping of immune cells. The use of flow cytometry (MACSQuant Analyzer 10, Miltenyi Biotec) to determine the activation frequency and magnitude of cell populations that are characteristic of the innate and adaptive response in RMs will be performed based on methods previously published (77) and optimization of the phenotyping strategy as described by our group (8, 10, 12) and showed in preliminary results (fig 4). One particular addition on this application will be the addition of the CD27 and IgD antibodies as previously described (105) to delineate naïve (CD3- CD20+ CD27- IgD+), memory (CD3- CD20+ CD27- IgD-) as well as Ki67 to identify proliferating cells. In summary, we will characterize the Monocytes (classical MON or non-classical activated MON), the Natural Killer (NK) and analyzed by the expression of NK cell markers CD16, CD8, CD56, NKG2A, NKp30, and NKp46, the dendritic cells (DCs) will be separated in two populations by the expression of CD123 (plasmacytoid DCs) or CD11c (myeloid DCs) in the HLA-DR$^+$ CD3$^-$ CD14$^-$ CD16$^-$ CD20$^-$ CD8$^-$ NKG2A$^-$ population as we previously reported (8, 10).

Experiment 1.9: Multiplex cytokine profile analysis. The cytokine profile post-DENV and ZIKV challenges will be evaluated to compare differential expression among the cohorts. A total of 32 cytokines and/or chemokines: B lymphocyte chemoattractant (BLC, CXCL13), B cell-activating factor (BAFF), eotaxin (CCL11), GROα (CXCL1), interferon alpha (IFN-α), IFN-γ, interleukin-1 beta (IL-1β), IL-1 receptor antagonist (IL-1RA), IL-2Ra, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-18, IL-22, IL-23, IFN-γ-induced protein 10 (IP-10, CXCL10), IFN-inducible T-cell alpha chemoattractant (I-TAC, CXCL11), monocyte chemoattractant protein 1 (MCP-1, CCL2), macrophage migration inhibitory factor (MIF), macrophage inflammatory protein I-alpha (MIP-1a, CCL3), MIP-1b (CCL4), perforin, regulated on activation, normal T cell expressed and secreted (RANT ES, CCL5), tumor necrosis factor-alpha and -beta (TNF-α TNF-β), and soluble CD40 ligand (sCD40L) will be measured (pg /ml^{-1}) by Luminex. This panel of cytokines showed to be comprehensive and to provide critical information on the immune response induced after the challenges. This experiment will be conducted as previously described by out group (8, 10).

Experiment 1.10: Immune cells functional response assessment. With these studies we will define the *breadth, functionality, magnitude,* and *durability* of the virus specific CD4$^+$ and CD8$^+$T cell responses post-DENV or ZIKV infection. The cellular immune response will be measured after the primary DENV or ZIKV infection to determine the DENV or ZIKV-specific CD4$^+$ and CD8$^+$T cells responses. We will then complete experiments after the secondary heterologous infection to characterize the cross-reacting CD4$^+$ and CD8$^+$T cells responses vs the naïve response. The experimental procedures used to understand the antigen specific response will be similar to procedures we have successfully performed in our previous work (8, 10). ***Breadth*:** By performing multicolor flow cytometry using methods similar to those described by Meyer et al. we will study the breadth of the virus specific immune response. Using RM PBMCs stimulated with either live virus or individual peptide pools. 1-Using live ZIKV or DENV we will be able to detect the total CD4+ or CD8+ T cell response to viral pathogen at the specific time points as we described before(8, 10). 2-To specifically target antigenic regions of the flaviviruses we also stimulate T cells with peptide pools. The peptide pools are designed as 15-mers peptides overlapping by 10 amino acids. For DENV the peptide pools will be based on amino acid sequence from the homologous DENV 2 strain New Guinea C. The peptide pools are comprising the full length of the Envelope, Capsid, NS1, NS3 or NS5 proteins creating five DENV peptide pools. Previously it has been showed that DENV-specific CD4+ and CD8+T lymphocytes target the NS1, NS3 and NS5 proteins in infected Indian rhesus macaques (84). We chose to add the Envelope

and C proteins for comparison with the ZIKV peptide stimulation because previously we demonstrated that both CD4+ and CD8+T cells shows a specific or cross-response to the Envelope-derived peptides from DENV or ZIKV (fig 2 and 5). For ZIKV a complete ZIKV peptide library based on amino acid sequences from ZIKV-PRVABC59 has previously been obtained by our collaborators, Dr. Pinto and Dr. Brien and described in Hassert, M et al. (18). The library consists of 683 15-mer peptides, overlapping by 10 amino acids spanning the entire ZIKV polyprotein. For these studies the individual pools cover ZIKV E protein, C, NS1, NS3 or NS5. ***Functionality:*** Intracellular cytokine staining of RM PBMCs will be performed by multicolor flow cytometry using methods similar to those described by Meyer et al. (106). Cytokine expression will be determined by the percent CD4+ or CD8+ cells, and then will be stained positive for the cytokine IFN-γ and TNF-α. CD107a and granzyme B expression and perforin production will be also measured in these populations to confirm functional cytotoxicity. Antigen-specific CD4+ and CD8+ T cell effector responses will be measured 7, 15, 30 dpi to identify the impact of previous DENV exposure in the ZIKV-specific functional response from these cells. For all studies the responses will be compared to the naïve animals prior to infection. After stimulation, lymphocytes will be gated based on their characteristic forward and side scatter pattern, T cells will be selected with a second gate on the CD3+ population and excluding CD20+ cells. CD8+T cells will be defined as CD3+CD20-CD8+ and CD4+T cells as CD3+CD20-CD4+ Further analysis will also be performed to examine CD28 and CD95 expression on the lymphocytes populations to determine the contribution of central and effector memory cell populations. ***Magnitude*:** PBMCs will be counted prior to start of the assay and the percentages of responding T cell populations as determined by IFN-γ will be used to identify the magnitude of the response in the individual responding NHPs following specific flavivirus infection at each timepoint. ***Durability*:** To assess the durability of the functional effector response we will compare our results from the acute responses, days 7 and 30 with the responses seen on days 60, 90, 180, 270 and 360 dpi. The peptides arrays will be obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH.

Association to MHC haplotypes: As a first approach to identify T cells epitopes restricted by the MHC in RM in the flavivirus field, we will correlate the MHC class I and II haplotypes frequencies from each single animal with the breath, the functionality and the magnitude of the CD4+ and CD8+ T cells response respectively.

