PI: **Lu, Lenette**	Title: Antibody Mediated Mechanisms of Immune Modulation in Tuberculosis	
Received: 06/10/2016	FOA: PA16-191	Council: 01/2017
Competition ID: FORMS-D	FOA Title: MENTORED CLINICAL SCIENTIST RESEARCH CAREER DEVELOPMENT AWARD (PARENT K08)	
1 K08 AI130357-01	Dual: HL	Accession Number: 3944298
IPF: 4907701	Organization: MASSACHUSETTS GENERAL HOSPITAL	
Former Number:	Department:	
IRG/SRG: MID-B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted] Year 2: [redacted] Year 3: [redacted] Year 4: [redacted] Year 5:	Animals: N Humans: Y Clinical Trial: N Current HS Code: 30 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Lenette Lu	MASSACHUSETTS GENERAL HOSPITAL	PD/PI
SARAH FORTUNE M.D.	Harvard School of Public Health	Other Professional-Mentor
GALIT ALTER	MASSACHUSETTS GENERAL HOSPITAL	Other Professional-Co-Mentor
Facundo Batista	Massachusetts General Hospital	Other (Specify)-Advisory Committee
Douglas Hayden Ph.D	MASSACHUSETTS GENERAL HOSPITAL	Other (Specify)-Collaborator
Akiko Iwasaki	Yale University School of Medicine	Other (Specify)-Advisory Committee
Falk Nimmerjahn	Friedrich-Alexander University Erlangen-Nurenberg	Other (Specify)-Advisory Committee
Blanca Restrepo Ph.D	The University of Texas	Other (Specify)-Collaborator
Eric Rubin	Harvard School of Public Health	Other (Specify)-Advisory Committee

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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
GRANT12188275

5. APPLICANT INFORMATION **Organizational DUNS*:** [redacted]

Legal Name*: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL
Department: Medicine
Division: Infectious Diseases
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: First Name*: Tristienne Middle Name: Last Name*: McCarthy Suffix:
Position/Title: Senior Grant Administrator
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* M: Nonprofit with 501C3 IRS Status (Other than Institution of Higher Education)

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*
Antibody Mediated Mechanisms of Immune Modulation in Tuberculosis

12. PROPOSED PROJECT

Start Date*	Ending Date*
04/01/2017	03/31/2022

13. CONGRESSIONAL DISTRICTS OF APPLICANT
MA-008

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Lenette Middle Name: L. Last Name*: Lu Suffix:
Position/Title: Assistant in Medicine/Instructor
Organization Name*: MASSACHUSETTS GENERAL HOSPITAL
Department:
Division: Infectious Diseases
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02114-2696
Phone Number*: 216-650-1307 Fax Number: Email*: LLU0@mgh.harvard.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$[redacted]
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$[redacted]
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*: Harry Middle Name: W. Last Name*: Orf Suffix: Ph.D
Position/Title*: Senior Vice President of Research
Organization Name*: MASSACHUSETTS GENERAL HOSPITAL
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: [redacted] Email*: [redacted]

Signature of Authorized Representative*
Tristienne McCarthy

Date Signed*
06/10/2016

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:FINAL_cover_letter.pdf

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

OMB Number: 4040-0010
Expiration Date: 06/30/2016

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: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL
Duns Number: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: MA-008

Project/Performance Site Location 1

❍ 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: Harvard School of Public Health
DUNS Number: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: MA-007

File Name

Additional Location(s)

Funding Opportunity Number: PA-16-191. Received Date: 2016-06-10T09:57:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

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

IRB Approval Date:

Human Subject Assurance Number [redacted]

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

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*	FINAL_Project_Summary.pdf
8. Project Narrative*	06072016_FINAL_Project_Narrative.pdf
9. Bibliography & References Cited	06102016_Bibliography.pdf
10.Facilities & Other Resources	FINAL_Facilities_and_Resources.pdf
11.Equipment	FINAL_Equipment.pdf

Funding Opportunity Number: PA-16-191. Received Date: 2016-06-10T09:57:43.000-04:00

Project Summary/Abstract

This proposal presents a five year research career development program focused on the study of antibody mediated immune modulation in tuberculosis to expand the breadth and depth of understanding the role of humoral immunity in this disease. The candidate is currently an Instructor of Medicine at Harvard Medical School in the Division of Infectious Diseases at the Massachusetts General Hospital. The outlined proposal builds on the candidate's previous research and clinical experience in host pathogen interactions by integrating two new domains of expertise represented by her mentor team of Drs Sarah Fortune and Galit Alter at the Harvard School of Public Health and the Ragon Institute of MGH, MIT and Harvard: tuberculosis and antibody mediated mechanisms of innate immune effector functions. The proposed experiments and didactic work will position the candidate with a unique set of cross disciplinary skills that will enable her transition to independence as a physician scientist in antibody mediated host pathogen interactions in tuberculosis.

One third of the world's population carries the burden of tuberculosis (TB). Efforts to reduce this burden have been hindered by the lack effective diagnostics and a protective vaccine underpinned by gaps in the understanding of the immune response in TB disease. While cellular immunity is important, the antibody (Ab) or humoral landscape is poorly understood. Ab function is determined by the combination of antigen specificity via the Fab and ability to recruit functional responses via the Fc domain. Ab Fc mediated recruitment of cellular responses is a promising underexplored potential for immune control. The foundation for this proposal is based on preliminary studies evaluating differences in antibody profiles from a systems serological approach in a cohort of individuals with latent and active TB that suggest a potential protective role for antibodies in TB disease. How exactly antibodies might function in this context and its physiological relevance are questions that this proposal begins to address. More specifically, the aims of this proposal are 1: Define the antigen specificity of functional *M. tuberculosis (Mtb)* specific antibodies, 2: Dissect the role for Fc/FcR mediated intracellular *Mtb* restriction and 3: Identify the macrophage effector mechanisms through which Ab restrict intracellular *Mtb*. The scientific objective of this proposal is to begin to define the Ab Fab and Fc features with the capacity to mediate effector function against intracellular *Mtb* with the vision of targeted transition into an appropriate animal model to generate hypotheses that inform the direction and design of subsequent human studies to expand the repertoire for immune correlates/diagnostics and rational vaccine design.

Project Narrative

One third of the world's population carries the burden of tuberculosis. Efforts to reduce this burden have been hindered by the lack effective diagnostics and a protective vaccine underpinned by gaps in the understanding of the immune response in tuberculosis disease. The proposed research will explore the role of humoral immunity in tuberculosis disease with the vision that antibody features may be harnessed to expand the repertoire for immune correlates, diagnostics and rational vaccine design.

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Facilities and Resources

The proposed projects will be conducted at the Ragon Institute of MGH, MIT and Harvard and also the Harvard School of Public Health.

Ragon Institute of MGH, MIT and Harvard

Laboratory: The Ragon Institute of MGH, MIT and Harvard in Kendall Square, Cambridge, MA and the Section of Retroviral Therapeutics in the Partners Research Building, Cambridge, MA, together encompass more than 80,000 square feet of research and office space. Facilities include BL2+ containment tissue culture rooms with adequate laminar flow hood space along with standard tissue culture equipment including incubators, freezers, centrifuges, automated cell counters and computer access within the tissue culture room. The laboratory is specifically designed to allow the biosafety containment necessary for work with HIV positive specimens and cultivation of virus stocks at the BL2+ safety level. There is a clean room for PCR adjacent to the tissue culture room and bench space in the open lab available for molecular biology and protein work. Dr Lu has a dedicated tissue culture hood for use on this project, along with shared space in the open benches in addition to adequate space in incubators, refrigerators, -20 freezers, cold and warm room, and dedicated -80 degree and liquid nitrogen storage space.

Animal: Not applicable

Computer/Office space: Full computer support for Dr Lu is available; currently she has dedicated access to a Macintosh laptop outfitted with both a solid state hard drive for high speed processing of applications and an additional spinning hard drive with a terabyte of data storage availability. She has access to the Partners Health Care Systems Network with associated secure email service, and access to all Partners and Harvard electronic library and research resources. In addition, the Dr Lu has the necessary software for data analysis including FloJo, GraphPad, MatLab, Microsoft Office, R, Stata, Cell Profiler and Endnote. The laboratory has additional computing resources including a Partners networked computer within the BL2+ lab space for protocol access. Dr Lu has a shared office and dedicated desk as well as a phone. There is open access to fax and photocopy services, along with administrative assistance.

Core resources: Dr Lu has access to the core facilities including the flow cytometry core with 3 LSR2 flow cytometers and a Fortessa 5 laser cytometer in addition to cell sorting with the BD FACSAria High speed sorter. Additional core resources include an AutoMACS for cell separation, BioRad Bioplex 3D, Biacore, an AMNIS Imagestream, and CYTOF in addition to a fluorescence microscopy core. Additional equipment includes multiple PCR platforms including the Roche LightCycler (2) and Fluidigm. Sequencing, virus cultivation and primer synthesis are all available through the core. Illumina NextSeq system is also available. Technical support in flow cytometry and imaging is available to the Dr Lu through the core.

Biosafety Level 3 Facility: Dr Fortune is a Director of the Ragon Institute TB Program. The Ragon Institute BSL-3 laboratory is run as a core facility. This is a 2240 square foot state-of-the-art BSL-3 facility, with dedicated adjacent bench and BSL-2 space, as well as flexible office space. The BSL3 facility includes six biosafety cabinets for tissue culture, an enclosed Aria FACS instrument for live cell sorting, a dedicated microscopy room for live cell imaging with a NIKON TiPFFS3 Perfect Focus System Inverted Microscope for time lapse imaging, and a vivarium for conventional and BLT humanized mouse models. The microscope NIKON TiPFFS3 Perfect Focus System Inverted Microscope is for time lapse imaging.

Safety: The safety of the BL-2 and BL-3 laboratories is managed by a dedicated biosafety officer. All experiments conducted in the BSL3 facility at the Ragon Institute of MGH, MIT and Harvard is per institutional protocols approved by the Partners Institutional Biosafety Committee. All work is in compliance with local (Cambridge), state and federal regulations, as well as the NIH *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* and the CDC *Biosafety in Microbiological and Biomedical Laboratories* guidance document. All personnel have training covering bloodborne pathogens and biosafety, and will be required to demonstrate competency in BSL3 laboratory work before receiving approval for unescorted access. All BSL3 personnel will also be required to be medically cleared by the Partners occupational medicine department prior to being allowed to access the BSL3 laboratories.

Harvard School of Public Health

Laboratory: Dr Fortune shares ~2,000 square feet of BL-2 space in the Harvard School of Public Health with one other investigator and a BL-3 containment facility with three other investigators. The Fortune lab has the following pieces of major equipment: an Illumina MiSeq, a Applied Precision Instruments Deltavision High Precision Live Cell Imaging System, a chemostat, PCR machines, a Nanodrop, a BioRad Gel Doc, -80 degree C freezers, -20 degree C freezers, tissue and bacterial culture facilities. In addition, the laboratory shares access to an ABI Real-Time PCR system, Miltenyi Biotec flow cytometer, fluoresence-activated cell sorter, Agilent bioanalyzer, a fluorescent/luminescent plate reader, high performance liquid chromatography systems, electron microscope-scanning and transmission, confocal microscopy, autoclaving and dish washing facilities. . Dr Lu has a dedicated bench space in addition to adequate space in tissue culture hood, incubators, refrigerators, -20 freezers, cold and warm room, and dedicated -80 degree and liquid nitrogen storage space.

Animal: Not applicable

Computer/Office space: Full computer support for Dr Lu is available; currently she has dedicated access to a PC desktop outfitted with both a solid state hard drive for high speed processing of applications including image processing and an additional spinning hard drive with a terabyte of data storage availability. She has access to the Harvard Medical School Network with associated image storage and processing services, and access to all Partners and Harvard electronic library and research resources. In addition, Dr Lu has the necessary software for data analysis including FloJo, GraphPad, MatLab, Microsoft Office, R, Stata, Cell Profiler and Endnote. Dr Lu has a dedicated desk. There is open access to fax and photocopy services, along with administrative assistance.

Biosafety Level 3 Facility: The BL-3 is a ~1200 square foot facility that is currently used by four investigators for work on M. tuberculosis. The facility is divided into four procedure rooms, an equipment room, an animal holding room and an office (with computer and internet access). The facility is maintained under negative pressure by dedicated exhaust fans with redundancy to compensate for failure of one or both fans. The laboratory processes like HVAC system, vacuum line, gas lines etc are exclusive to the suite. The regular power is backed by a generator to supply emergency power. All functions are integrated into the schools operation department's mainframe and are under continuous monitoring both locally and remotely. All systems undergo annual checks and certifications. All maintenance work is done by trained and dedicated tradesmen/engineers in the Operation's department.

Security: The laboratory area is physically secured at three tier system. To enter the school you need ID (issued by university). To enter the floor you need swipe access on your ID (Granted by PI). To enter BL3 suite you need swipe access on your ID card (issued by Laboratory director/manager).

Safety: The safety of the BL-2 and BL-3 laboratories is managed by a full time, dedicated biosafety officer. All protocols are approved by the Committee on Microbiologic Safety (University's IBC) and work in the facility is overseen by a committee of users and by the BL-3 manager. All investigators undergo intensive training and testing before starting work. The training includes knowledge of pathogen/s, risk assessment, risk mitigation, PPEs, biosafety, good laboratory practices, evacuation, spill control and clean up, incident reporting and health monitoring. All new hires are registered into an occupational health program. This requires initial and annual screenings (health and specifically skin test for TB). The laboratory manager/director has more than 10 years experience of working and managing BL2 and BL3 laboratories. Most users including manager have post graduate degrees in microbiology and are active members of ASM and or ABSA.

Additional resources

-Microarray cores are available at a variety of Harvard associated facilities with associated support services: Harvard Partners Center for Genetics and Genomics/ Partners HealthCare Center for Personalized Genetic Medicine Microarray Facility, Microarray Core Facility at Dana Farber Cancer Center.

-Statistical support is available at Massachusetts General Biostatistics Core supported by Harvard Catalyst.

-High throughput imaging facilities are available at the Department of Systems Biology at Harvard Medical School, the Physical Property Measure System (PPMS) for the Laboratory of Systems Pharmacology with both live and fixed cell Operetta High-Content Imaging Systems.

-CellProfiler image analysis platform and support is available at the Broad Institute at MIT.

Equipment

Ragon Institute of MGH, MIT and Harvard

Equipment available include Laminar Flow Hoods (80), Fume Hood (6), Inverted Microscopes (13), Microcentrifuge (10) ABI 3130xl Automated Sequencer, BD FACS Calibur Flow Cytometer, BD LSR 2 Flow Cytometer (3), BD FACSAria High Speed Cell Sorter, BD Fortessa 5 laser flow cytometer, C.T.L ELISPOT reader, SUNRISE TECKAN– p24 reader (2) Nucleocounters (7), LN2 Storage Freezers (16), AMNIS Imagestream, CYTOF, Perkin Elmer Thermal Cycler (4), Tissue Culture Incubators (100), Sequencing Power Pack and Gel Apparatus, TopCount Beta Plate Reader (1), Beckman Centrifuges (12) Beckman Table top Ultracentrifuge (1), Thermo floor ultracentrifuges (3), -80oC Freezers (30), AID ELISpot Reader System, Stratagene Mx3000P qPCR machine (2) BioSource Luminex platform, MJ Research PTC-200 PCR Machine (3), LUMINEX- BIOPLEX200 system, BioRad BioPlex 3D system, ZEISS LSM510 Microscope, ZEISS MIRAX MIDI Slide Scanner, ZEISS AXIO OBSERVER Microscope, ZEISS A1 Microscope, TissueFAXS Slide Scanning Microscope, Leica RM2205 cryostat, Dako Slide hybridizer, GS Junior, Qiaxtractor, Agilent Bionanalyzer 2100, Viia7, Roche LightCycler 96 well, Roche LightCycler 384 well, Nanodrop 8000, Applied Biosystems 9800, Fluidigm.

The Ragon BSL3 facility contains an enclosed Aria FACS instrument for live cell sorting and a NIKON TiPFFS3 Perfect Focus System Inverted Microscope with Cellasic fluidic system for live cell imaging, 8 biosafety hoods, CO2 incubators, 37 ºC incubators and shakers, centrifuges, microscopes, stomachers, 2 autoclaves, an aerobiology suite with aerosol machine for mice infection, refrigerators, -80 0C freezers and a cryostat. The BSCs are certified biannually and rest of the equipment is certified annually. All equipment is backed by emergency power. There is written SOP for each equipment use and maintenance. The maintenance is performed after decontamination of the equipment.

Harvard School of Public Health

Dr. Sarah Fortune has access to the following: The BSL-2 laboratory is equipped with a fume hood, 7 biosafety hoods, CO2 incubators, a 4 ºC cold room, a –20 °C freezer room, a 37 °C warm room, refrigerated centrifuges, incubators and shakers for bacterial cultures, microscopes, electrophoretic equipment, thermocyclers, hybridization oven, UV multilinker, spectrophotometer, vacufuge, sonicator, polytron, gel and chemiluminescence documentation system, automated microarray hybridization station, microarray scanner and real-time PCR machine. In addition, the laboratory has an Illumina MiSeq, a Applied Precision Instruments Deltavision High Precision Live Cell Imaging System, a chemostat, a Nanodrop, an ABI Real- Time PCR system, Miltenyi Biotec flow cytometer, fluoresence-activated cell sorter, Agilent bioanalyzer, a fluorescent/luminescent plate reader, high performance liquid chromatography systems, electron microscope-scanning and transmission, confocal microscopy, autoclaving and dish washing facilities.

The Harvard School of Public Health BSL-3 facility contains 6 biosafety hoods, CO2 incubators, 37 ºC warm room and shakers, centrifuges, microscopes, stomachers, 2 autoclaves, an aerobiology suite with aerosol machine for mice infection, refrigerators, -80 0C freezers and a cryostat. The BSCs are certified biannually and rest of the equipment is certified annually. All equipment is backed by emergency power. There is written SOP for each equipment use and maintenance. The maintenance is performed after decontamination of the equipment.

Additional resources include:

-Microarray cores are available at a variety of Harvard associated facilities with associated support services: Harvard Partners Center for Genetics and Genomics/ Partners HealthCare Center for Personalized Genetic Medicine Microarray Facility, Microarray Core Facility at Dana Farber Cancer Center.

-Statistical support is available at Massachusetts General Biostatistics Core supported by Harvard Catalyst.

-High throughput imaging facilities are available at the Department of Systems Biology at Harvard Medical School, the Physical Property Measure System (PPMS) for the Laboratory of Systems Pharmacology with both live and fixed cell Operetta High-Content Imaging Systems.

-CellProfiler image analysis platform and support is available at the Broad Institute at MIT.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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Degree Type: Degree Year:

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Country*: [redacted]
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: E-Mail*: [redacted]

Credential, e.g., agency login:

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

Degree Type: Degree Year:

File Name
Attach Biographical Sketch*: Final_Blanca_Restrepo_Biosketch.pdf
Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: First Name*: Eric Middle Name Last Name*: Rubin Suffix:

Position/Title*: Professor of Immunology and Infectious Diseas
Organization Name*: Harvard School of Public Health
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: E-Mail*: [redacted]

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Advisory Committee

Degree Type: Degree Year:

File Name
Attach Biographical Sketch*: FINAL_Eric_Rubin_Biosketch.pdf
Attach Current & Pending Support:

Tracking Number: GRANT12189025 Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

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: Lu, Lenette

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Assistant in Medicine/Instructor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Swarthmore College, Swarthmore, Pennsylvania	BA	05/2002	Biology and Asian Studies
Case Western Reserve University School of Medicine, Cleveland, Ohio	MD	06/2010	Medicine
Case Western Reserve University School of Medicine, Cleveland, Ohio	PHD	06/2010	Molecular Virology
New York Presbyterian-Weill Cornell, New York, New York	Resident	06/2013	Internal Medicine
Massachusetts General Hospital and Brigham and Women's Hospital, Boston, Massachusetts	Fellow	07/2015	Infectious Disease clinical fellowship

A. Personal Statement

My research is focused on host-pathogen interactions, more specifically the role of humoral immunity in tuberculosis infection. My initial work as a postdoctoral fellow characterized divergent humoral profiles in patients with different disease states that hint at a protective role for antibodies. However, the mechanisms of action within macrophages for bacterial restriction and other innate immune cells remain to be elucidated. My overall research goal is to define and dissect the effector cellular pathways and immune repertoire of antibody Fc mediated protection with the vision that robust Fc features may be harnessed to expand the repertoire for immune correlates for critically needed improved diagnostics and next generation vaccine design to address the global burden of tuberculosis disease.

1. Lu LL, Chung AW, Rosebrock TR, Yu W, Ghebremichael M, Schoen MK, Mahan AE, Draghi M, Sips M, Kumar M, Tedesco J, Robinson H, Tkachenko E, Freedberg KJ, Tafesse F, Martin C, Lauffenburger D, Day C, Restrepo B, Fortune S, Alter G. Unique functional antibody profiles in active and latent tuberculosis infection. Keystone Symposia: Tuberculosis Co-Morbidities and Immunopathogenesis; 2016; Keystone, CO.

B. Positions and Honors

Positions and Employment

2010 - 2011 Intern, New York Presbyterian-Weill Cornell, New York, NY
2011 - 2013 Resident, New York Presbyterian- Weill Cornell, New York, NY
2013 - 2015 Clinical and Research Fellow, Massachusetts General Hospital and Brigham and Women's Hospital, Boston
2014 - Postdoctoral Research Fellow, Harvard School of Public Health, Boston, MA
2015 - Teachers Assistant- Medical Microbiology, graduate student section leader, Harvard University Extension School, Cambridge, MA

2015 -	Assistant in Medicine/Instructor, Massachusetts General Hospital/Harvard Medical School, Boston, MA

Other Experience and Professional Memberships

2006 - 2007	Associate Member, American Society of Virology
2008 -	Associate Member, American College of Physicians
2013 -	Member in training, Infectious Disease Society of America
2013 -	Member, Massachusetts Medical Society
2014 -	Trainee Member, American Association of Immunologists

Honors

2000	Nomination, Sigma Xi
2002	High Honors in Honors Program, Swarthmore College
2008	AAAS/Science Program for Excellence in Science, Case Western Reserve University School of Medicine
2008	ShowCASE Grand Prize Poster Award, Case Western Reserve University School of Medicine

C. Contribution to Science

1. Cell cycle regulation of DNA replication in Saccharomyces cerevisiae

   My undergraduate research under the mentorship of Elizabeth Vallen in the Biology Honors Program focused on cell cycle regulation in Saccharomyces cerevisiae, specifically protein-protein interactions of Sid2p, a protein required for DNA replication. Cyclin-dependent kinase inhibitors are important in the maintenance of genome integrity and frequently mutated in human cancers. In a screen for genes that interact with the cyclin dependent kinase inhibitor Sic1, the novel gene SID2 was identified. I helped characterize its essential functions in DNA replication and repair, specifically using field inversion gel electrophoresis to look for replication forks and bubbles in mutant strains. To assess protein-protein interactions that might elucidate mechanism, I used the yeast two hybrid system to conduct a screen looking for novel interacting partners from a library and to address specific hypotheses predicted from Saccharomyces pombe homologs. I found that although SID2 is an ortholog of more functionally characterized S. pombe genes, the specific mechanisms underlying its role in DNA replication may diverge.

   a. Jacobson MD, Muñoz CX, Knox KS, Williams BE, Lu LL, Cross FR, Vallen EA. Mutations in SID2, a novel gene in Saccharomyces cerevisiae, cause synthetic lethality with sic1 deletion and may cause a defect during S phase. Genetics. 2001 Sep;159(1):17-33. PubMed PMID: 11560884; PubMed Central PMCID: PMC1461789.

2. dsRNA Signaling in Innate Immunity and Viral Inhibition

   My graduate work under the mentorship of Ganes Sen focused on host pathogen interactions in the setting of virus infection and innate immune responses. dsRNA is a molecular pattern associated viral infections that activate transcription factors such as interferon regulatory factor 3 (IRF3) to mediate the expression of interferon stimulated genes that can prevent viral replication. To dissect the complex regulation of IRF3, I constructed a cell survival assay to identify both activators and inhibitors of signaling and found that V proteins encoded by select RNA Paramyxoviridae viruses could inhibit IRF3 activation. The mechanism underlying this phenomenon I elucidated to involve V proteins acting as alternative substrates for the IRF3 kinases inhibitor of κ B kinase epsilon (IKKe) and TANK binding kinase (TBK1). I extended this approach to the DNA viruses cytomegalovirus and murine gammaherpesvirus 68 and identified additional novel open reading frames that could inhibit IRF3 activation in a distinctly different manner. This work argues for dynamic host-pathogen relationships in the evolutionary arms race of molecular interactions that lie central to the pathogenesis of disease and

ultimately, prevention and cure.

a. Lu LL, Puri M, Horvath CM, Sen GC. Select paramyxoviral V proteins inhibit IRF3 activation by acting as alternative substrates for inhibitor of kappaB kinase epsilon (IKKe)/TBK1. J Biol Chem. 2008 May 23;283(21):14269-76. PubMed PMID: 18362155; PubMed Central PMCID: PMC2386944.

3. Clinical infectious disease

My clinical training in infectious diseases has led to several publications on clinical presentation, diagnosis and management of infections.

a. Lu LL, Weil AW, Wiederhold N, Sutton DA, Chesnut L, Lindner J, Fan H, Tingpej B, El-Khoury J, Kwon DS. Probable case of Cephalotheca foveolata bloodstream infection. JMM Case Reports. 2015 January 08;
b. Lu L, Milner D. Diagnostic Pathology: Infectious Diseases. 1st ed. Philadelphia, PA: Elsevier; 2015. Anaplasmosis and Ehrlichiosis; p.II-1-2.
c. Lu L, Milner D. Diagnostic Pathology: Infectious Diseases. Philadelphia, PA: Elsevier; 2015. Babesiosis; p.IV-1-22.
d. Pecora N, Lu L. Diagnostic Pathology: Infectious Diseases. Philadelphia, PA: Elsevier; 2015. Primary Gram-Negative Respiratory Pathogen Infections; p.II-2-40.

D. Research Support

Ongoing Research Support

2T32AI007387, NIH Kuritzkes, Daniel (PI) 08/01/90-01/07/20
MULTIDISCIPLINARY AIDS TRAINING PROGRAM
Role: TA

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: Sarah M. Fortune

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Professor of Immunology and Infectious Diseases, Harvard School of Public Health

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
Yale University	BS	1990	Biology
Columbia University College of Physicians & Surgeons	MD	1996	Medicine
Brigham & Women's Hospital, Dept. of Medicine		2000	Internship/Residency
Massachusetts General Hospital & Brigham Women's Hospital, Division of Infectious Diseases		2001	Clinical Fellowship

A. Personal Statement. My laboratory focuses on the molecular basis of population heterogeneity in *Mycobacterium tuberculosis* (*Mtb*) and the extent to which differences between mycobacterial cells contribute to differences in disease and treatment outcomes. We use high density whole genome sequencing, RNAseq and quantitative live cell imaging to define the molecular mechanisms by which *Mtb* generates diversity and how this diversity enables the bacterium to survive subsequent selective forces including antibiotics and immune selection. My work engages a broad network of collaborators including experts in technologies to assess single cell behavior at MIT and MGH, experts in sequencing methodologies at the Broad Institute and experts in human immunology at the Ragon Institute, where I am the Director of the TB Program. Lenette's K08 proposal provides a unique perspective that complements and builds on my current approaches to define the mechanisms underlying heterogeneity in tuberculosis disease and treatment outcomes.

B. Positions and Honors.

Positions and Employment

1990 – 1991 Researcher, Memorial Sloan Kettering Cancer Center, Laboratory of Dr. Robert DeLotto, New York City NY, USA
1991 – 1993 Researcher, Columbia University College of Physicians & Surgeons, Laboratory of Dr. Seth Lederman, New York City NY, USA
1996 –1997 Researcher, Wellcome Trust Clinical Research Unit, Ho Chi Minh City, Vietnam
2001 – 2006 Research Associate, Harvard School of Public Health, Boston, MA, USA
2006 – 2012 Assistant Professor of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA, USA
2011 – Present Associate Member, Broad Institute
2011 – Present Associate Member, Ragon Institute; Director of the Ragon Institute TB Program
2012 – 2015 Associate Professor of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA, USA
2015 – Present Professor of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA, USA

Honors

1989 Yale Community Service Award
1990 Phi Beta Kappa
1993 Howard Hughes Medical Student Research Fellowship

1994	Howard Hughes Medical Student Fellowship for Return to Medical Studies
1996	Alpha Omega Alpha
2001	Harvard Medical School Teaching Award for Residents in Internal Medicine
2004	The Maxwell Finland Award, Massachusetts Infectious Disease Society
2007	Howard Hughes Medical Institute Early Career Award
2007	New Innovator Award from the National Institutes of Health
2010	PopTech Science and Public Leadership Fellow
2010	Doris Duke Clinical Scientist Development Award
2012	Burroughs Wellcome Foundation Investigator in the Pathogenesis of Infectious Diseases
2012	Melvin J. and Geraldine L. Glimcher Associate Professorship of Biological Sciences
2013	Alice B. Hamilton Award, Harvard School of Public Health
2013	Diane Taylor Lecturer in Microbiology, University of Alberta

C. Contribution to Science

Mechanisms and consequences of genetic variation in Mycobacterium tuberculosis. In many infections, pathogens genetically diversify to escape clearance by the immune system or antibiotics. *Mtb* was predicted to have limited capacity for genetic diversification during infection because it replicates so slowly *in vivo*. However, *Mtb* rapidly acquires de novo mutations leading to multidrug resistance. My laboratory has elucidated both the dynamics and mechanisms of mutation *in vivo* working in both the nonhuman primate model developed by Dr. JoAnne Flynn, in human transmission chains and at the molecular level. We have shown that *Mtb* acquires genetic diversity in a time as opposed to replication dependent fashion. To understand these findings, we have sought to define the molecular determinants of both time and replication dependent mutation *Mtb*.