Aim 2: Characterize the contribution of ZIKV-crossreactive CD4+ or CD8+ T cells in the heterologous protection against DENV (Years 2-4). Rational: Currently (January 2019) few is known about the contribution of previous ZIKV cellular immunity to DENV immune response and pathogenesis. This is highly relevant as new DENV outbreaks, in countries with recent ZIKV epidemics, it is just matter of time. One work in mice reported that ZIKV-specific and ZIKV/DENV cross-reactive epitopes showed a protective role for epitope-specific CD8+ T cells against ZIKV (17). The protective role was confirmed by CD8 T cells depletion. Another work has showed that ZIKV primed CD4+ T cells responded to homologous sequences of DENV 1-4 but that responses could confer immune deviation resulting in IL-17 an immunity often associated with exacerbated immunopathogenesis (107). Recently George *et al.*, showed that a short-term immunity to ZIKV can enhance DENV infection in rhesus macaques inducing a pro-inflammatory cytokine profile and higher peak of DENV viremia (11). However, the role of the cross primed T cells was not evaluated. On the other hand, our results show that previous ZIKV immunity can modulate the immune response against DENV but does not result in enhancement (12) (fig 3). In addition, we also showed that the ZIKV-primed T cells plays a relevant role in the modulation of the immune response to DENV, but it is unclear if the role is protective or not (figs 4,5). More interesting is that the role of the ZIKV-reactive T cells was more relevant and associated to a trend to control DENV viremia after 10 months of the primary ZIKV infection but not after two months (12)(fig 3). On this aim, we will assess DENV replication, the clinical presentation, the serological and cytokine profiles and the cellular immune response in the presence of an intact or a blunted cellular immune response to a previous ZIKV infection.

Table 4: Schedule of events for tasks 2.1

Aim 2 (Task 2.1)																																												
Procedures	Cohorts																																											
CD4 depletion	8																																							x	x	x		
CD8 depletion	9																																							x	x	x		
Infections	8-10		ZIKV																																									DENV2*
Depletion Follow up																																								x	x	x	x	
Measurements (all cohorts)																																												
Temperature		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x		x		x		x		x		x		x	x	x	x	x	x	x					
Chemistry/Hematology		x							x				x					x															x											
Viremia				x	x	x	x	x	x	x	x	x	x		x		x		x		x		x		x		x		x		x		x											
Antibodies		x						x		x								x															x	x	x	x	x	x	x					
Neutralization		x																x															x	x	x	x	x	x	x					
Cellular Assays		x		x						x								x															x	x	x	x	x	x	x					
B Memory Cells		x		x						x								x															x						x					
Monocytes, NK and DCs		x		x	x	x				x			x					x																					x					
Cytokines		x		x	x	x				x			x					x															x						x					
Days		-30	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	60	90	150	180	270	360	-1	-3	-7	-15	0

* Heterologous challenge will observe same schedule from day 1 till day 60

Tasks 2.1, 2.2 and 2.3 will use new animal groups (total 42) to reproduce the experimental design under Aim 1 but where the initial infecting agent will be ZIKV followed by DENV as secondary infection.

In **Task 2.1** we will determine the contribution of either ZIKV-cross reactive CD4+ or CD8+ T cells in the outcome of DENV infection. For this, three groups of 6 Indian origin rhesus macaques (cohorts 8, 9 and 10, total 18) will be infected with ZIKV. One year later all groups will be exposed to DENV2. Previous to DENV infection one group will be CD4+ or CD8+ T cells depleted, and a third group will serve as control group with intact T cells compartments (Table 4).

Task 2.2 will serve to complement the results from Task 2.1 by infecting ZIKV-immune animals, with a blunted CD4+ T

cells response, with DENV2. Similar to previous Task 1.1, twelve (12) animals will be initially infected with ZIKV but after being depleted of CD4+ T cells. Animals will be divided in two groups (cohorts 11 and 12) of 6 subjects each. After one year will be infected with DENV2 virus. However, one group will be CD4+Tcells depleted again before the DENV infection while the other group will preserve the ZIKV-naïve CD4+ T cells, that replenished the depleted compartment after the initial ZIKV infection, one year earlier (Table 5). By comparing the groups with double depletion before the secondary heterologous flavivirus infection with the group with the single initial depletion, will allow for testing the contribution, if any, of the naïve CD4+ T cells that were replenish after the initial depletion one year earlier. Also results from this task will serve as a confirmation of the protective role of the CD4+ T cells cross-priming addressed under task 1.1.

Task 2.3 will be similar to Task 2.2 but animals will be CD8+ T cells depleted (cohorts 13 and 14) (Table 5). By comparing the groups with double depletion before the secondary heterologous flavivirus infection with the group with the single initial depletion, will allow for testing the contribution, if any, of the naïve CD8+ T cells that were replenish after the initial depletion one year earlier. Also results from this task will serve as a confirmation of the protective role of the CD8+ T cells cross-priming addressed under task 1.1.

Table 5: Schedule of events for tasks 2.2 and 2.3.

Aim 2 (Tasks 2.2 and 2.3)																																																
Procedures	Cohorts																																												Cohorts			
CD4 depletion	11,12		x	x	x																																							11	11	11		
CD8 depletion	13,14		x	x	x																																							13	13	13		
Infections	11-14						ZIKV																																									DENV2*
Depletion Follow up			x	x	x	x																																						x	x	x	x	
Measurements (all cohorts)																																																
Temperature		x						x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x		x		x		x		x		x		x		x	x	x	x	x	x	x					
Chemistry/Hematology		x											x				x					x															x											
Viremia								x	x	x	x	x	x	x	x	x	x		x		x		x		x		x		x		x		x		x		x											
Antibodies		x										x		x								x															x	x	x	x	x	x	x					
Neutralization		x																				x															x	x	x	x	x	x	x					
Cellular Assays		x						x						x								x															x	x	x	x	x	x	x					
B Memory Cells		x						x						x								x															x						x					
Monocytes, NK and DCs		x						x	x	x				x			x					x																					x					
Cytokines		x						x	x	x				x			x					x															x						x					
Days		-30	-1	-3	-7	-15	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	60	90	150	180	270	360	-1	-3	-7	-15	0

* Heterologous challenge will observe same schedule from day 1 till day 60

Experiments on this AIM will follow the same experiments sequence (From 1.1. to 1.10) as described under Aim 1 taking in to account that the primary infecting virus will be ZIKV followed by a secondary DENV infection. Samples collection schedule for this series of experiments are described in tables 4 and 5 above and under Vertebrate Animals section.