1. Ford CB, Lin PL, Chase MR, Shah RR, Iartchouk O, Galagan JE, Ioerger TR, Mohaideen N, Sacchettini J, Lipsitch M, Flynn JL and **Fortune SM**. Use of whole genome sequencing to estimate the mutation rate of *Mycobacterium tuberculosis* during latent infection. *Nature Genetics.* 2011 May; 43(5):482-6. Epub 2011 Apr 24. PMCID: PMC3101871.
2. Ford CB, Shah RR, Kato-Maeda M, Gagneux S, Murray MB, Cohen T, Johnston JC, Gardy J, Lipsitch M, **Fortune SM**. Mycobacterium tuberculosis mutation rate estimates from different lineages predict substantial differences in the emergence of drug resistant tuberculosis. *Nature Genetics*, Epub 2013 Jun, PMCID: PMC3777616.
3. Rock JR*, Lang UF*, Chase MR, Ford CB, Gerrick ER, Gawande R, Coscolla M, Gagneux S, **Fortune SM***, Lamers MH* (*co-first, co-corresponding authors). Replication fidelity in *Mtb* is mediated by an ancestral prokaryotic proofreader. *Nature Genetics, 2015 Apr 20. doi: 10.1038/ng.3269. PubMed PMID: 25894501.* PMCID: PMC4449270.

Mechanisms and consequences of phenotypic variation in Mtb. In eukaryotes, there are multiple epigenetic mechanisms by which heritable phenotypic diversity is maintained. It is unclear how prokaryotes create and maintain phenotypic diversity except through genetic mechanisms. My laboratory has elucidated the mechanisms and biologic relevance of both epigenetically regulated variation and high frequency variation created through asymmetric growth and division of the mycobacterial cell. Our data suggest that there are multiple mechanisms by which bacterial cells phenotypically diversify, creating phenotypically distinct subpopulations of different sizes and stabilities and with different sensitivities to drug.

1. Aldridge BB, Fernandez-Suarez M, Heller D, Ambravaneswaran V, Irimia D, Toner M, **Fortune SM**. Asymmetry and aging of mycobacterial cells leads to variable growth rates and antibiotic susceptibility, *Science,* 2012 Jan 6;335(6064):100-4. Epub 2011 Dec 15. PMCID: PMC3397429.
2. Shell SS, Prestwich EG, Baek SH, Shah RR, Sassetti CM, Dedon PC and **Fortune SM.** DNA methylation regulates gene expression and enhances hypoxic survival of *Mycobacterium tuberculosis. PLoS Pathogens*, 2013, Jul;9(7):e1003419. PMCID: PMC3701705.
3. Shell SS, Wang J, Lapierre P, Mir M, Chase MR, Pyle MM, Gawande R, Ahmad R, Sarracino DA, Ioerger TR, **Fortune SM**, Derbyshire KM, Wade JT, Gray TA. Leaderless Transcripts and Small Proteins Are Common Features of the Mycobacterial Translational Landscape. *PLoS Genetics.* 2015 Nov 4;11(11):e1005641. doi: 10.1371/journal.pgen.1005641. eCollection 2015 Nov. PMCID: PMC4633059

Within host variability in TB infection outcome: uncommon events predict clinical disease. A third of the world's population is infected with *Mtb* yet only 10% of these individuals will develop clinical disease. Here

we sought to define determinants of the variable outcome of *Mtb* infection in the nonhuman primate model, the only animal model that recapitulates the variability of human TB. In collaboration with Dr. JoAnne Flynn, who developed the macaque model, we showed that infection is highly geographic. We demonstrated that each lesion arises from a single bacterium and follow the same early course with the same extent of bacterial growth. After the onset of adaptive immunity, however, differences emerge. In all animals, most lesions are actually fully resolved. In animals that develop active TB, one site of infection progresses despite the fact that the host response is broadly able to control infection at other sites in the same animal. These findings imply that disease progression is an uncommon event and that we need to understand this rare event to understand how to protect against disease.

1. Lin PL, Ford CB, Coleman MT, Myers AJ, Gawande R, Ioerger T, Sacchettini J, **Fortune SM*,** Flynn JL* (co-corresponding authors). Sterilization of granulomas is common in both active and latent tuberculosis despite extensive within-host variability in bacterial killing. *Nature Medicine*, 2014, Jan;20(1):75-9. PMCID: PMC3947310.
2. Gideon HP, Phuah JY, Myers AJ, Bryson BD, Rodgers MA, Coleman MT, Maiello P, Rutledge T, Marino S, **Fortune S**, Kirschner DE, Lin PL, Flynn JL. Variability in tuberculosis granuloma T cell responses exists, but a balance of pro- and anti-inflammatory cytokines is associated with sterilization. *PLoS Pathogens.* 2015 Jan 22;11(1):e1004603. doi: 10.1371/journal.ppat.1004603. eCollection 2015 Jan. PMCID: PMC4303275.

Alternative paradigm for the role of the ESX1 secretion system in virulence: Many lines of evidence point to the central importance of a specialized secretion system, called ESX1, in mycobacterial virulence. It has emerged repeatedly as the critical determinant of Mtb's ability to survive in vivo. However, we do not understand its function. It has been widely speculated that ESX1 functions to secrete a pore-forming toxin. We have analyzed ESX1 function through a genetic and proteomic approaches, demonstrating that ESX1 secretory function per se is not required for virulence. Rather, we find that ESX1 has a homeostatic role in cell wall integrity and that mutants that perturb ESX1's cell wall function are attenuated for virulence, leading to an alternative paradigm for the role of ESX1 in virulence.

1. **Fortune SM**, Jaeger A, Sarracino DA, Chase MR, Sassetti CM, Sherman DR, Bloom BR and Rubin EJ. Mutually-dependent secretion of proteins required for mycobacterial virulence. *Proc Natl Acad Sci U S A*, 2005 Jul 26;102(30):10676-81. PMCID: PMC1176248.
2. Garces A, Atmakuri K, Chase MR, Woodworth JS, Krastins B, Rothchild AC, Ramsdell TL, Lopez MF, Behar SM, Sarracino DA, **Fortune SM**. EspA acts as a critical mediator of ESX1-dependent virulence in Mycobacterium tuberculosis by affecting bacterial cell wall integrity. *PLoS Pathogens*. 2010 Jun 24; 6(6):e1000957. PMCID: PMC2891827.
3. Wirth SE, Krywy JA, Aldridge B, **Fortune SM**, Fernandez-Suarez M, Gray TA, Derbyshire SM. Polar assembly and scaffolding of the virulence associated ESX-1 secretory apparatus in mycobacteria, *Mol Micro*, 2012 Feb;83(3):654-64. PMCID: PMC3277861.
4. Ramsdell TL*, Huppert LA*, Sysoeva TA, **Fortune SM***, Burton BM* (*co-first, co-corresponding authors). Linked Domain, Architectures Allow for Specialization of Function in the FtsK/SpoIIIE ATPases of ESX Secretion Systems. *J Mol Biol.* 2014 Jun 27. pii: S0022-2836(14)00306-4. doi: 10.1016/j.jmb.2014.06.013. PMCID: PMC4277743.

Translating bench science into new tools. The tools that we need to stop the TB epidemic are deceptively simple--drugs that shorten treatment, cheap and rapid diagnostics and a vaccine that blocks transmission. I have undertaken research collaborations specifically towards these goals including collaborations to develop a human-challenge model of TB, to build high-resolution molecular tools for the nonhuman primate low-dose challenge model being developed for TB vaccine testing and to develop a point-of-care diagnostic for TB.

1. Liong M, Fernandez-Suarez M, Issadore D, Min C, Tassa C, Reiner T, **Fortune SM**, Toner M, Lee H, Weissleder R. Specific pathogen detection using bioorthogonal chemistry and diagnostic magnetic resonance. *Bioconjug Chem*. 2011 Dec 21;22(12):2390-4. doi: 10.1021/bc200490r. Epub 2011 Nov 7. PubMed PMID: 22043803; PubMed Central PMCID: PMC3263317
2. Liong M, Hoang AN, Chung J, Gural N, Ford CB, Min C, Shah RR, Ahmad R, Fernandez-Suarez M, **Fortune SM**, Toner M, Lee H, Weissleder R. Magnetic barcode assay for genetic detection of pathogens. *Nature Communications,* 2013 Apr 23: 41752. PMCID: PMC3635151.
3. Ma S, Bryson BD, Sun C, **Fortune SM**, Lu C. RNA Extraction from a Mycobacterium under Ultrahigh Electric Field Intensity in a Microfluidic Device. Anal Chem. 2016 May 17;88(10):5053-7. doi: 10.1021/acs.analchem.6b00381. Epub 2016 Apr 27. PMCID: PMC4872636 [Available on 2017-04-15]

URL to complete bibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1zWMixfTHHl5z/bibliography/49099123/public/?sort=date&direction=ascending.

D. Research Support

Ongoing Research Support

[redacted]

[redacted]

R01 AI097191-04 (Derbyshire, K.) 05/15/12-04/30/17
NIH
A Community Mycobacterial Systems Resource
The major goal of this project is to study the phenotypes of Mycobacterium smegmatis mutants.
Role: Subcontract Principal Investigator

No Award No. (Walker, B.) 07/01/15-06/30/17
Ragon Institute of MGH, MIT, and Harvard
Engineering a model of transmitted M. tuberculosis
We propose to develop a droplet based system to reproducibly capture individual Mtb cells delivery as an inocula in nonhuman primate (NHP) infections.
Role: Subcontract Principal Investigator

[redacted]

[redacted]

U19 AI107774-03 (Rubin, E.) 07/02/13-06/30/18
NIH/NIAID
Decoding the roles of critical genes of unknown function in M. tuberculosis
The major goal of this project is to experimentally define the roles of genes. We will concentrate on a particularly important class: those that are required for the optimal growth of M. tuberculosis.
Role: Co-Investigator

[redacted]

[redacted]

U19 AI109755-02 (Murray, M.) 03/01/14-02/28/19
NIH/NIAID
CETR: Discovery and validation of drug resistance mutations
The goal of this project is to identify and validate which of the resistance-associated mutations are causally connected to either high-level drug resistance or an increased risk of developing resistance.
Role: Subcontract Principal Investigator

R01 AI111871-02 (Lin, P.) 03/01/14-02/28/19
NIH/NIAID
HIV-TB Co-infection: Tracking TB emergence after asymptomatic (latent) infection
The goal of this project is to use new molecular tools in the nonhuman primate model of tuberculosis to establish infection dynamics in the presence and absence of SIV infection.
Role: Subcontract Principal Investigator

P30 AI060354-12 (Walker, B.) 08/01/14-07/31/19
NIH/NIAID
HU CFAR (Center for AIDS Research)
Harvard University Center for AIDS Research: Core B Developmental Core
The goal of this Core is to promote HIV/AIDS research activities at Harvard University.
Role: Subcontract Principal Investigator

R01 AI114674-01 (Fortune, S. and Flynn, J.) 07/01/15-12/31/19
NIH/NIAID
The Consequences of Reinfection with M. tuberculosis
The Fortune lab will be responsible for the proposed studies to generate tagged libraries of various M. tuberculosis strains as proposed in Aims 1 and 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: Alter, Galit

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Associate Professor in 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
McGill University, Montreal, Canada	BSc	05/1999	Microbiology and Immunology
McGill University, Montreal, Canada	PhD	05/2003	Experimental Medicine
Research Fellow, Massachusetts General Hospital	Post-doctorate	02/2007	Partners AIDS Research Center

A. Personal Statement

Galit Alter received her PhD in experimental medicine from McGill University, and is currently an Associate Professor in Medicine at Harvard Medical School and a faculty member at the Ragon Institute of MGH, MIT, and Harvard. Over the past 8 years her research has focused on understanding the role of the innate immune response to chronic viral infections, with a focus on defining the role of Natural Killer cells in antiviral control. Recently, these studies focused on defining the mechanism by which these innate immune effector cells may be harnessed through vaccination or immunotherapeutic strategies. Her current research interests lie at the intersection of the innate immune response and the adaptive humoral immune response, defining the role of innate immune recruiting antibodies in providing specificity to kill virally infected cells. In this capacity, Dr. Alter has developed a suite of antibody profiling assays that gain a deeper appreciation of the correlates of humoral immune activity. Most recently, Dr. Alter's team adapted the antibody screening technologies to screen for potential antibody correlates of protective immunity against *Mycobacterium tuberculosis*, demonstrating for the first time the presence of highly divergent Fc-effector profiles among latently infected compared to actively infected subjects. Thus, linked to antigen-specific B cell profiling efforts, Dr. Alter's laboratory seeks to contribute to define the potential role of functional antibodies in the control/clearance of Mtb with the goal of developing more effective diagnostics, therapeutics as well as provide insights for the design of next generation Mtb vaccines. Thus, Dr Alter is uniquely suited as a co-mentor in Dr. Lu's K08 application.

B. Positions and Honors

Positions

2007-2008 Instructor in Medicine, Harvard University, Boston, MA.
2008-2012 Assistant Professor in Medicine, Harvard Medical School, Boston, MA.
2012-present Associate Professor in Medicine, Harvard Medical School, MA

Fellowships

2005 Fellowship Award - MGH fellowship for Medical Discovery
2005 Fellowship Scholar Award - Harvard Medical School Center for AIDS Research
2006 K99/R00 Path to Independence, NIH.

Awards

2007 Merck Young Scientist Award, Philadelphia, PA.
2009 Duke Young Scientist CFAR Award

2012 Krane Award, MGH
2012 MGH Scholars Award, MGH

C. Contribution to Science

1. **Defining a role for NK cells in HIV control**: Dr. Alter's early post-doctoral studies focused on the role of innate immune cells in the control of HIV infection, and pointed to a critical role of NK cells in HIV specific control in the setting of protective KIR/HLA genotypes. Furthermore, these studies led to the elucidation of NK cell driven footprints along the HIV genome, highlighting the direct antiviral effect that these innate immune killer cells.

a. **Alter G**, Martin MP, Teigen N, Carr WH, Suscovich TJ, Schneidewind A, Streeck H, Waring M, Meier A, Brander C, Lifson JD, Allen TM, Carrington M, Altfeld M. Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes. J Exp Med. 2007 Nov 26;204(12):3027-36. PMCID: PMC2118524
b. Luteijn R, Sciaranghella G, van Lunzen J, Nolting A, Dugast AS, Ghebremichael MS, Altfeld M, **Alter G**. Early viral replication in lymph nodes provides HIV with a means by which to escape NK-cell-mediated control. Eur J Immunol. 2011 Sep;41(9):2729-40. doi: 10.1002/eji.201040886. Epub 2011 Aug 12. PMID: 21630248
c. **Alter G**, Heckerman D, Schneidewind A, Fadda L, Kadie CM, Carlson JM, Oniangue-Ndza C, Martin M, Li B, Khakoo SI, Carrington M, Allen TM, Altfeld M. HIV-1 adaptation to NK-cell-mediated immune pressure. Nature. 2011 Aug 3;476(7358):96-100. doi: 10.1038/nature10237. PMCID: PMC3194000
d. Sips M, Sciaranghella G, Diefenbach T, Dugast AS, Berger CT, Liu Q, Kwon D, Ghebremichael M, Estes JD, Carrington M, Martin JN, Deeks SG, Hunt PW, **Alter G**. Altered distribution of mucosal NK cells during HIV infection. Mucosal Immunol. 2011 Oct 12. doi: 10.1038/mi.2011.40. PMCID: PMC3740353
e. Pelak K, Need AC, Fellay J, Shianna KV, Feng S, Urban TJ, Ge D, De Luca A, Martinez-Picado J, Wolinsky SM, Martinson JJ, Jamieson BD, Bream JH, Martin MP, Borrow P, Letvin NL, McMichael AJ, Haynes BF, Telenti A, Carrington M, Goldstein DB, **Alter G**; on behalf of NIAID Center for HIV/AIDS Vaccine Immunology (CHAVI). Copy Number Variation of KIR Genes Influences HIV-1 Control. PLoS Biol. 2011 Nov;9(11):e1001208. Epub 2011 Nov 29. PMCID: PMC3226550

2. **Delineating the evolution of functional antibodies against HIV**: While a lucky subset of HIV-infected individuals benefit from the direct antiviral activity of NK cells, through the expression of particular NK cell receptor genotypes, strategies to harness the antiviral activity of NK cells more broadly could enhance antiviral control of HIV at a global level. As NK cells express Fc-receptors, the next phase of Dr. Alter's research focused on exploring the role of antibody dependent cellular cytotoxicity (ADCC) in providing specificity to NK cells to kill HIV infected cells, which enabled her to develop a unique set of expertise and a novel area of research in the setting of non-neutralizing antibody biology.

a. Liu Q, Sun Y, Rihn S, Nolting A, Tsoukas PN, Jost S, Cohen K, Walker B, **Alter G**. Matrix Metalloprotease inhibitors restore impaired NK cell mediated Antibody dependent cellular cytotoxicity in human immunodeficiency virus-1 infection. J Virol. 2009 Jun 24. PMCID: PMC2738177
b. Dugast AS, Tonelli A, Berger CT, Ackerman ME, Sciaranghella G, Liu Q, Sips M, Toth I, Piechocka-Trocha A, Ghebremichael M, **Alter G**. Decreased Fc receptor expression on innate immune cells is associated with impaired antibody-mediated cellular phagocytic activity in chronically HIV-1 infected individuals. Virology. 2011 Jul 5;415(2):160-7. Epub 2011 May 12. PMCID: PMC3112178
c. Dugast AS, Stamatatos L, Suscovich TJ, Licht AF, Mikell I, Ackerman ME, Streeck H, Klasse PJ, Moore JP, **Alter G** Independent evolution of Fc- and Fab-mediated HIV-1-specific antiviral antibody activity begins during acute infection. European Journal of Immunology. 2014 Oct;44 (10):2925-37

3. **Defining the profile of protective antibodies against HIV**: Beyond ADCC, antibodies are able to recruit a wider array of antiviral functions. Thus in an effort to gain a broader appreciation for the full spectrum of antiviral functions of antibodies, Dr. Alter has developed a comprehensive platform of high throughput assays that interrogate the functional profile of antibodies against any antigen of interest. In these studies, Dr. Alter was able to identify a novel correlate of protection against infection in the first moderately protective HIV vaccine trial, RV144, as well as apply these methodologies to non-human primate vaccine and protection studies.

a. Ackerman ME, Dugast AS, McAndrew EG, Tsoukas S, Licht AF, Irvine DJ, **Alter G**. Enhanced phagocytic activity of HIV-specific antibodies correlates with natural production of immunoglobulins with skewed affinity for FcγR2a and FcγR2b. J Virol. 2013 Mar 6. PMCID: PMC3648186
b. Barouch DH, Stephenson KE, Borducchi EN, Smith K, Stanley K, McNally AG, Liu J, Abbink P, Maxfield LF, Seaman MS, Dugast AS, **Alter G**, Ferguson M, Li W, Earl PL, Moss B, Giorgi EE, Szinger JJ, Eller LA, Billings EA, Rao M, Tovanabutra S, Sanders-Buell E, Weijtens M, Pau MG, Schuitemaker H, Robb ML, Kim JH, Korber BT, Michael NL. Protective efficacy of global HIV-1 mosaic vaccine against heterologous SHIV challenges in rhesus monkeys. Cell. 2013 October 24. 155(3):531-9.
c. Chung AW, Ghebremichael M, Robinson H, Brown E, Choi I, Lane S, Dugast AS, Schoen MK, Rolland M, Suscovich TJ, Mahan AE, Liao L, Streeck H, Andrews C, Rerks-Ngarm S, Nitayaphan S, de Souza MS, Kaewkungwal J, Pitisuttithum P, Francis D, Michael NL, Kim JH, Bailey-Kellogg C, Ackerman ME, **Alter G** (2014) Polyfunctional Fc-effector profiles mediated by IgG subclass selection distinguish RV144 and VAX003 vaccines. Sci Transl Med 6: 228-238.
d. Dugast AS, Chan Y, Hoffner M, Licht A, Nkolola J, Li H, Streeck H, Suscovich TJ, Ghebremichael M, Ackerman ME, Barouch DH, **Alter G** (2014) Lack of Protection following Passive Transfer of Polyclonal Highly Functional Low-Dose Non-Neutralizing Antibodies. PLoS One 9: e97229.

4. **Exploring antibody glycosylation as a means to control antiviral antibody activity**: Beyond subclass variation, alterations in antibody glycosylation within the Fc-region results in a rapid change in the affinity of the antibody for Fc-receptors and therefore altered Fc-effector functions. Along these lines, Dr. Alter developed a high throughput method to interrogate these alterations on antigen-specific antibodies and performed the first studies to define the role of altered antibody glycosylation in natural HIV control.

a. Ackerman ME, Crispin M, Yu X, Baruah K, Boesch AW, Harvey DJ, Dugast AS, Heizen EL, Ercan A, Choi I, Streeck H, Nigrovic PA, Bailey-Kellogg C, Scanlan C, **Alter G**. Natural variation in Fc glycosylation of HIV-specific antibodies impacts antiviral activity. J Clin Invest. 3013 Apr 8 doi:pii: 65708. 10.1172/JCI65708. PMCID: PMC3637034
b. Mahan AE, Tedesco J, Dionne K, Baruah K, Cheng HD, De Jager PL, Barouch DH, Suscovich T, Ackerman M, Crispin M, **Alter G**. A method for high-throughput, sensitive analysis of IgG Fc and Fab glycosylation by capillary electrophoresis. J Immunol Methods. 2015 Feb;417:34-44. doi: 10.1016/j.jim.2014.12.004. Epub 2014 Dec 15. PMID: 25523925
c. Mahan AE, Jennewein MF, Suscovich T, Dionne K, Tedesco J, Chung AW, Streeck H, Pau M, Schuitemaker H, Francis D, Fast P, Laufer D, Walker BD, Baden L, Barouch DH, **Alter G**. Antigen-Specific Antibody Glycosylation Is Regulated via Vaccination. PLoS Pathog. 2016 Mar. PMID: 26982805

5. **Developing next generation monoclonal therapeutic strategies for HIV "cure":** The cancer monoclonal therapeutics field has explored the role of improving antibody Fc-effector functions extensively to improve treatment outcomes. Thus Dr. Alter's most recent work aims to harness the antiviral power of the Fc-region of antibodies to gain more efficient control of HIV and drive reservoir eradication.

a. Chung AW, Robinson H, Crispin M, Gorny MK, Bailey-Kellog C, Ackerman ME, Burton DR, Scanlan C, Zolla-Pazner S, **Alter G** Identification of antibody glycosylation structures that predict monoclonal antibody Fc-effector function. AIDS. 2014 Aug 26.
b. Euler Z, **Alter G**. Exploring the Potential of Monoclonal Antibody Therapeutics for HIV-1 Eradication. AIDS Res Hum Retroviruses. 2015 Jan;31(1):13-24. PMID: 25385703

Complete list of published work in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/41145215/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

5 P30 AI060354-11 (Head of CFAR Core Immunology Core) 08/01/2014-07/31/2019
NIH/NIAID
Harvard Medical School Center for AIDS Research

The major goals of this project are to 1) to consolidate and expand existing collaborations among the members of two existing, highly successful HMS-affiliated CFARs and 2) to promote new interactions and research initiatives capable of more effectively addressing key AIDS research questions. Dr. Alter will serve as the Head of the CFAR Immunology Core at MGH.

R01 AI102660-03 (Alter) 07/01/12-06/30/16
NIH
Tuning Fc-effector functions of HIV-specific antibodies
This proposal aims to define the mechanim(s) regulating antibody glycosylation in B cells. These studies may identify new strategies to specifically tune B cell responses during vaccination to induce antibodies with specific antiviral activity.

1 R21 AI110165-01 (Alter) 12/01/2013-11/30/2018
NIH
Killing the Reservoir with Antibodies
The goal of this award if to develop a monoclonal therapeutic approach, using Fc-enhanced antibodies, to kill reactivated latently infected cells.

2R01AI080289-06A1 (Alter) 09/01/2014 – 08/31/2019
NIH/NIAID
Demystifying the antiviral activity of the IgG3+ antibody response
The goal of this project is to explore and define the specificity/functionality of IgG3+ B cell responses and the mechanism, by which the immune system programs such potent antiviral humoral immunity.

Completed Research Support

2U19 A166345 (Lauer) 08/01/2010-07/31/2015
NIH
Immune Control and Evasion during Acute HIV Infection – Project 2
This project aims to determine the role of NK cells in acute HCV infection, providing new potential therapeutic avenues to enhance the activity of particular NK cell populations to drive HCV clearance.
Project Leader

R01 AI080289 (Alter) 04/01/2009-03/31/2015
NIH/NIAID
Cytolytic Antibodies, Bridging the Gap Between Innate and Adaptive Immune Responses
This grant aims to define the role and mechanism of antibody mediated cellular cytotoxicity in the natural control of HIV-1 infection.

5 P30 AI060354-10 (Walker) 08/01/2009-07/31/2014
NIH/NIAID
Harvard Medical School Center for AIDS Research
The major goals of this project are to 1) to consolidate and expand existing collaborations among the members of two existing, highly successful HMS-affiliated CFARs and 2) to promote new interactions and research initiatives capable of more effectively addressing key AIDS research questions. Dr. Alter will serve as the Head of the CFAR Immunology Core at MGH.

NIH R01 AI 90867-04 (Pillai) 07/01/2010-05/31/2014
Inducing Neutralizing antibodies to HIV by inhibiting SIAE
This project aims to examine assays for humoral responses and assays for neutralizing antibodies in immunized *Siae* mutant mice and in *Siae* knockdown humanized mice.

R01 AI080289 Supplement (Alter) 09/01/2010-03/31/2014
NIH/NIAID
Cytolytic Antibodies, Bridging the gap between innate & adaptive immune responses
This project is geared towards defining whether a combined FcR/Lectin microarray can provide a high-throughput, cost-effective alternative to readout the innate immune recruiting properties of antibodies.

1R56 AI095078-01 (Alter) 09/20/2011-08/31/2012
NIH/NIAID
The Innate Immune Signals on B Cells that Induce ADCC Antibodies
In this proposal we intend to dissect the innate immune danger signals, elicited by pattern recognition receptors, on B cells that drive the induction of innate immune recruiting antibodies, such as those that mediate ADCC, that may be critical for antiviral control of HIV infection.

R01 AI090866-02 (Sundberg) 07/01/2010-6/30/2012
NIH/University of Maryland
Molecular Basis of ADCC-mediated HIV Protection
This project aims to examine ADCC functional assays on patient samples as well as recombinant antibodies. The assays include NK cell degranulation assays and Antibody dependent cellular viral inhibition assays.

3R01 AI080289-02S1 (Alter) 05/24/2010-04/30/2012
NIH/NIAID
Cytolytic Antibodies, Bridging the Gap Between Innate and Adaptive Immune Responses
This project is geared towards defining the potential role of innate immune recruiting antibodies in providing sterilizing protection in the peripheral circulation or mucosa of highly exposed men who resist HIV infection despite persistent exposure to virus.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

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: BATISTA, Facundo

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

POSITION TITLE: Associate Director of the Ragon Institute; Professor, Harvard Medical School

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 Buenos Aires, Argentina	BSc	1989–1991	Biology
International School of Advance Studies, Italy	MSc	1992–1993	Biology
International School of Advance Studies, Italy	PhD	1993–1995	Biology
International Centre for Genetic Engineering and Biotechnology, Italy	Postdoctoral Fellow	1995–1996	Immunology
MRC Molecular Biology Laboratory, Cambridge University, UK	Senior Postdoctoral EMBO Fellow	1996–2002	Immunology

A. Personal Statement

Vaccination is an important tool in the medical armamentarium and relies on the in vivo production of effective antibodies by B cells. The effectiveness of antibodies is known to depend on the affinity of the B cell receptor for its cognate antigen. My long-term research interests have been the study of B cell activation, including the role of Toll-like receptors in antigen presentation. In the work accomplished in the Lymphocyte Interaction Laboratory, I have sought to address fundamental questions concerning the molecular and cellular events underlying B cell activation using a range of techniques including cutting-edge imaging, genetic dissections and in vivo methodology. These techniques and approaches will complement Lenette Lu's K08 proposal specifically in terms of imaging and cell biology.

B. Positions and Honors

Positions

2002-present	Honorary Professor	Immunology	University College London
2013-2015	Professor	Medicine	Imperial College London
2014-present	Honorary Professor	Immunology	Kings College London
2016-present	Professor	Microbiology & Immunobiology	Harvard Medical School
2016-present	Professor	Medicine	Harvard Medical School

2002–2016 Group Leader, Lymphocyte Interaction Laboratory, Cancer Research UK, London Research Institute, UK
1996–2001 Senior Postdoctoral Fellow, MRC Laboratory of Molecular Biology, Cambridge University, UK
1992–1995 PhD Student, International Centre for Genetic Engineering and Biotechnology, Italy
1989–1991 Undergraduate Research Student, University of Buenos Aires, Argentina

Honors

Fellow of the Academy of Medical Sciences (2013)
EMBO Member (2009)
The Royal Society Wolfson Research Merit Award (2009)
EMBO Young Investigator Award (2004)

Arthritis Research Campaign, Project Grant (1999)
EMBO Long Term Postdoctoral Fellowship (1996–1997)
Cancer Research Institute Fellowship (1995)
UNIDO-International Centre for Genetic Engineering and Biotechnology Fellowship (1993–1995)
Ministero degli Affari Esteri of Italy Fellowship (1991–92)
University of Buenos Aires, Undergraduate Research Fellowship (1989–91)

C. Contribution to Science

1. Investigating B cell activation in vivo

B cell activation occurs in SLOs, including lymph nodes and the spleen, locations where the architecture maximizes the probability of a lymphocyte to encounter its cognate antigen. However, the exact location of these interactions within the SLOs was unknown. In vivo investigations were needed to probe the highly dynamic nature of these immune interactions, contrasting with the previous investigations using immunohistochemistry and electron microscopy. Using fluorescent particles or bacteria we observed that after administration, antigen localized in the subcapsular sinus (SCS) of the periphery of the draining lymph node. Moreover, using multiple approaches including adoptive transfer, immunohistochemistry and multi-photon microscopy (MPM) we visualized the dynamic distribution of B cells to find that that antigen-specific B cells accumulate and are retained in close-proximity to CD169+ macrophages in the SCS (Fig 1). During this time cognate B cells acquire antigen and within 24 hours become activated and migrate to the B—T cell boundary to present antigen to T cells. Furthermore, the SCS macrophages were shown by us in collaboration with Vincenzo Cerundolo in Oxford to be implicated in initiating innate immune responses, specifically in natural killer T cells (NKT), where the SCS macrophages appear to internalize and present lipid antigen to the NKT cells. To facilitate our in vivo work, we developed an innovative strategy involving direct conjugation of antigen with various immuno-stimulatory ligands. This approach not only revealed a role for iNKT cells and B-cell intrinsic TLR9 in providing assistance for B cell activation, but also has enormous potential clinical significance in terms of designing vaccination strategies and generating monoclonal antibodies (Patent Application WO PCT/GB2009/001111). The approach attracted external investment and the formation of the spinout company BliNK Therapeutics Ltd.
In vivo investigations into the effects of infection on the immune system revealed that the architecture of the lymph node, critical in bringing antigen and B cells together, is altered on primary infection. We found that the SCS macrophage layer being temporarily disrupted, leading to a reduction in immune responses against subsequent challenge until such time as the lymph node architecture is restored. These results may go some way towards providing a mechanism for the increased susceptibility to secondary pathogens that is often seen in clinical practice.