AIM 3: To determine the net contribution of a previous cross-reactive cellular immunity, in the presence of a blunted humoral response, to the heterologous protection against ZIKV in DENV-immune subjects and vice versa (Years 3.5-5). Rational: It has been suggested that CD4+ cells are not essential for the induction of DENV-specific CD8+ T cells or antibodies but they are required to induce protection after vaccination in mice (19). The role of CD4+ T cells in combination with the IFN-γ and the antibodies showed to be essential to control ZIKV replication and neurovirulence. A switch towards IgG2 was linked to protective response (101). These results as many others on T cells cross protection (15-17, 108, 109) have been obtained in interferon deficient mice. However, both type I and II IFNs have an adjuvant effect during infection by promoting class switching (100, 110) and as consequences they have an impact in the antibody subclasses to DENV functional immune responses (62, 99, 111, 112). More caution is needed while interpreting results from those IFN-deficient mice as it has been documented, precisely in mice, that the IFNs are essential to control dengue (113-115), and ZIKV replications (116-119). Furthermore, by using samples from humans exposed to DENV, after natural infection or vaccination, it was suggested that the production of effector cytokines such as IFN-γ and IL2 by a strong T cell response, was associated with better protection against an heterologous DENV infection (120, 121) and ((116-119). A recent report using IFN-immunodeficient mice model, showed that maternally acquired ZIKV antibodies were able to enhance DENV severity in the animals (20). However, the impact of the cellular immune response in the quality of the antibodies was not examined in those works. Another work on the flavivirus serocomplex cross-reactivity immunity shown that the key capability of the CD4+ T cells providing B cell help and refining antibody responses during a secondary DENV1 infection, was associated to the enhancement of the DENV1 antibodies-binding capacity as consequences of the induction of heightened numbers of activated CD4+ TEM cells by a previous Japanese Encephalitis Virus (JEV) vaccination (88). This type of interaction has not been addressed before in the context of DENV and ZIKV cross-reactive immunity. With this aim, we do expect to complement the results from Aims 1 and 2 and to dissect the contribution of the CD4+ T cells to the quality, quantity and properties of the Abs generated after the primary and the secondary infections with DENV or ZIKV and vice versa, in the presence of an intact innate immune response (interferon-competent animals). By comparing the results from the primary versus the secondary heterologous infection will allows for weighting the protective role of the CD4+ T cells and their contribution to the functionality of the Memory B cells (MBC).

Task 3.1. We will proceed deplete B cells in the experimental groups before the primary or secondary infections with DENV or ZIKV virus. Four groups, of 6 new animals each (cohorts 15-18, total 24), will be used (Table 6). Results from this task will be compared with results from previous tasks. Experiments on this AIM will follow the same experiments sequence (From 1.1. to 1.10) as described under Aim 1 considering dengue as primary and ZIKV as secondary infection (cohorts 15 and 16) and vice versa (cohorts 17 and 18). Samples collection schedule for this series of experiments are described in table 6 and under Vertebrate Animal section.

D.2.1 Statistical Methods: Statistical analyses will be performed using GraphPad Prism 7.0 software (GraphPad Software, San Diego, CA, USA). For viral burden analysis, the log titers and levels of viral RNA were analyzed by one-way ANOVA. A p value of <0.05 indicate statistically significant difference. The statistical significance between the groups evaluated was determined using one-way analysis of variance (ANOVA) or unpaired t-test to compare the population means.

D.3 Anticipated Problems and Alternative Strategies

Table 6: Schedule of events for tasks 3.1.

Aim 3 (Task 3.1)																																																
Procedures	Cohorts																																												Cohorts			
Bcells depletion	15, 17		x	x	x																																							16, 18	16, 18	16, 18		
Infections	15, 16						DENV2																																											ZIKV*
Infections	17, 18						ZIKV																																											DENV2*
Depletion Follow up			x	x	x	x																																						x	x	x	x	
Measurements (all cohorts)																																																
Temperature		x						x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x		x		x		x		x		x		x	x	x	x	x	x	x					
Chemistry/Hematology		x											x				x					x															x											
Viremia								x	x	x	x	x	x	x	x	x	x		x		x		x		x		x		x		x		x		x		x											
Antibodies		x										x		x								x															x	x	x	x	x	x	x					
Neutralization		x																				x															x	x	x	x	x	x	x					
Cellular Assays		x						x						x								x															x	x	x	x	x	x	x					
B Memory Cells		x						x						x								x															x						x					
Monocytes, NK and DCs		x						x	x	x				x			x					x																										
Cytokines		x						x	x	x				x			x					x															x						x					
Days		-30	-1	-3	-7	-15	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	60	90	150	180	270	360	-1	-3	-7	-15	0

* Heterologous challenge will observe same schedule from day 1 till day 60

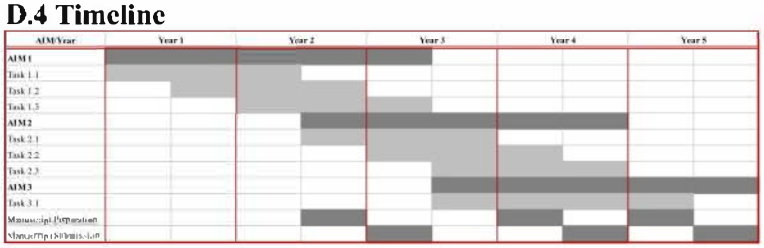
D.3.1 Natural disasters and countermeasures during the execution of these experiments: As it is widely known, on September 20, 2017 Puerto Rico was swamped by the category 5-4 major hurricane Maria. The CPRC and the Animals Resources Center (ARC) facilities were heavily impacted for this natural event. However, no animals were lost because the direct impact of the hurricane or in the aftermath. Nevertheless, a major ZIKV experiment in course was affected, as we were unable to collect samples from days 7 to 30 after the hurricane (8, 10, 23, 105, 122, 123). To mitigate this natural threat, we have modified our Standard Operation Procedures and in the eventuality of another similar devastating hurricane (which occurs 75 to 100 years apart), at least one Veterinarian and one research associated technician will stay overnight in the main building where the ARC is located. This building is very safe and with a solid construction. Located in the major Hospitals area, the main building has backup generators an enough water reserve to supply water to the ARC for at least two weeks. These services were available after the hurricane Maria. In addition, the PI on this application, on November 2018 was granted with a C06 application entitled "Beyond Hurricane Maria: Rebuilding and Renovation of the HIV/AIDS Non-Human Primates Research Facility". With this construction grant we are remodeling essential areas (NHP housing, HVAC, Flooring, etc.) to bring them to higher construction standards resistant to higher winds forces and heavy rain. If this application is granted, the essential services in support of the experiments proposed here will be available, improved and less susceptible to be impacted by such natural eventuality.

D.3.2. Limitations of the NHP model: After several years working with NHP as a model, the PI and the team involved on this application are aware that dengue infection in NHP does not results in the development of symptoms like humans. In several cases these animals respond to the infection with certain clinical manifestations and laboratory abnormalities manifestations like rash, increase in the body temperature, leukopenia, and changes in the liver enzymes (62, 64, 65, 81, 124, 125). The scenario is pretty similar after ZIKV infection, but the rash and the hematological and laboratory changes are more frequent (8, 10, 23, 105, 122, 123). However, this drawback of the model will not impact the aims of this application which are more focus in the qualitative and quantitative characterization of immune response and its correlation with the viremia and induction or not of effective dengue or ZIKV-specific neutralizing antibodies.

D.3.3. Limitations Related to Procedures and Experiments: One potential risk of this application is losing animals during the cell's depletion procedures. We have performed these procedures in the past for HIV/SIV related experiments (data no published) and for ZIKV-dengue-related experiments as described in section C.4. However, we never lost any animal because the first administration of the specific monoclonal antibodies were administered sub-cutaneous instead of i.v. to avoid anaphylactic reactions. In addition, the proper veterinarian care from experimented staff is available (see Vertebrate Animals' section). Additionally, we are planning to use 6 animals per group. In the eventuality some animals died, collecting data from at least 4 animals per group will allow to get enough data for statistical analysis (64). Furthermore, we have the institutional commitment to have access to additional animals in the case we need to add replacements.

D.4. Future Plans: Is of our interest to perform all the work proposed here but with pregnant and non-pregnant females, which are an important part of the population at risk of complications by ZIKV infection. The impact on the fetus development in the background of pre-exposition to different flaviviruses and after homo or heterogeneous secondary flaviviral infections will be evaluated. These plans will be the subject of another major application.

D.4 Timeline



PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	○ Yes ● No
Is the Project Exempt from Federal regulations?	○ Yes ○ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	○ Yes ● No
Other Requested information	

VERTEBRATE ANIMAL SECTIONS PROJECT

F. VERTEBRATE ANIMALS:

RHESUS MACAQUES

1. Animal use description.

All the research involving studies with macaques will take place at the Animal Resources Center (ARC), Caribbean Primate Research Center (CPRC), UPR, San Juan, Puerto Rico. The CPRC/ARC are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). CPRC houses approximately 1,500 rhesus macaque monkeys in Cayo Santiago and 3,900 macaques in Sabana Seca Field Station for research and breeding. Approximately 350 infants are born every year.

Species:	**Macaca mulatta**
Source:	Caribbean Primate Research Center
Age:	Juvenile (between 2 and 4 years of age)
Sex:	Male
Number:	
Aim1:	Task 1.1: Three groups of six, total 18.
	Task 1.2: Two groups of six, total 12.
	Task 1.3: Two groups of six total 12.
	Total animals: 42.
Aim 2:	Task 2.1: Three groups of six, total 18.
	Task 2.2: Two groups of six, total 12.
	Task 2.3: Two groups of six, total 12.
	Total animals: 42.
Aim 3:	Task 3.1: Four groups of six, total 24.
	Total animals Aims 1-3: 108.

However, in the eventuality we need to replace some animals, we will have access to a surplus of animals.

Serology Status:

Prior to DENV challenge all RMs will be subjected to quarantine period. All cohorts will be bled for baseline and tested for DENV and ZIKV antibodies. Other flavivirus like Yellow Fever and West Nile viruses are not circulating in Puerto Rico. However, to rule out any chance of previous flavivirus exposition, animals will also be tested for WNV and YFV before entering in to the protocol at least 30 days before entering to the protocol.

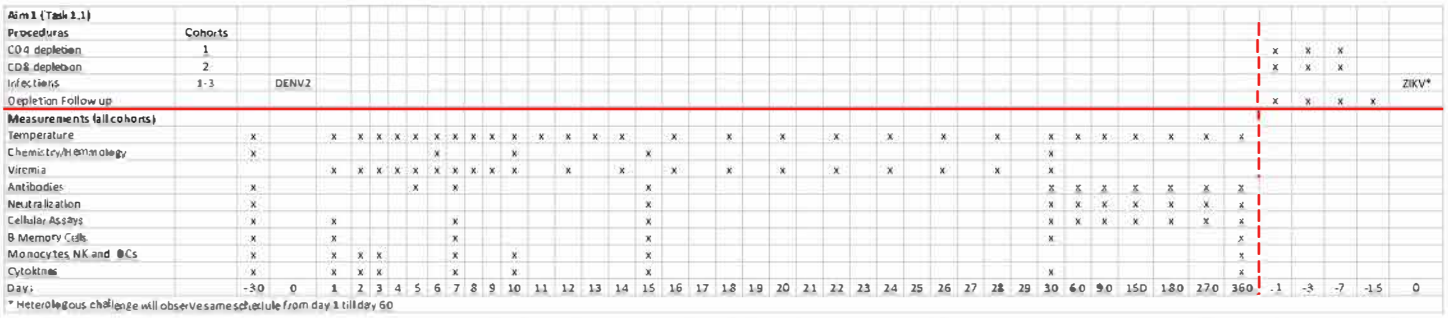
Viral Challenges:

Animals will be challenge, subcutaneously with either 5x10^5 pfu/500 ul of DENV-2 New Guinea 44 strain or 1 x 10^6 of ZIKV PRVABC59. As we described before, those doses and the route of administration proven to induce detectable viremia and immune response in these animals (8, 9). The DENV-2 New Guinea 44 (NGC) strain (kindly provided by Steve Whitehead, NIH/NIAID) and the ZIKV PRVABC59 (Accession number KU501215.1) obtained through BEI Resources, NIAID, NIH, NR-50240.