(a) Barral, P., Eckl-Dorna, J., Harwood, N. E. *et al* 2008. B cell receptor-mediated uptake of CD1d-restricted antigen augments antibody responses by recruiting invariant NKT cell help in vivo. *Proceedings of the National Academy of Sciences.*
(b) Eckl-Dorna, J. & Batista, F. D. 2009. BCR-mediated uptake of antigen linked to TLR9 ligand stimulates B-cell proliferation and antigen-specific plasma cell formation. *Blood.*
(c) Barral, P., Polzella, P., Bruckbauer, A. *et al* 2010. CD169(+) macrophages present lipid antigens to mediate early activation of iNKT cells in lymph nodes. *Nat. Immunol.*
(d) Gaya, M., Castello, A., Montaner, B. *et al* 2015. Inflammation-induced disruption of SCS macrophages impairs B cell responses to secondary infection. *Science,* 347**,** 667-672.

2. *Investigating B cell activation at the molecular level and understanding the underlying molecular mechanisms*

It was particularly satisfying that we had managed to confirm in vivo that B cells do indeed recognize antigen on the surface of presenting cells, especially given the long-term commitment to developing tools to investigate this mode of B cell activation at a molecular resolution. We have also established the importance of the context in which antigen is presented to B cells by identifying and characterizing a role for integrins in lowering the threshold for B cell activation. We have detected a novel cellular response involving a global reorganization of the cytoskeleton such that the B cell spreads across the antigen-presenting surface, providing a mechanistic basis for affinity discrimination by B cells. We were the first to report the rapid formation of numerous BCR antigen microclusters, which are sites of active signaling and drive the propagation of B cell spreading. We also revealed an essential role for the B cell coreceptor CD19 in mediating B cell activation, offering an elegant

explanation for the immunodeficiency that has been reported both in CD19-deficient mice and in human patients with mutations in CD19.

(a) Carrasco, Y. R. and F. D. Batista (2006). B cell recognition of membrane-bound antigen: an exquisite way of sensing ligands. **Curr. Opin. Immunol**. 18: 286-291
(b) Fleire, S. J., et al. (2006). B cell ligand discrimination through a spreading and contraction response. **Science**. 312: 738-741.
(c) Arana, E., et al. (2008). Activation of the small GTPase Rac2 via the B cell receptor regulates B cell adhesion and immunological-synapse formation. **Immunity**. 28: 88-99.
(d) Depoil, D., et al. (2008). CD19 is essential for B cell activation by promoting B cell receptor-antigen microcluster formation in response to membrane-bound ligand. Nat. Immunol. 9: 63-72.

Our observations proved pivotal in establishing the importance of cytoskeleton reorganization during B cell activation. We developed single particle tracking methods, which showed that the actin cytoskeleton restricts BCR diffusion and signaling, suggesting a role for the cytoskeleton in tonic signaling, B cell survival and activation. Additionally, we found that the microtubule network and its associated motor protein, dynein, participate in the gathering of antigen that is required for presentation to T cells and therefore maximal B cell activation. We also found that accumulated antigen is maintained in a polarized distribution leading to asymmetric segregation of antigen during B cell division, which may have implications in B cell fate determination.

(a) Weber, M., et al. (2008). Phospholipase C-gamma2 and Vav cooperate within signaling microclusters to propagate B cell spreading in response to membrane-bound antigen. **J. Exp. Med.** 205: 853-868.
(b) Treanor, B., et al. (2010). The membrane skeleton controls diffusion dynamics and signaling through the B cell receptor. **Immunity**. 32: 187-199.
(c) Treanor, B., et al. (2011). Dynamic cortical actin remodeling by ERM proteins controls BCR microcluster organization and integrity. **J. Exp. Med.** 208: 1055-1068.
(d) Schnyder, T., et al. (2011). B cell receptor-mediated antigen gathering requires ubiquitin ligase Cbl and adaptors Grb2 and Dok-3 to recruit dynein to the signaling microcluster. Immunity. 34: 905-918.

Effective production of antibodies requires effective signaling via the B cell receptor. Much of our work has been aimed at increasing our understanding of the mechanisms and downstream molecular pathways following BCR triggering. In 2010 we identified a role for an ezrin-defined actin network in restricting BCR diffusion and signaling in the resting B cell membrane. This work showed that CD19 was essential for mediating BCR signaling in response to cytoskeleton disruption also led us to also study the role of inhibitory co-receptors in restraining B cell signaling using a combination of high resolution imaging and more classical biochemistry methods. We initially characterized the diffusion dynamics of CD22 in the membrane of resting and activated B cells by single particle tracking, observing how this molecule co-operates with the cortical cytoskeleton to restrain BCR signaling. Brownian simulations and ex vivo experiments indicated that both the nanoscale organization fast diffusion of CD22 enabled a global BCR surveillance at the plasma membrane which contributed to its inhibitory function.

Our investigation into BCR co-receptor molecules such as CD19 have also led us to examine the effects of other receptors involved in BCR signaling on immune responses at both the molecular and cellular levels. Mice deficient in the Wiskott-Aldrich Syndrome Interacting Protein (WIP) exhibited multiple B cell defects including chemotaxis, survival, and differentiation, leading to diminished antibody production. Additionally, several receptors, namely the BCR, BAFFR, CXCR4, CXCR5, CD40, and TLR4, were impaired in promoting CD19 co-receptor activation and subsequent PI3 kinase (PI3K) signaling in the absence of WIP. The underlying mechanism was due to a distortion in the actin and tetraspanin networks that led to altered CD19 cell-surface dynamics. The small Rho GTPase Cdc42, is known to interact with Wiskott-Aldrich syndrome (WAS) protein, and is an important regulator of actin remodeling. Genetic ablation of Cdc42 exclusively in the B cell lineage, rendered mice being unable to mount antibody responses, so much so that they did not form germinal centers or generate plasma cells upon either viral infection or immunization. This was caused by multiple and profound B cell abnormalities, highlighting Cdc42 as key regulator of B cell physiology.

(a) Treanor, B., et al. (2010). The membrane skeleton controls diffusion dynamics and signaling through the B cell receptor. Immunity. 32: 187-199.
(b) Gasparrini, F., et al. (2015). "Nanoscale organization and dynamics of the siglec CD22 cooperate with the cytoskeleton in restraining BCR signalling." EMBO J. [Epub ahead of print]

(c) Burbage, M., et al. (2015). Cdc42 is a key regulator of B cell differentiation and is required for antiviral humoral immunity. J. Exp. Med. 212: 53-72.
(d) Keppler, S. J., et al. (2015). "Wiskott-Aldrich Syndrome Interacting Protein Deficiency Uncovers the Role of the Co-receptor CD19 as a Generic Hub for PI3 Kinase Signaling in B Cells." Immunity 43(4): 660-673.

Publications

A list of publications can be found at:

https://www.crick.ac.uk/research/a-z-researchers/researchers-a-c/facundo-d-batista/publications/

D. Research Support

Current:

UM1 AI100663 Batista (PI) 2014-2015
NIH/The Scripps Research Institute (CHAVI-ID) Administrative Supplement
Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery. The overall mission of the CHAVI-ID is to define immunogens and immunization regimens that induce HIV cross-protective B cell and CD4+ T cell responses in preclinical models and thereby guide product development strategies for a preventive human AIDS vaccine. This supplement is aimed at developing in vitro strategies to evaluate GP120 as immunogen and my responsibilities were the scientific direction and general overseeing of the project.
Role: PI

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Douglas Hayden, PhD

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Assistant Professor of Medicine Harvard Medical School

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 Massachusetts, Boston, MA	BA	06/1983	Mathematics
Boston University, Boston, MA	MA	06/1986	Mathematics
Boston University, Boston, MA	PhD	06/2012	Mathematics

A. Personal Statement

I am a biostatistician with training in pure mathematics and twenty-seven years of professional experience in clinical research, including seven years as a research mathematician in the biomedical technology industry, sixteen years as a master's level statistician at the Massachusetts General Hospital Biostatistics Center. I was the statistician for the NHLBI ARDS Network Clinical Coordinating Center and for the Computational Analysis and Modeling Core of the NIGMS Inflammation and Host Response to Injury Glue Grant. I spent ten years on both projects as well as providing statistical support for sixteen years for the MGH General Clinical Research Center (now Harvard Catalyst). I was lead statistician on the ARDS Network Lower Tidal Volume Trial and the Fluid and Catheter Treatment Trial performing the statistical analysis for the papers reporting the final study results. I also pioneered the use of software algorithms for monthly computer generated patient by patient on-target reports based on daily ventilator settings and physiologic parameters to provide the clinical sites with nearly real time feedback to improve study protocol compliance. I have co-authored over 70 clinical papers and first authored original methodology papers in causal inference and genomics. I currently serve as the biostatistician on a U01, P50, and P01 grant and continue as a biostatistician for the Harvard CTSA grant.

My experience in experimental design, statistical analysis, and close collaboration with physician-scientists will add additional strength to Lenette Lu's study. In particular, I have provided statistical support for studies of sepsis, inflammation, and genomics and understand the analytic challenges associated with high dimensional datasets.

1. Xiao W, Mindrinos MN, Seok J, Cuschieri J, Cuenca AG, Gao H, Hayden DL, Hennessy L, Moore EE, Minei JP, Bankey PE, Johnson JL, Sperry J, Nathens AB, Billiar TR, West MA, Brownstein BH, Mason PH, Baker HV, Finnerty CC, Jeschke MG, López MC, Klein MB, Gamelli RL, Gibran NS, Arnoldo B, Xu W, Zhang Y, Calvano SE, McDonald-Smith GP, Schoenfeld DA, Storey JD, Cobb JP, Warren HS, Moldawer LL, Herndon DN, Lowry SF, Maier RV, Davis RW, Tompkins RG. A genomic storm in critically injured humans. J Exp Med. 2011 Dec 19; 208(13):2581-90. PMID: 22110166; PMCID: PMC3244029.

2. Cobb JP, Hayden DL, Schoenfeld DA. Novel diagnostics for sepsis: a decade of promise for gene expression profiling. Crit Care Med. 2011 Nov; 39(11):2579-81. PMID: 22005238.

3. Warren HS, Fitting C, Hoff E, Adib-Conquy M, Beasley-Topliffe L, Tesini B, Liang X, Valentine C, Hellman J, Hayden D, Cavaillon JM. Resilience to bacterial infection: difference between species could be due to proteins in serum. J Infect Dis. 2010 Jan 15; 201(2):223-32. PMID: 20001600; PMCID: PMC2798011.

4. Hayden D, Lazar P, Schoenfeld D. Assessing statistical significance in microarray experiments using the distance between microarrays. PLoS One. 2009; 4(6):e5838. PMID: 19529777; PMCID: PMC2691999.

B. Positions and Honors

Positions and Employment

1986-1987	Math Teacher, Winthrop High School, Boston, MA
1989-1995	Mathematician, Oculon Corporation, Boston, MA
1995-current	Biostatistician, Massachusetts General Hospital, Boston, MA
2008-current	Consulting Statistician, Harvard University, Boston, MA
2012-current	Lecturer, Harvard Medical School, Boston, MA
2013-current	Assistant Professor, Harvard Medical School, Boston, MA

Honors

2010	Poster of Distinction, MGH Clinical Research Day, Boston, MA
2010	Partners in Excellence Award

C. Contribution to Science

1. Conduct of Clinical Research: I have a published methodology paper on the use of counterfactuals for the analysis of secondary outcomes in survivors in randomized clinical trials. Despite random allocation to treatment, survivors in randomized clinical trials are not necessarily balanced on potential confounders due to differential probability of mortality on the two treatments. Baseline covariates can be used to rebalance surviving patients by weighting by the estimated probability of survival on the un-assigned treatment.

    a) Hayden D, Pauler DK, Schoenfeld D. An estimator for treatment comparisons among survivors in randomized trials. **Biometrics**. 2005; 61(1):305-10. PMID: 15737107
    b) Hayden D, Lazar P, Schoenfeld D, Inflammation and the Host Response to Injury Investigators. Assessing statistical significance in microarray experiments using the distance between microarrays. **PLoS One**. 2009; 4(6):e5838. PMID: 19529777
    c) Healy BC, Hayden DL, Sampat MP, Bakshi R, Guttmann CR. Unbiased treatment effect estimates by modeling the disease process of multiple sclerosis. **J Neurol Sci**. 2009; 278(1-2):54-9. PMID: 19121526
    d) Healy BC, Arora A, Hayden DL, Ceccarelli A, Tauhid SS, Neema M, Bakshi R. Approaches to Normalization of Spinal Cord Volume: Application to Multiple Sclerosis. **J Neuroimaging**. 2012; 22 (3): e12-9. PMID: 21854479

2. ARDS research: For nearly 20 years, I have worked extensively in the area of pulmonary and critical care research and served as a biostatistician for the NHLBI ARDS network. In conjunction with multiple investigators, I have published numerous ancillary trials that utilized the extensive ARDS network database. As a result, I am very familiar with the intricacies of critical care research.

    a) Rice TW, Wheeler AP, Bernard GR, Hayden DL, Schoenfeld DA, Ware LB. Comparison of the SpO2/FIO2 ratio and the PaO2/FIO2 ratio in patients with acute lung injury or ARDS. **Chest.** 2007; 132(2):410-7. PMID 17573487
    b) Hager DN, Krishnan JA, Hayden DL, Brower RG. Tidal volume reduction in patients with acute lung injury when plateau pressures are not high. **Am J Respir Crit Care Med**. 2005; 172(10):1241-5. PMID 16081547
    c) Brower RG, Morris A, MacIntyre N, Matthay MA, Hayden D, Thompson T, Clemmer T, Lanken PN, Schoenfeld D. Effects of recruitment maneuvers in patients with acute lung injury and acute respiratory

distress syndrome ventilated with high positive end-expiratory pressure. **Crit Care Med**. 2003; 31(11):2592-7. PMID: 14605529

d) Eisner MD, Thompson T, Hudson LD, Luce JM, Hayden D, Schoenfeld D, Matthay MA. Efficacy of low tidal volume ventilation in patients with different clinical risk factors for acute lung injury and the acute respiratory distress syndrome. **Am J Respir Crit Care Med**. 2001; 164(2):231-6. PMID 11463593

3. Clinical Trials Management: I have been fortunate to serve as the biostatistician on several high impact multi-center clinical trials. Relevant to this proposal, I was the lead biostatistician on the NHLBI ARDS network Fluid and Catheter Treatment Trial (FACTT).