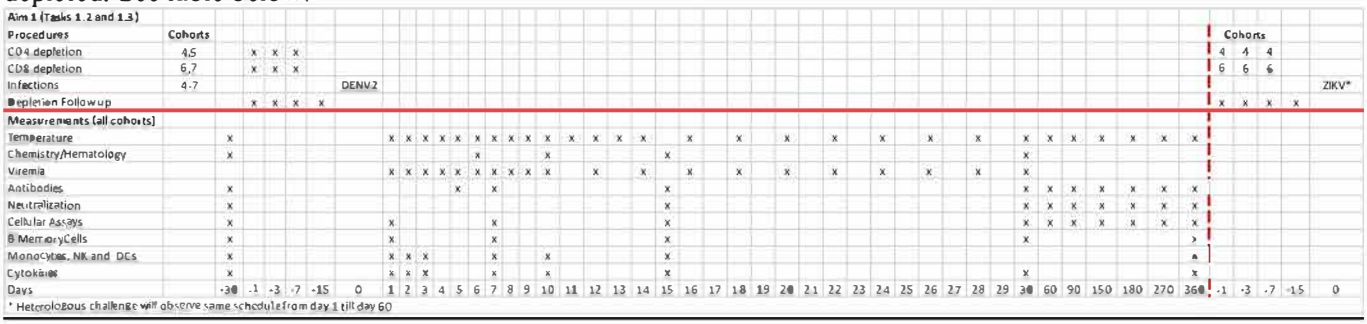
Procedures:

Aim 1, Tasks 1.1, 1,2 and 1.3

We will determine the contribution of either DENV-cross reactive CD4+ or CD8+ T cells in the outcome of ZIKV infection. For this, three cohorts (Cohorts 1,2 and 3) of 6 Indian origin rhesus macaques (total 18) will be infected with DENV-2. One year later all groups will be exposed to ZIKV. Previous to ZIKV infection one group will be CD4+ or CD8+ T Cells depleted, and a third group will serve as control group with intact T cells compartments. See details on depletion procedures and sample collection in the table below.



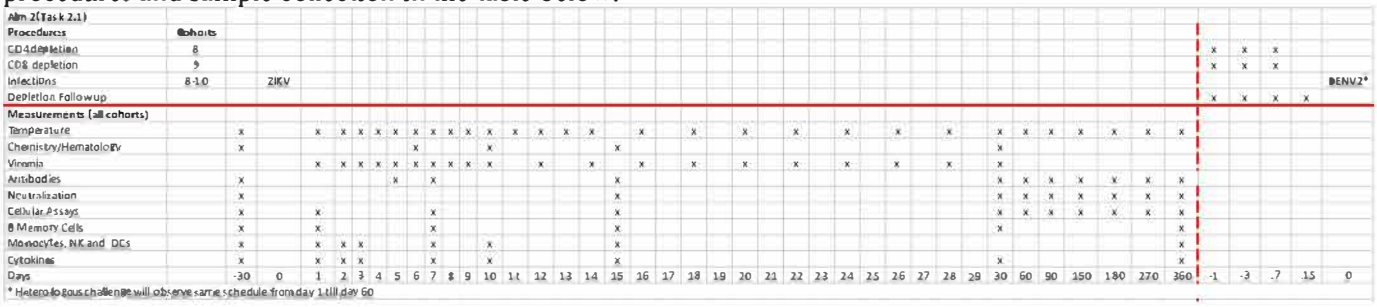
Task 1.2 will serve to complement the results from Task 1.1 by infecting DENV-immune animals with a blunted CD4+ T cells response, with ZIKV. Similar to previous Task 1.1, twelve (12) animals will be initially infected with DENV-2 but after being depleted of CD4+ T cells. Animals will be divided in two cohorts (cohorts 4 and 5) of 6 subjects each. After one year will be Infected with ZIKV virus. However, one group will be CD4+Tcells depleted again before the ZIKV infection while the other group will preserve the DENV naïve CD4+ T cells, that replenished the depleted compartment after the DENV infection, one year earlier. **Task 1.3** will be similar to Task 1.2 but animals (cohorts 6 and 7) will be CD8+ T cells depleted. See table below.



Aim 2, Tasks 2.1, 2.2, 2.3

On this aim, we will assess DENV replication, the clinical presentation, the serological and cytokine profiles and the cellular immune response in the presence of an intact or a blunted cellular immune response to a previous DENV infection.

In **Task 2.1** we will determine the contribution of either ZIKV-cross reactive CD4+ or CD8+ T cells in the outcome of DENV infection. For this, three groups of 6 Indian origin rhesus macaques (cohorts 8, 9 10, total 18) will be infected with ZIKV. One year later all groups will be exposed to DENV2. Previous to DENV infection one group will be CD4+ or CD8+ T cells depleted, and a third group will serve as control group with intact T cells compartments. See details on depletion procedures and sample collection in the table below.



Task 2.2 will serve to complement the results from Task 2.1 by infecting ZIKV-immune animals, with a blunted CD4+ T cells response, with DENV2. Similar to previous Task 1.1, twelve (12) animals will be initially infected with ZIKV but after being depleted of CD4+ T cells. Animals will be divided in two groups (cohorts 11 and 12) of 6 subjects each. After one year will be Infected with DENV2 virus. However, one group will be CD4+Tcells depleted again before the DENV infection while the other group will preserve the ZIKV-naïve CD4+ T cells, that replenished the depleted compartment after the ZIKV infection, one year earlier.

Task 2.3 will be similar to Task 2.2 but animals will be CD8+ T cells depleted (cohorts 13 and 14). See details on depletion procedures and sample collection in the table below.

Aim 2 (Tasks 2.2 and 2.3)																																																
Procedures	Cohorts																																												Cohorts			
CD4 depletion	11,12		x	x	x																																							11	11	11		
CD8 depletion	13,14		x	x	x																																							13	13	13		
Infections	11-14						ZIKV																																									DENV2*
Depletion Follow up			x	x	x	x																																						x	x	x	x	
Measurements (all cohorts)																																																
Temperature		x						x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x		x		x		x		x		x		x	x	x	x	x	x	x					
Chemistry/Hematology		x											x				x					x															x											
Viremia								x	x	x	x	x	x	x	x	x	x		x		x		x		x		x		x		x		x		x		x											
Antibodies		x										x		x								x															x	x	x	x	x	x	x					
Neutralization		x																				x															x	x	x	x	x	x	x					
Cellular Assays		x						x						x								x															x	x	x	x	x	x	x					
B Memory Cells		x						x						x								x															x						x					
Monocytes, NK and DCs		x						x	x	x				x			x					x																					x					
Cytokines		x						x	x	x				x			x					x															x						x					
Days		-30	-1	-3	-7	-15	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	60	90	150	180	270	360	-1	-3	-7	-15	0

* Heterologous challenge will observe same schedule from day 1 till day 60

Aim 3, Task 3.1

Task 3.1. We will proceed to B cells depletion in the experimental groups before the primary or secondary infections with DENV or ZIKV virus. Four groups, of 6 new animals each (cohorts 15-18, total 24), will be used (Table 6). Results from this task will be compared with the previous tasks. See details on depletion procedures and sample collection in the table below.