a) Cudkowicz ME, Titus S, Kearney M, Yu H, Sherman A, Schoenfeld D, Hayden D, Shui A, Brooks B, Conwit R, Felsenstein D, Greenblatt DJ, Keroack M, Kissel JT, Miller R, Rosenfeld J, Rothstein JD, Simpson E, Tolkoff-Rubin N, Zinman L, Shefner JM. Safety and efficacy of ceftriaxone for amyotrophic lateral sclerosis: a multi-stage, randomised, double-blind, placebo-controlled trial. **Lancet Neurol**. 2014; 13(11):1083-91 PMID 25297012

b) Wiedemann HP, Wheeler AP, Bernard GR, Thompson BT, Hayden D, deBoisblanc B, Connors AF, Hite RD, Harabin AL. Comparison of two fluid-management strategies in acute lung injury. **N Engl J Med.** 2006; 354(24):2564-75. PMID 16714767

c) Smith MR, McGovern FJ, Zietman AL, Fallon MA, Hayden DL, Schoenfeld DA, Kantoff PW, Finkelstein JS. Pamidronate to prevent bone loss during androgen-deprivation therapy for prostate cancer. **N Engl J Med**. 2001; 345(13):948-55. PMID 11575286

d) Grinspoon S, Corcoran C, Parlman K, Costello M, Rosenthal D, Anderson E, Stanley T, Schoenfeld D, Burrows B, Hayden D, Basgoz N, Klibanski A. Effects of testosterone and progressive resistance training in eugonadal men with AIDS wasting. A randomized, controlled trial. **Ann Intern Med**. 2000; 133(5):348-55. PMID 10979879

4. Epidemiological Research: I have also participated in epidemiological trials in the area of critical care medicine including burn, trauma, and cardiology. Several of these studies have been published in high impact journals such as the New England Journal of Medicine.

a. Klein MB, Goverman J, Hayden DL, Fagan SP, McDonald-Smith GP, Alexander AK, Gamelli RL, Gibran NS, Finnerty CC, Jeschke MG, Arnoldo B, Wispelwey B, Mindrinos MN, Xiao W, Honari SE, Mason PH, Schoenfeld DA, Herndon DN, Tompkins RG. Benchmarking outcomes in the critically injured burn patient. **Ann Surg**. 2014; 259(5):833-41. PMID: 24722222

b. Truong QA, Hayden D, Woodard PK, Kirby R, Chou ET, Nagurney JT, Wiviott SD, Fleg JL, Schoenfeld DA, Udelson JE, Hoffmann U. Sex differences in the effectiveness of early coronary computed tomographic angiography compared with standard emergency department evaluation for acute chest pain: the rule-out myocardial infarction with Computer-Assisted Tomography (ROMICAT)-II Trial. **Circulation.** 2013; 127(25):2494-502. PMID 23685743

c. Hoffmann U, Truong QA, Schoenfeld DA, Chou ET, Woodard PK, Nagurney JT, Pope JH, Hauser TH, White CS, Weiner SG, Kalanjian S, Mullins ME, Mikati I, Peacock WF, Zakroysky P, Hayden D, Goehler A, Lee H, Gazelle GS, Wiviott SD, Fleg JL, Udelson JE. Coronary CT angiography versus standard evaluation in acute chest pain. **N Engl J Med.** 2012; 367(4):299-308. PMID: 22830462

Publications List http://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/47952679/

D. Research Support

Ongoing Research Support

1U01HL123009 (Schoenfeld) 04/01/14–03/31/21
NIH / NHLBI
Clinical Coordination Center for a NHLBI Prevention and Early Treatment of Acute Lung Injury (PETAL) Network
The major goals of this project are the coordination, design, and statistical analysis of clinical trials in Petal.
Role: Biostatistician

1UL1TR001102 (Orf) 10/01/13-9/30/18
NIH/NCRR
Harvard Clinical and Translational Science Center
Provide enriched resources to educate and develop the next generation of researchers trained in the complexities of translating research discoveries into clinical trials and ultimately into practice. Design new and improved clinical research informatics tools for analyzing research data and managing clinical trials. Support outreach to underserved populations, local community and advocacy organizations, and health care providers. Assemble interdisciplinary teams and forge new partnerships with private and public health care organizations.
Role: Biostatistician

2P50GM02100 (Tompkins) 06/01/13-05/31/18
NIH
Burn Trauma Center
The host response to burns and trauma is a collection of biological and pathological processes that depends critically upon the regulation of the human metabolic response. Over the years of P50 funding, our Center has had a tremendous and unique research opportunity to study the physiology of metabolism after injury. Since its inception, the Center has focused on the metabolic aspects of the patient's immuno-inflammatory reaction to injury.
Role: Biostatistician

2P01HL018646 (Madsen) 09/01/14–08/31/19
NIH/NIAD
New Approaches to Cardiothoracic Tolerance Induction
The unifying goal of this program project is to combine mixed chimerism with novel strategies designed to amplify the contributions of Tregs in order develop a clinical tolerance protocol that can be rapidly translated to human recipients of heart and lung allografts.
Role: Biostatistician

Completed Research Support

HHSN268200536179C (D. Schoenfeld, PhD) 9/30/05 – 02/28/2014
NIH / NHLBI
Clinical Coordination Center for a Clinical Research Network for the Treatment of Acute Lung Injury and Acute Respiratory Distress Syndrome The major goals of this project are the coordination, design, and statistical analysis of clinical trials in ARDS.

5U01 NS049640-08 (Cudkowicz) 07/01/10-06/30/14
NIH
Clinical Trial of Ceftriaxone in ALS
The major goal of this project is to provide statistical support for clinical studies.
Role: Biostatistician

5R01 HL092022-03 (D. Schoenfeld, PhD) 09/15/09 – 06/30/14
NIH / NHLBI
Rule-Out Myocardial Infarction Using Computed Assisted Tomograpy –ROMICAT II, DCC The major goals of this project are the coordination, design, and statistical analysis of the multicenter trial in 1000 subjects with acute chest pain. The objective is to determine whether rate of discharge from the Emergency Department is increased by the use of Cardiac Computer Assisted Tomography.
Role: Biostatistician

5R01-GM081524-04 (Cobb) 09/03/09 – 12/31/13 (NCE)

Plasticity of the Systemic Inflammation Response To develop a strategy for blood immunomonitoring that can be used as a novel diagnostic and prognostic tool, thereby improving the care of critically ill patients at risk for sepsis.
Role: Biostatistician

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: Iwasaki, Akiko

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Professor of Immunobiology and Molecular Cellular and Developmental Biology

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 Toronto, Toronto, Canada	B. Sc	1994	Biochemistry and Physics
University of Toronto, Toronto, Canada	Ph.D	1998	Immunology
National Institutes of Health, Bethesda, MD	Postdoctoral	1998-2000	Mucosal Immunity

A. Personal Statement

Over the course of the past 16 years as an independent investigator, I have had the opportunity to mentor 11 postdoctoral fellows and 11 graduate students, 7 of which have already establish their own laboratories as junior faculty at various academic institutions, including an associate professor in Tokyo University (Takeshi Ichinohe), assistant professors in Osaka University (Miwa Sasai), KAIST (Heung Kyu Lee), Fred Hutchinson Cancer Research Center (Jennifer Lund), University of Washington (Hong Shen) and Washington University (Haina Shin). My lab is well funded/equipped to support the development of trainees in research in innate and adaptive immunity to viruses. In summary, I have a demonstrated record of successful and productive research program, and my expertise and experience in innate immunity have prepared me to serve on Lenette Lu's K08 committee.

1. Shin, H. and **Iwasaki A.** A vaccine strategy that protects against genital herpes by establishing local memory T cells. Nature 2012 Nov 15;491(7424):463-7. doi: 10.1038/nature11522, PMCID:PMC3499630.
2. Iijima N and **Iwasaki A.** T cell memory. A local macrophage chemokine network sustains protective tissue-resident memory CD4 T cells. Science (2014) Oct 3;346(6205):93-8. PMCID: PMC4254703.
3. Nakanishi, Y., Lu, B., Gerard, C., **Iwasaki, A.** CTL mobilization to virus-infected tissue requires CD4+ T cell help. Nature, 2009 462(7272):510-3. PMCID: PMC2789415.
4. Sasai M, Linehan MM, **Iwasaki A.** Bifurcation of Toll-like receptor 9 signaling by adaptor protein 3. Science 2010 Sep 17;329(5998):1530-4, PMCID:PMC3063333.

B. Positions and Honors

Positions and Employment

1993-1998 Ph.D. Research Project, Supervisor: Professor Brian H. Barber, Department of Immunology, University of Toronto
1998-2000 Postdoctoral research fellow, Laboratory of Clinical Investigation, NIH,NIAID
2000-2004 Assistant Professor of Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT
2004-2006 Assistant Professor of Immunobiology, Yale University School of Medicine, New Haven, CT
2006-2009 Associate Professor of Immunobiology, Yale University School of Medicine, New Haven, CT
2009-2011 Associate Professor with Tenure of Immunobiology and Molecular, Cellular and Developmental Biology, Yale University School of Medicine, New Haven, CT

2011-2016 Professor with Tenure of Immunobiology and Molecular, Cellular and Developmental Biology, Yale University School of Medicine, New Haven, CT
2014- Investigator, Howard Hughes Medical Institute
2016- Waldemar von Zedtwitz Professor of Immunobiology and of Molecular, Cellular and Developmental Biology, Yale University School of Medicine, New Haven, CT

Other Experience and Professional Memberships

Associate Editor for Mucosal Immunity (NPG) and Trends in Immunology. Ad hoc reviewer for Nature, Science, Cell, Immunity, J. Immunology, J. Virology, J. Experimental Medicine, J. Leukocyte Biology, J. Cellular Immunology, J. Clinical Investigation, Blood, Proc. Natl. Acad. Sci., PLOS, PLOS Pathogen, Cell Host & Microbe, Nature Immunology, Nature Review in Immunology.

Honors

1990-1993 Faculty Scholar
1991-1993 St. Michael's College Scholarship
1993 Silver Medalist at St. Michael's College
1993-1994 Massey College Fellow
1993-1996 Connaught Scholarship
1996-1998 Ontario Graduate Scholarship
1998 Natural Sciences and Engineering Research Council of Canada Fellowship Award
1998-2001 Medical Research Council of Canada Fellowship
2000 The Fellows Award for Research Excellence
2000 Canadian Society for Immunologist Travel Award
2002 Young Investigator Award from Society for Mucosal Immunology
2000-2003 Burroughs Wellcome Fund Career Award in Biomedical Sciences
2003 Ethel Donaghue Women's Health Program Investigator Award
2003 Wyeth-Lederle Infectious Disease Society of America Young Investigator Award
2005-2010 Burroughs Wellcome Fund Investigator in Pathogenesis in Infectious Diseases
2011 American Associations of Immunologists (AAI) BD Biosciences Investigator Award
2012 Eli Lilly and Company Research Award, American Society for Microbiology
2013 Master of Arts *Privatum*, Yale University
2014 Appointment, HHMI Investigator

C. Contribution to Science

C1. Innate recognition of viruses

My laboratory discovered that viral nucleic acids are recognized by Toll-like receptors (TLR) 9 (dsDNA) and TLR7 (ssRNA) within the endosomes. The endosomal recognition helps to distinguish viral from self nucleic acids. We also found that viruses that enter cytosol upstream of endosomal compartment are still detected by TLRs, but in this case viral replication intermediates have to be delivered to the TLRs by autophagy. We identified a lysosome-related organelle from which both TLR7 and TLR9 traffic to signal for interferon production and demonstrated that TLR traffic to this compartment is mediated by the adaptor protein AP-3.

a. Lund J, Sato A, Akira S, Medzhitov R and **Iwasaki A**. Toll-like receptor 9-mediated recognition of herpes simplex virus-2 by plasmacytoid dendritic cells. Journal of Experimental Medicine 2003 Aug 4;198(3):513-20. PMCID: PMC2194085.
b. Lee HK, Lund JM, Ramanathan B, Mizushima N and **Iwasaki A**. Autophagy-dependent viral recognition by plasmacytoid dendritic cells. Science, 2007 Mar 9;315(5817):1398-401, PMID: 17272685.
c. Sasai M, Linehan MM, **Iwasaki A.** Bifurcation of Toll-like receptor 9 signaling by adaptor protein 3. Science 2010 Sep 17;329(5998):1530-4, PMCID:PMC3063333
d. Pillai PS, Molony RD, Martinod K, Dong H, Pang IK, Tal MC, Solis AG, Bielecki P, Mohanty S, Trentalange M, Homer RJ, Flavell RA, Wagner DD, Montgomery RR, Shaw AC, Staeheli P, and **Iwasaki A**. Mx1 Reveals Innate Pathways to Antiviral Resistance and Lethal Influenza Disease. Science, in press.

C2. Role of dendritic cells in priming immune responses

Pathogen recognition by dendritic cells (DCs) triggers activation of adaptive immune responses, but how this occurs in the context of natural viral infections is poorly understood, in part because virus infection impairs the function of DCs. We demonstrated that TLR signals in both virally infected stromal cells and non-infected DCs are required for optimal T cell immunity following herpes virus infection. We further demonstrated that DCs use autophagy machinery for processing of viral antigens for MHC class II presentation. Our studies demonstrated the role of various DC subsets in generating different classes of immune responses in vivo.

a. Sato A and **Iwasaki A**. Induction of antiviral immunity requires Toll-like receptor signaling in both stromal and dendritic cell compartments. Proc. Natl. Acad. Sci. 2004 Nov 16;101(46):16274-9. PMCID: PMC528964.
b. Kumamoto Y, Mattei LM, Sellers S, Payne GW, **Iwasaki A**. CD4+ T cells support cytotoxic T lymphocyte priming by controlling lymph node input. Proc Natl Acad Sci 2011 May 24;108(21):8749-54. PMCID: PMC3102372.
c. Lee HK, Mattei LM, Steinberg BE, Alberts P, Lee YH, Chervonsky A, Mizushima N, Grinstein S, **Iwasaki A**. In Vivo Requirement for Atg5 in Antigen Presentation by Dendritic Cells. Immunity. 2010 Feb 26;32(2):227-239. PMCID:PMC2996467.
d. Kumamoto Y, Linehan M, Weinstein JS, Laidlaw BJ, Craft JE and **Iwasaki A**: CD301b(+) Dermal Dendritic Cells Drive T Helper 2 Cell-Mediated Immunity. Immunity. 2013 Oct 17;39 (4) :733-43. Epub 2013 Sep 26. PMCID: PMC3819035

C3. Innate link to adaptive immunity

Infection with a live pathogen typically results in the generation of multiple PAMPs. Yet, not all innate signals are equal with respect to their ability to program adaptive immune responses. Using respiratory influenza virus infection model in mice, we demonstrated that influenza virus M2 ion channel triggers the activation of NLRP3 inflammasomes. This activity results in the secretion of IL-1β. We showed that pro-IL-1β expression depended on signals from the microbiota. Notably, this NLRP3/caspase-1/IL-1b axis, and not the TLR7 or RIG-I recognition, is required for the generation of B and T cell responses to flu. We showed that infected cells are not able to stimulate CD8 T cells but the bystander activation of DCs through IL-1R provides a key signal for migration and priming of CD8 T cells in vivo. These studies highlight the possible hierarchy of PAMPs in eliciting adaptive immunity.

a. Ichinohe, T., Lee, H.K., Ogura, Y., Flavell, R., **Iwasaki A.** Inflammasome recognition of influenza virus is essential for adaptive immune responses. Journal of Experimental Medicine 2009 Jan 16;206(1):79-87. PMCID: PMC2626661.
b. Ichinohe T, Pang IK, **Iwasaki A.** Influenza virus activates inflammasomes via its intracellular M2 ion channel. Nature Immunology 2010 May;11(5):404-10. PMCID: PMC2857582
c. Pang, IK, Ichinohe, T, **Iwasaki, A.** IL-1R signaling in dendritic cells replaces pattern recognition receptors to promote CD8+ T cell responses to influenza A virus. Nature Immunology, 2013 Jan 13;14(3):246-53, PMCID: PMC3577947.
d. Ichinohe, T., Pang, IK, Kumamoto, Y., Peaper, DR, Ho, JH, Murray, TS, and **Iwasaki, A.** Microbiota regulates immune defense against respiratory tract influenza A virus infection. Proc. Natl. Acad. Sci. 2011 Mar 29;108(13):5354-9. PMCID: PMC3069176.