Aim 3 (Task 3.1)																																																
Procedures	Cohorts																																												Cohorts			
B cells depletion	15, 17		x	x	x																																							16, 18	16, 18	16, 18		
Infections	15,16						DENV2																																									ZIKV*
Infections	17, 18						ZIKV																																									DENV2*
Depletion Follow up			x	x	x	x																																						x	x	x	x	
Measurements (all cohorts)																																																
Temperature		x						x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x		x		x		x		x		x		x	x	x	x	x	x	x					
Chemistry/Hematology		x											x				x					x															x											
Viremia								x	x	x	x	x	x	x	x	x	x		x		x		x		x		x		x		x		x		x		x											
Antibodies		x										x		x								x															x	x	x	x	x	x	x					
Neutralization		x																				x															x	x	x	x	x	x	x					
Cellular Assays		x						x						x								x															x	x	x	x	x	x	x					
B Memory Cells		x						x						x								x															x						x					
Monocytes, NK and DCs		x						x	x	x				x			x					x																					x					
Cytokines		x						x	x	x				x			x					x															x						x					
Days		-30	-1	-3	-7	-15	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	60	90	150	180	270	360	-1	-3	-7	-15	0

* Heterologous challenge will observe same schedule from day 1 till day 60

Cells Depletion:

CD8 depletion will be completed by using the Anti-CD8 (MT807R1) monoclonal antibody as recommended by the Non-Human Primates Reagent Resources (http://nhpreagents.bidmc.harvard.edu) with modification according previous experience of our group (See preliminary results, section C.4.).
Concentration: 10.60 mg/ml
D1: 10 mg/kg 100 mg = 9.40 ml s.c.
D3: 5 mg/kg 50 mg =4.50 ml i.v.
D7: 5 mg/kg 50 mg =4.50 ml i.v
Total= 18.8 ml per animal

CD4 depletion will be completed by using the Anti-CD4 (CD4R1) monoclonal antibody as recommended by the Non-Human Primates Reagent Resources (http://nhpreagents.bidmc.harvard.edu) with modification according previous experience of our group (See preliminary results, section C.4.).
Concentration: 10.65 mg/ml
Dose
D1: 50 mg/kg 500 mg = 46.94 ml s.c.
D3: 10 mg/kg 50 mg =9.2 ml i.v
D7: 10 mg/kg 50 mg =9.2 ml i.v
Total= 56,14 ml per animal

CD20 depletion will be completed by using the Anti-CD20 (2B8) monoclonal antibody as recommended by the Non-Human Primates Reagent Resources (http://nhpreagents.bidmc.harvard.edu) with modification according previous experience of our group (See preliminary results, section C.4.).
Concentration: 10.2 mg/ml
Dose
D1: 20 mg/kg 200 mg = 19.6 ml s.c.
D3: 20 mg/kg 200 mg = 19.6 ml i.v.
D7: 20 mg/kg 200 mg = 19.6 ml i.v.
Total= 58,8 ml per animal

Mock depletion
Animals will receive an isotype-matched mouse-human chimeric monoclonal antibody directed against Respiratory Syncytial Virus (MedImmune, Inc., Gaithersburg, MD)
The first dose will be administered s.c. (50 mg/kg) followed by two i.v. similar dose

Bleeding:
Bleeding will be performed following the schedules provided in the tables above.
Volume of samples and type of tubes are described in the table below.

Tubes	Heparine	Red Top	EDTA	CPT
Volume*	2 ml	8 ml	2ml	**8 ml**
Procedure	Citometry	Serology	Hematology	Cellular Assay
	analysis	Neutralization		(PBMC)
		CMP		
		Viremia		
		Cytokines		
*Volume of CPT (PBMC) will be increased to 16 ml on days 30 to 360				

For all bleeding processes, blood will be obtained via the saphenous or femoral vein using 21-23 gauge vacutainer sterile needle set-up after the area is wiped with alcohol. Animals will be under short term anesthesia (Ketamine at a dose of 10-20mg/kg). Animals are monitored closely by the veterinary team until fully recovered. The blood collected will be the minimum necessary, with the maximum allowable amount of blood to be collected calculated using the formula: 6.6ml/kg every 21 days. However, when more blood is required, the possibility of additional blood collection will be assessed by the veterinarian based on the animal's health status and using procedures in which animals will be supplemented with subcutaneous fluids (30-50 mls; 0.9% saline), iron (10mg/kg), and injectable vitamins.

2. Justification for Animal Selection. Rhesus macaques are susceptible to DEN and ZIKV infection, developing viremia, some clinical and laboratory abnormalities (rash, fever, changes in liver profile, changes in the percentage of monocytes, lymphocytes and platelets) and a robust humoral and cellular immune response that can be measured in magnitude and duration. There is no alternative to the use of animals in these studies. No amount of mathematical modeling can provide the desired information on the effectiveness and efficacy of DENV vaccines. Over the past 6 years, we have learned that neutralizing Ab responses in mice and people target different epitopes on DENV. Therefore, the mouse model is not suitable for assessing the functional significance of the flaviviruses immune response. Most of the dengue vaccines in the pipeline have been tested using this animal model before going to clinical trials. There is not an in vitro model to test for this. The minimal number suggested of animals to achieve statistical significance have been set to 4-6 animals per group. To achieve statistical significance in comparing between vaccine formulations, numerous dengue vaccine researchers have reported the use of 4-6 macaques per group (1-3 PMID:12093182, PMID:17560694, PMID:19913867). A review covering key aspects on the optimal use of animals in dengue protocols have been recently published (4 PMCID 4174039). Differences in the fold increase in antibody titers will be compared using Mann-Whitney analysis. Differences in responses among groups or between two groups of animals will be determined using One-way ANOVA and differences between time points will be determined by post-hoc analysis using Tukey's multiple comparisons test. A $p < 0.05$ will be considered significant.