C4. Adaptive immune mechanism of protection at the female genital mucosa

My laboratory has made contributions to our understanding of the innate and adaptive mechanisms of protection in the genital mucosa following HSV-2 infection. In particular, we found that CD4 T helper cells are critical for entry of effector CD8 T cells into the genital mucosa, and unexpectedly, that they do so indirectly through IFN-γ secretion and induction of local chemokine production by the infected tissue. We demonstrated, using a parabiosis system, that effective protection against genital HSV-2 infection requires the presence of local "tissue-resident" memory T cells (TRM). The precursors of these cells are recruited into the mucosal tissue during the initial infection, and the TRM that subsequently develop remain there for extended periods of time. Our results strongly suggest that successful T cell based vaccines against sexually transmitted viruses must establish a local memory T cell pool. To this end, we have developed a novel approach, called “prime and pull”, in which we are able to manipulate the immune system to establish protective TRM in a safe and effective manner.

a. Nakanishi, Y., Lu, B., Gerard, C., **Iwasaki, A.** CTL mobilization to virus-infected tissue requires CD4+ T cell help. Nature, 2009 462(7272):510-3. PMCID: PMC2789415.
b. Shin, H. and **Iwasaki A.** A vaccine strategy that protects against genital herpes by establishing local memory T cells. Nature 2012 Nov 15;491(7424):463-7. doi: 10.1038/nature11522, PMCID:PMC3499630.
c. Iijima, N., Linehan. M.M., Zamora, M., Butkus, D., Dunn R., Kehry, M.R., Laufer, T.M., **Iwasaki, A.** Dendritic cells and B cells maximize mucosal Th1 memory response to herpes simplex virus. Journal of Experimental Medicine, 2008 Dec 22;205(13):3041-52. PMCID: PMC2605233.
d. Iijima N and **Iwasaki A.** T cell memory. A local macrophage chemokine network sustains protective tissue-resident memory CD4 T cells. Science (2014) Oct 3;346(6205):93-8. PMCID: PMC4254703.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/akiko.iwasaki.1/bibliography/40635062/public/?sort=date&direction=ascending.

D. **Research Support**
List both selected ongoing and completed research projects for the past three years (Federal or non-Federally-supported). *Begin with the projects that are most relevant to the research proposed in the application.* Briefly indicate the overall goals of the projects and responsibilities of the key person identified on the Biographical Sketch. Do not include number of person months or direct costs.

Ongoing Research Support

5 R01 AI054359 (Iwasaki) 12/01/2003 - 03/31/2019
NIH/NIAID
Immunity to Genital Herpes Simplex Virus 2
The major goals of this project are to determine the mechanisms by which dendritic cells initiate CD4+ T cell immunity in the vaginal mucosa.

Abbvie Yale Collaboration In Immunobiology 05/20/2013 – 06/30/2017
Role of endogenous retroviruses in lupus pathogenesis
The goal of the proposed project is to understand the disease pathogenesis of SLE and possibly develop a new strategy to diagnose, prevent and/or treat SLE in humans.

11R41AI120269-01 (Bilsel) 08/15/2014- 08/14/2016
NIH/NIAID
Restimulating memory T-cell responses in elderly by a novel, live influenza vaccine
In this proposal we will test the hypothesis that engaging non-inflammasome dependent innate pathways by M2SR virus results in robust restimulation of memory CD4 and CD8 T cells in older humans without causing pathological inflammation.

Abbvie Yale Collaboration In Immunobiology 07/01/2015 – 06/30/2019
Identification and targeting of Tfh-inducing dendritic cells
The goal of the proposed project is to understand the disease pathogenesis of SLE and possibly develop a new strategy to diagnose, prevent and/or treat SLE in humans.

Howard Hughes Medical Institute (Iwasaki) 05/15/2014 – 05/14/2019
To understand the cellular and molecular mechanisms of innate virus recognition and in elucidating innate signals that lead to the generation of protective immune responses and to apply such understanding to create effective vaccines.

Completed Research Support (last three years)

Women's Health Research at Yale Pilot Project Grant (Iwasaki) 07/01/2013 – 06/30/2014
A New Therapeutic Vaccination Strategy for Genital Herpes

The major goals of this project are to test efficacy of Prime and Pull method to protect the host against recurrent HSV-2 infection in guinea pig model of genital herpes.

[redacted]

1R01AI102625-01 (Haase) 11/15/2012 – 06/30/2014
University of Minnesota, Twin Cities - IVHD Pilot Project Grant
Pilot project to find optimal timing for prime and pull to recruit plasma cells (Iwasaki)
This project proposes to obtain preliminary data in mice to find optimal timing for prime and pull to recruit plasma cells.

[redacted]

5 R01 AI062428-08 (Iwasaki) 07/01/2004 – 11/30/2015
NIH/NIAID
Antiviral CTL Mobilization to the Genital Mucosa
The major goals of this project are to examine the mechanism by which cytotoxic T lymphocytes are recruited to the genital mucosa during natural infection and design vaccine strategies based on this knowledge.

5 R01 AI 064705 (Iwasaki) 04/01/2005 – 01/31/2016
Viral Recognition by Plasmacytoid Dendritic Cells
The major goals of this project are to: examine the mechanism of type 1 IFN induction by live viruses in pDCs; determine the cellular mechanisms utilized by pDCs for innate recognition of DNA and RNA viruses; and to examine the in vivo role of pDCs in innate and adaptive immunity following mucosal viral infections.

5 R01 AI 081884 (Iwasaki) 05/01/2010 – 04/30/2015
NIH/NIAID
Autophagy in Antiviral Immunity
The goal of this project is to examine the importance of autophagy in both innate and adaptive antiviral immune responses using well-established genetic, biochemical and cell biological tools as well as in vivo animal models of both RNA and DNA virus infections.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

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: Nimmerjahn, Falk

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

POSITION TITLE: Full Professor of Immunology and Chairman of the Institute of Genetics

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
Friedrich-Alexander University Erlangen-Nuremberg, Germany	Diploma (Master)	1993-1998	Immunology, genetics
Ludwig-Maximilians University Munich	PhD	1999-2002	Tumor immunology
National Research Center for Environment and Health (GSF), Munich, Germany	scientist	2002-2004	Immunology, Virology
The Rockefeller University, New York, USA	Postdoc	2004-2007	Immunology, autoimmunity

A. Personal Statement

I have a long standing research interest in the mechanisms underlying antibody and Fc receptor activity. We were one of the first groups showing that differentially glycosylated IgG variants not only modulate the pro- but also the anti-inflammatory activity of IgG (1, 2). Moreover, our work on how different mouse and human IgG subclasses mediate their activity may of major relevance for polyclonal and complex antibody responses induced by bacterial infections (3,4). Thus, I feel confident that my area of expertise may be of use in the Scientific Advisory Committee for the K08.

Publications:

1) Schwab, I., Lux, A., and Nimmerjahn, F. (2015). Pathways Responsible for Human Autoantibody and Therapeutic Intravenous IgG Activity in Humanized Mice. Cell Rep *13*, 610-620.
2) Kaneko, Y., Nimmerjahn, F., and Ravetch, E.V. (2006). Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science *313*, 670-673.
3) Lux, A., Yu, X., Scanlan, C.N., and Nimmerjahn, F. (2013). Impact of immune complex size and glycosylation on IgG binding to human FcgammaRs. J Immunol *190*, 4315-4323.
4) Nimmerjahn, F., and Ravetch, J.V. (2005). Divergent immunoglobulin G subclass activity through selective Fc receptor binding. Science *310*, 1510-1512.

B. Positions and Honors

Positions

2007-2010: Associate professor of immunology, Medical Department 3, University of Erlangen-Nuremberg, Germany
Since 2010: Full professor and chairman, Institute of Genetics, Department of Biology, University of Erlangen-Nuremberg, Germany

Fellowships

2005-2007 Fellow of the Cancer Research Institute, New York
2007-2012 Fellow of the Bavarian Genome Research Network
Since 2006 Fellow of the German society of immunology
Since 2009 Fellow of the Henry G. Kunkel society, New York

Awards

2003 Ph.D. award at the National Research Center for Environment and Health for the best Ph.D thesis in immunology and molecular biology
2008 BD prize for excellence in research from the European Macrophage and Dendritic Cell society
2008 Pro-Scientia award of the Eckhart Buddecke foundation
2009 Paul Ehrlich and Ludwig Darmstädter award

C. Contribution to Science

1) My group has a long standing interest in deciphering how cellular Fc-receptors contribute to the activity of immunoglobulin G (IgG) antibodies. Over the last 12 years we have published some of the key papers demonstrating that cytotoxic IgG antibodies mediate their activity mainly via cellular Fc-receptors and not via the activation of the complement pathway. These findings have been reproduced by many other groups and have led to the development of second generation therapeutic antibodies with an enhanced affinity to cellular Fc-receptors.

Key publications:

- Schwab, I., Lux, A., and Nimmerjahn, F. (2015). Pathways Responsible for Human Autoantibody and Therapeutic Intravenous IgG Activity in Humanized Mice. Cell Rep 13, 610-620.
- Hamaguchi, Y., Xiu, Y., Komura, K., Nimmerjahn, F., and Tedder, T.F. (2006). Antibody isotype-specific engagement of Fc gamma receptors regulates B lymphocyte depletion during CD20 immunotherapy. Journal of Experimental Medicine 203, 743-753.
- Nimmerjahn, F., and Ravetch, J.V. (2005). Divergent immunoglobulin G subclass activity through selective Fc receptor binding. Science 310, 1510-1512.
- Nimmerjahn, F., Bruhns, P., Horiuchi, K., and Ravetch, J.V. (2005). Fc gamma RIV: A novel FcR with distinct IgG subclass specificity. Immunity *23*, 41-51.

2) Another key aspect of our research is to understand which effector cells are responsible for mouse and human IgG subclass activity. This work has led to a change in our current models of how target cells become depleted by cytotoxic antibodies. While the long standing assumption was that natural killer cells are the main cell type involved in antibody dependent cell mediated cytotoxicity (ADCC) it has become established that at least in classical and humanized mouse model systems not NK cells but rather cells of the mononuclear phagocytic system, including tissue resident macrophages and monocytes are key effector cells in this pathway.

Key publications:

- Lux, A., Seeling, M., Baerenwaldt, A., Lehmann, B., Schwab, I., Repp, R., Meidenbauer, N., Mackensen, A., Hartmann, A., Heidkamp, G., et al. (2014). A humanized mouse identifies the bone marrow as a niche with low therapeutic IgG activity. Cell Rep 7, 236-248.
- Biburger, M., Aschermann, S., Schwab, I., Lux, A., Albert, H., Danzer, H., Woigk, M., Dudziak, D., and Nimmerjahn, F. (2011). Monocyte subsets responsible for immunoglobulin G-dependent effector functions in vivo. Immunity 35, 932-944.

3) A third focus of our work, which has resulted in a new understanding of how the pro-inflammatory activity of IgG is modulated, is to define how glycosylation of IgG impacts its activity. We were able to show that IgG antibodies lacking fucose residues in their sugar domain have a more than ten-fold increased affinity for mouse FcRIV and human FcRIIIa, respectively. This increased affinity translated into enhanced FcR dependent effector functions and this concept has now been translated into the clinic in the form of second generation therapeutic antibodies with increased affinity for activating and reduced affinity for the inhibitory FcRIIb. Further along these lines we were able to show that lack of galactosylation does not impact the pro-inflammatory activity of IgG. In contrast the size of an immune complex is a major factor determining the pro-inflammatory activity of IgG subclasses and may even diminish the influence of differentially glycosylated IgG variants with respect to enhanced or decreased binding to individual activating FcRs.

Key publications

- Kao, D., Danzer, H., Collin, M., Gross, A., Eichler, J., Stambuk, J., Lauc, G., Lux, A., and Nimmerjahn, F. (2015). A Monosaccharide Residue Is Sufficient to Maintain Mouse and Human IgG Subclass Activity and Directs IgG Effector Functions to Cellular Fc Receptors. Cell Rep 13, 2376-2385.
- Lux, A., Yu, X., Scanlan, C.N., and Nimmerjahn, F. (2013). Impact of immune complex size and glycosylation on IgG binding to human FcgammaRs. J Immunol *190*, 4315-4323.
- Nimmerjahn, F., Anthony, R.M., and Ravetch, J.V. (2007). Agalactosylated IgG antibodies depend on cellular Fc receptors for in vivo activity. Proceedings of the National Academy of Sciences of the United States of America 104, 8433-8437.
- Nimmerjahn, F., and Ravetch, J.V. (2005). Divergent immunoglobulin G subclass activity through selective Fc receptor binding. Science 310, 1510-1512.

4) In addition to defining parameters influencing the pro-inflammatory activity we are studying how glycosylation impacts the anti-inflammatory activity of IgG. Pooled serum IgG preparations are used frequently in the form of intravenous immunoglobulins (IVIg) to treat a great variety of chronic inflammatory or autoimmune diseases. We were the first group to show that glycosylation is critical for the anti-inflammatory activity of IgG. More in depth studies elucidated the role of individual residues of the IgG sugar moiety in this process. Thus, terminal sialic acid residues were essential for IVIg mediated suppression of a autoimmune diseases such as ITP, nephrotoxic nephritis, inflammatory arthritis and epidermolysis bullosa acquisita. More recently we were able to show that enhancing sialylation levels on commercially available IVIg preparations increases the anti-inflammatory activity. Apart from sialylation we could also show that enhanced levels of galactosylation and sialylation may impact complement mediated pro-inflammatory processes as well.