3. Veterinary Care. The laboratory animal care program at the Animal Resources Center (ARC), Unit of Comparative Medicine, Medical Sciences Campus, University of Puerto Rico is fully accredited by the American Association for Accreditation of Laboratory Animal Care International (AAALAC). The ARC has one full time Clinical/Research Veterinarian who provides Veterinary Care and research animal professional services. This individual is also appointed as the institutional attending veterinarian for regulatory compliance.

The ARC provides service in support of the animal care program. All major surgical procedures on nonhuman primates are performed by or under the direction of a veterinarian in dedicated aseptic surgery facilities. Diagnostic pathology support is provided by a veterinarian who is certified as a Diplomat by the American College of Veterinary Pathologists. Laboratory animal care is provided by the ARC Veterinarian and four Veterinary Technologist. Animal husbandry care and technical and administrative support is provided by a complement of trained and competent individuals. The majority of the Veterinary Technologist are certified by the American Association for Laboratory Animal Science at the Assistant Laboratory Animal Technician, Laboratory Animal Technician, or Laboratory Animal Technologist level and all have college degrees and State Animal Health Technician certification. All animals are observed at least three times a day by trained animal technicians and animal care staff. Animals are reported and treated according to accepted veterinary medical practices. Records documenting all medical procedures as well as experimental use, reproductive history, and demographic

data are maintained for the life of the animal. While on active protocols all nonhuman primates are weighted at least monthly and TB tested semi-annually. The animal husbandry program includes once daily cleaning of the waste collection pans. Cages are sanitized at least once every 2 weeks. Nonhuman primates are provided fresh water and routinely fed commercially prepared primate feed milled within the past 6 months. This feed is supplemented daily with fruit and special diets prepared onsite. Ketamine HCl - 10 - 20mg/kg will be used to induce anesthesia for all routine non-invasive clinical procedures associated with the study protocols such as blood and therapy administration as well as clinical examinations or treatment. Blood sample volumes will be limited to 6.6ml/kg of total body weight in any 21-day period of time.

The Veterinarian will be available 24/7 for any emergency and in contact with Dr. Sariol in case his intervention is required.

4. Animal Discomfort, Distress, or Pain Management. All animal handling will be performed under Ketamine anesthesia (10-20 mg/kg, i.m.) to avoid injury to our personnel and discomfort, distress, or pain to the animal. Pain management will be provided through preemptive analgesia given using Buprenorphine (.01mg/kg IM every 12 hours) or Ketoprofen (2mg/kg IM once a day) and continue for at least two more days. Animals will be monitor closely thru the serial bleeding period and supplemented with fluids, vitamins and iron as needed to minimize discomfort. Some distress to the animal will take place while the animal is briefly restrained in the cage, during ketamine administration. In addition, steps will be taken to ameliorate suffering in accordance with the recommendations of the Weatherall report, "The Use of Non-human Primates in Research".

5. Method and Decision for Euthanasia. The method for euthanasia is based on the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. We do not expect any fatal outcome during the execution of this protocol. However, animals will be necropsied in the case their disease has reached a point at which is feared that its well-being is jeopardized or that they will die unexpectedly, or as described in the protocol after IACUC approval. The necropsy will be performed at the time of sacrifice. All necropsies will be performed under a safety cabinet. Animals will be anesthetized with Ketamine (10-20mg/kg) IM. Blood and samples requested by the investigator will be obtained prior to death. As a minimum data base all animals will be bled for CBC and serum chemistry if required by the research. If extensive bleeding is to be performed a combination of Xylazine (.25mg/kg) IM and Ketamine (20mg/kg) IM will be used. An IV catheter line will be placed to establish access to the vein. If required when tissues of the Nervous System will be collected, a perfusion will be performed as follows: Under ketamine effect, most of the hair is then clipped from the body, followed by appropriate disinfections of the skin. Pentobarbital (25mg/kg) is infused to effect thru the IV catheter until the animal is deeply anesthetized (loss of corneal reflex and deep pain perception). The thorax is then opened, and the animal is perfused with a pump using PBS or LR solution or any other preservative as specified in the IACUC approved protocol. Otherwise, animals will be euthanized with Fatal Plus 1cc/10lbs IV to effect.

If necropsy is performed at any time, in animals being exposed to ZIKV, we will collect samples like spinal fluid, brain, liver and testis to assess ZIKV replication and to corelate the results with the treatment previously applied (Cells depletion).

6. Regulatory Compliance. All procedures will be reviewed and approved by the Institute's Animal Care and Use Committee at Medical Sciences Campus, University of Puerto Rico (IACUC-UPR-MSC), and according to existing regulations as described by USDA, OLAW. Procedures will be performed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Animal Welfare Assurance Number: A3421.

During the time of the protocol, animals will also be under the Environmental Enrichment program of the facility, also approved by IACUC.

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Universidad de Puerto Rico, Recinto de Ciencias Médicas
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Oficina del Rector
Chancellor's Office

January 17, 2019

Carlos A. Sariol, M.D., MS.
FACP, ASM, ASTMH
Director
Unit of Comparative Medicine
Virology Laboratory, CPRC
UPR-School of Medicine.

Reference: RFA-PA-19-056

Project Title: "DENV and Zika: Correlates of Cross-Protection in Non-Human Primates"

To whom it may Concern,

This letter confirms that the appropriate program and administrative personnel at the University of Puerto Rico Caribbean Primate Research Center are committed to support the work proposed on this application for the performance period of October 1st, 2019 to September 30, 2024.

The work to be performed by the Caribbean Primate Research Center, Medical Sciences Campus, University of Puerto Rico, includes 108 animal research subjects. The Caribbean Primate Research Center and the Animal Resources Center will support the NHP work to be performed on this application and the PI is Dr. Carlos A. Sariol. The estimated cost of the proposed subcontract will not exceed $3,497,527 and includes appropriate direct ($ 2,485,627) and indirect ($1,011,901) costs.

Furthermore, by submission of this commitment letter the Caribbean Primate Research Center, Medical Sciences Campus, University of Puerto Rico and its Principal Investigator (PI) certify (1) that the information submitted within the application is true, complete and accurate to the best of the University of Puerto Rico Caribbean Primate Research Center and PI's knowledge; (2) that any false, fictitious, or fraudulent statements or claims may subject the University of Puerto Rico Caribbean Primate Research Center and PI to criminal, civil, or administrative penalties; and (3) that the PI agrees to accept responsibility for the scientific conduct of the project and to provide the required progress reports if an award is made as a result of the GVI application.

If you have any questions, please contact the undersigned at the Deanship of Investigation, phone number 787-758-2380.

Sincerely,

Segundo Rodriguez Quillichini, MD
Chancellor
Interim Chancellor

Melween I. Martínez, DVM
Director
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CARIBBEAN PRIMATE RESEARCH CENTER
UNIVERSITY OF PUERTO RICO, MEDICAL SCIENCES CAMPUS

January 17, 2019

Carlos A. Sariol, M.D., MS.
FACP, ASM, ASTMH
Director
Unit of Comparative Medicine
Virology Laboratory, CPRC
UPR-School of Medicine.

Dear Carlos,

This is an enthusiastic letter in support of your application "DENV and Zika: Correlates of Protection in Non-Human Primates". The Caribbean Primate Research Center (CPRC) and the Animal Resources Center (ARC) are animal research facilities of the Medical Sciences Campus. Both are under the Unit of Comparative Medicine.

The CPRC is an NIH-supported facility dedicated to provide high quality rhesus macaques in support of biomedical research. The Animal Resources Center (ARC) is a unit committed to the development and support of animal – based research and resources, which will ultimately contribute to improve human health and animal welfare. It provides researchers, faculty and students with an AAALAC accredited facility, suitably equipped and staffed, for the short- and long-term maintenance of laboratory animals used in research and/or education.

Our facilities and our group have previous experience conducting several dengue and Zika experiments lead by yourself. The work you are proposing are really interesting for the novel approach in order to establish correlates of protection that can support the design of more effective dengue and Zika vaccines. In the recent past we have published and generated strong data supporting the rationale behind this experimental design.

It is our pleasure to provide the support to this application and to continue be part of this enthusiastic group of researchers. We have the capabilities to support you and your team as specified in the LOI to get as much as possible from the experiments detailed in this proposal. Looking forward to seeing this application funded,

Sincerely,

Dr. Melween I. Martinez, DVM
Director
Caribbean Primate Research Center
Animal Resources Center

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SAINT LOUIS UNIVERSITY

Department of Molecular Microbiology & Immunology

January, 9th 2019

Carlos A. Sariol, M.D., MS.
FACP, ASM, ASTMH
Director
Unit of Comparative Medicine
Virology Laboratory, CPRC
UPR-School of Medicine.
carlos.sariol1@upr.edu

Dear Carlos,

This is an enthusiastic letter in support of the R01 application "DENV and Zika: Correlates of Cross-Protection in Non-Human Primates".

The introduction of Zika virus in the Americas has changed the epidemiological concepts of flavivirus infections and opened a new era of challenges for virologists and immunologists. Before Zika we were struggling to solve the issue of an effective vaccine against the four dengue serotypes. As secondary dengue infections result in severe dengue cases, the introduction of Zika virus into dengue endemic areas has driven questions of how Zika virus infections might alter the course of dengue disease severity. Conversely, for the first time we are now facing questions of how previous immunity Zika virus may alter the clinical outcomes following a secondary dengue infection. Understanding the immunological mechanisms that take place during sequential infections with heterologous flaviviruses is mandatory to design effective vaccine against Zika and dengue.

Our highly successful ongoing collaboration has allowed us to develop and standardize the assays proposed on this application and thus far has resulted in three manuscripts. One published in *Nature Communications* in 2017, another under consideration on the same journal and third that should be submitted at the time this application is completed. I am also very excited about the preliminary results from our ongoing collaboration in NHP, showing that the period of time between dengue and Zika infections and vice versa is very important modulating the immune response to the secondary infecting virus. These novel longitudinal studies are challenging many of the concepts of immunological memory and providing insight into the potential cross protective role that the T cell component may have between those two viruses. The experiments you are proposing in this application are required to understand the correlates of protection between flaviviruses and will open the door to better understand the correlates of protection among all flaviviruses.

My group and I are very excited to continue our collaboration with you and to be part of this application. The resources and the knowledge we do have will be available in support of this work. I am very happy to continue this collaboration and I want to confirm that our resources, personnel and experience will be available to support the experiments you are proposing. Your research into the immune response after multiple flavivirus infections has been very productive, and I look forward to continuing our discussions and collaboration.

Sincerely,

Amelia K. Pinto, Ph.D.
Assistant Professor
Molecular Microbiology & Immunology
Saint Louis University

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February 1, 2019

Carlos A. Sariol, M.D., MS. FACP, ASM, ASTMH
Director, Unit of Comparative Medicine
Virology Laboratory, CPRC
UPR-School of Medicine.

Dear Carlos.

I am writing to offer my strongest support and confirm my role as a collaborator on your application entitled "DENV and Zika: Correlates of Cross-Protection in Non-Human Primates". Despite large investments in Zika research and many publications about Zika immunology and pathogenesis, we still do not understand how cross-reactive immunity between dengue and Zika viruses influence pathogenesis. It has become difficult to address this question prospectively in human epidemiological studies because Zika transmission has plummeted in Latin America. Immunodeficient mouse models widely used in the field have serious limitations and limited relevance to human disease.

The experiments you propose with the non-human primate model developed at CPRC are best suited for understanding the role of cross-reactive immunity (both cellular and humoral) and to identify correlates of protection or immune enhanced disease. The work is timely because of the profound implications of cross-protective immunity in settings where dengue vaccines are currently being evaluated or deployed.

Our group has been engaged in a fruitful collaboration with you for the last 10 years. Our collaborations have established the value of non-human primates for dissecting the fine-specificity of B cell responses as well as the remarkable similarities between the human and macaque immune responses to dengue.

My group will provide you with advice and reagents required for the studies described in this application. We will also analyze samples at UNC using antibody depletion assays and recombinant viruses widely used in our work. We will also run the antibody-dependent enhancement assays to identify cross reactive antibodies with potential for exacerbating disease.

Good luck with your application. I look forward to our continued collaboration.

Sincerely,

Aravinda de Silva, PhD, MPH
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University of North Carolina School of Medicine
Department of Microbiology & Immunology
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RESOURCE SHARING PLAN

The Caribbean Primate Research Center (CPRC) will make available to the scientific community the results for the experiment proposed on this application including the raw data if requested. Results will be available as per-review publication or as partial results in case it will be requested.
All data will be available to any research group conducting similar or related research work in order to advance the science.