Key publications:

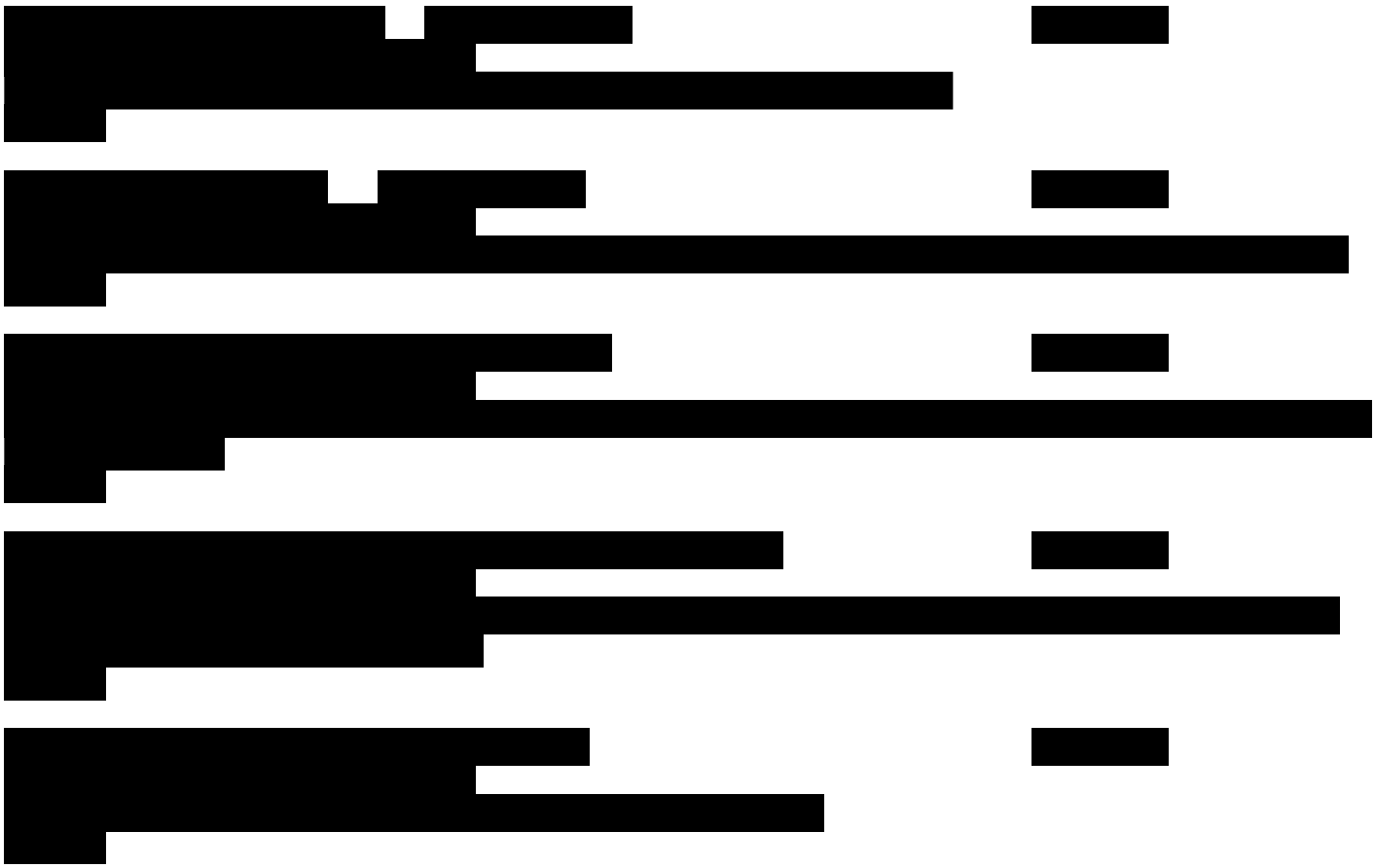
- Schwab, I., Lux, A., and Nimmerjahn, F. (2015). Pathways Responsible for Human Autoantibody and Therapeutic Intravenous IgG Activity in Humanized Mice. Cell Rep *13*, 610-620.
- Karsten, C.M., Pandey, M.K., Figge, J., Kilchenstein, R., Taylor, P.R., Rosas, M., McDonald, J.U., Orr, S.J., Berger, M., Petzold, D*., et al.* (2012). Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcgammaRIIB and dectin-1. Nat Med *18*, 1401-1406.
- Anthony, R.M., Nimmerjahn, F., Ashline, D.J., Reinhold, V.N., Paulson, J.C., and Ravetch, J.V. (2008). Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG fc. Science *320*, 373-376.
- Kaneko, Y., Nimmerjahn, F., and Ravetch, E.V. (2006). Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science *313*, 670-673.

5) Apart from understanding basic IgG biology we are developing novel approaches to inhibit the unwanted activity of autoantibodies. We have established approaches to specifically target the glycosylation of serum IgG antibodies to inhibit their binding to activating FcR. For this we are using enzymes derived from streptococcus pyogenes called endoglycosidase S (ENDO S) and IdeS. While EndoS targets the sugar moiety IdeS specifically cleaves the IgG Fc-portion of select IgG subclasses. Apart from targeting glycosylation we are investigating to which extend B cells and autoantibodies contribute to complex autoimmune diseases to develop novel options for treating these severely affected patients, such as children lacking regulatory T cells.

Key publications:

- Aschermann, S., Lehmann, C.H., Mihai, S., Schett, G., Dudziak, D., and Nimmerjahn, F. (2013). B cells are critical for autoimmune pathology in Scurfy mice. Proc Natl Acad Sci U S A *110*, 19042-19047.
- Albert, H., Collin, M., Dudziak, D., Ravetch, J.V., and Nimmerjahn, F. (2008). In vivo enzymatic modulation of IgG glycosylation inhibits autoimmune disease in an IgG subclass-dependent manner. Proceedings of the National Academy of Sciences of the United States of America 105, 15005-15009.

D. Additional Information: Research Support



OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

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: **Restrepo**, Blanca I

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Associate 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
Colegio Mayor de Antioquia, Medellin, Colombia	B.S.	12/86	Medical Technology
Univ Texas Health Science Center San Antonio	Ph.D.	06/94	Microbiology
Univ Texas Health Science Center San Antonio	Postdoctoral	03/97	Parasite Immunology
Univ Texas Health- Houston, School Public Health in Brownsville	Postdoctoral	08/03	Field Epidemiology

A. Personal statement

I am Hispanic, bilingual, bi-cultural, and a role model to Hispanics from South Texas, a population with severe health disparities. I joined UTHealth-School of Public Health campus in Brownsville in 2002 to launch a new tuberculosis (TB) research program. I quickly found that TB studies in this community had to take into account the contribution of type 2 diabetes (DM2). Today I lead a TB research program with most studies on the TB-DM2 co-morbidity.

My epidemiology studies have pioneered the current appreciation for the importance of the re-emerging association between TB and DM2 (Epidemiol Infect, 2007; Clin Infect Dis, 2007; Am J Trop.Med.Hyg. 2008; Scand.J Infect.Dis. 2008; Bull World Health Organ. 2011). The latter work received international recognition (see “Honors” section below). These studies have raised the attention of the World Health Organization (WHO), World Diabetes Foundation (WDF), and the International Union Against TB and Lung Diseases (IUATLD). I am a regular manuscript and grant reviewer on this topic, have been an invited speaker to national and international conferences. In fact, I was part of the steering committee, session chair and speaker at the recent NIH workshop (NIAID, NIDDK, Office of AIDS research) on TB, DM2 and HIV (May 2016).

My basic science studies on TB-DM2 complement our epidemiological findings. I was among the first to show in contemporary times the dysfunctional immune response in DM2 patients with TB (Clin Infect Dis, 2008; Int J Tub Lung Dis, 2011). I have extended the immunology studies to DM2 patients who are TB naïve or recent contacts of TB patients, and in all these study groups we find defective monocyte responses to *M. tuberculosis* (Tuberculosis, 2012; Tuberculosis, 2013; PlosONE, 2014; unpublished findings). Specifically, we find reduced phagocytosis and lower capacity to contain mycobacterial growth by DM2 monocytes (vs non-DM) which provides support for their higher TB risk.

Relevance to this proposal. Beyond my studies on TB and DM, I have also participated in various proof-of-principle studies in collaboration with TB scientists across the US who require TB patient specimens. My strategic location on the Texas-Mexico border provides access to more specimens than most other sites in the US. A recent and exciting collaboration is with Drs. Sarah Fortune and Galit Alter (PIs) and their post-doctoral fellow, Dr. Lu, who is the PI on the current proposal. For these studies I have provided a well-characterized collection of plasma specimens that have contributed to the initial discovery of differential Fc receptor effector functions and glycosylation patterns in sera from TB versus LTBI patients. For the current proposal I will continue to provide Dr. Lu with plasma specimens selected based on the scientific needs, and participate in the data analysis of these findings taking into account the participant characteristics.

- Lenette L. Lu, Amy W. Chung, Tracy Rosebrock, Musie Ghebremichael, Wen Han Yu, Matthew K. Schoen, Fikadu Tafesse, Constance Martin, Vivian Leung, Alison E. Mahan, Magdalena Sips, Jacquelynne Tedesco, Hannah Robinson, Elizabeth Tkachenko, Katherine J. Freedberg, Hendrik Streeck, Todd J. Suscovich, Monia Draghi, Douglas Lauffenburger, Blanca I. **Restrepo**, Cheryl Day, Sarah Fortune, Galit Alter. A functional role for antibodies in tuberculosis. Cell. (*submitted*)

Relevant expertise: My team's discovery of defective responses to *M. tuberculosis* by diabetic monocytes plus the literature reports of defects in autophagy in DM2 patients provide the foundation for this proposal. For this I have initiated a collaboration with Dr. Jagannath who is an expert on TB autophagy and whose laboratory is within driving distance from mine. This strategic partnership complements my expertise and access to DM2 patients at different stages of TB, with Dr. Jagannath's knowledge and technical experience on TB autophagy.

B. Positions and Honors

Positions and Employment:

1997-2001: Head, Molecular Parasitology Group, Corporación para Investigaciones Biológicas (CIB), Medellín, Colombia
2003-2007: Assistant Professor NTR. Univ Texas Health Houston- School of Public Health, Brownsville Regional Campus (UT-SPHB), Brownsville, TX
2007-2010: Assistant Professor of Epidemiology, Tenure track, UT-SPHB, Brownsville, TX
2010-present: Associate Professor of Epidemiology, Tenured. UT-SPHB, Brownsville, TX
2012-present: Adjunct Associate Professor of Microbiology, UT-Health Science Center at San Antonio in Edinburg, TX

Honors:

1995: Honorable Mention for Leadership Award. Women's Faculty Association. University of Texas Health Science Center at San Antonio
1998: Scholarship Award "Servicios y Cultivos LTDA" at CIB-Medellin. Award equivalent to US $16,500 to support salary
2000: Gorgas Memorial Institute Research Award. Travel and Grant Award for the project "Development of improved immunoassays for tuberculous meningitis: Antigen detection for diagnosis and prognosis". (US $20,000)
2003: Friends of Public Health Award, Texas Department of Health Region 11.
2009: Award of Merit. "Heads-Up Immunology module". International competition honoring excellence in film, television and videography. Dr. Restrepo's role: provide advice in this module's design; featured as Hispanic scientist role model. www.accoladecompetition.org. PI: Natalie Sessions.
2011: Manuscript "Cross-sectional assessment reveals high diabetes prevalence among newly-diagnosed tuberculosis cases. (Bull World Health Organ 2011 May 1)"
- Selected and evaluated by Anthony Harries, a Member of the Faculty of 1000 (F1000), placed publication in the F1000 library of top 2% of published articles in biology and medicine (http://f1000.com/11855966)
- Featured by WHO for media release (http://www.who.int/bulletin/releases/media_notes/en/index.html)
- Featured United Nations radio (http://www.unmultimedia.org/radio/spanish/detail/175949.html)

C. Contribution to Science

For each topic below I present selected publications. Full list available at My Bibliography (n=53) is at: http://www.ncbi.nlm.nih.gov/sites/myncbi/collections/public/1PGgVrbTknfffbOVCTJwNvTQM/?sort=date&direction=ascending.

1. **I discovered a novel DNA recombination mechanism that contributes to antigenic variation in a relapsing fever bacterium (**Ph.D. advisor Dr. Alan Barbour): The expression of a new variable surface protein resulting from the intra-molecular activation of its pseudogene, with deletion of the intervening DNA. Further deletions at the junction site contribute to additional antigenic variation in the newly-expressed surface protein. This novel mechanism in Borrelias is remarkably similar to that used by our own immune system to generate diversity of antibodies or T-cell receptors (intra-molecular recombination, deletion of intervening DNA, further diversity generation at the DNA junction site). Given that host antibodies neutralize the emerging serotypes during relapsing fever, this is an example of convergent evolution between bacteria and the immune cells of the host they are infecting.

- **Restrepo** BI, Barbour AG. Antigen diversity in the bacterium B. hermsii through "somatic" mutations in rearranged vmp genes. Cell 1994 Sep 9; 78(5):867-76
- Barbour AG, Dai Q, **Restrepo** BI, Stoenner HG, Frank SA. Pathogen escape from host immunity by a genome program for antigenic variation. Proc.Natl.Acad.Sci.U.S.A 2006 Nov 28; 103(48):18290-5. PMCID or free URL: PMCID: PMC1635980

2. **I did significant advances on diagnostics and on elucidation of the neuroimmunology underlying the pathogenesis of neurocysticercosis (NCC) by *Taenia solium***. During my post-doctoral training (Advisor Dr. Judy Teale) I switched topics to study parasite immunology to increase my knowledge breath. I continued these studies when I moved to my home country in Colombia where I had my own laboratory. For diagnostics I led the characterization of the protein and carbohydrate structures of the most antigenic and specific glycoprotein antigens of this flatworm. We demonstrated that their antigenic component lied in their carbohydrates- hence explaining the failure by other research groups to produce successful recombinant antigens. For neuroimmunology we provided the most detailed immunohistochemical characterization done to date on the immune responses in the human brains of NCC patients, which lead to brain tissue damage and their life-threatening symptoms. This was complemented and contrasted with studies in pig tissues where the parasite does not cause symptoms, and by the development of a mouse model of NCC for experimental testing.
   - Cardona AE, **Restrepo** BI, Jaramillo JM, Teale JM. Development of an animal model for neurocysticercosis: immune response in the central nervous system is characterized by a predominance of gamma delta T cells. J Immunol. 1999 Jan 15; 162(2):995-1002
   - **Restrepo** BI, Obregon-Henao A, Mesa M, Gil DL, Ortiz BL, Mejia JS, Villota GE, Sanzon F, Teale JM. Characterisation of the carbohydrate components of Taenia solium metacestode glycoprotein antigens. Int J Parasitol. 2000 May; 30(6):689-96
   - Obregon-Henao A, Gil DL, Gomez DI, Sanzon F, Teale JM, **Restrepo** BI. The role of N-linked carbohydrates in the antigenicity of Taenia solium metacestode glycoproteins of 12, 16 and 18 kD. Mol.Biochem.Parasitol. 2001 May; 114(2):209-15
   - **Restrepo** BI, Alvarez JI, Castano JA, Arias LF, **Restrepo** M, Trujillo J, Colegial CH, Teale JM. Brain granulomas in neurocysticercosis patients are associated with a Th1 and Th2 profile. Infect.Immun. 2001 Jul; 69(7):4554-60. PMCID or free URL: PMC98532
   - Alvarez JI, Colegial CH, Castano CA, Trujillo J, Teale JM, **Restrepo** BI. The human nervous tissue in proximity to granulomatous lesions induced by Taenia solium metacestodes displays an active response. J Neuroimmunol. 2002 Jun; 127(1-2):139-44
   - Haslam SM, **Restrepo** BI, Obregon-Henao A, Teale JM, Morris HR, Dell A. Structural characterization of the N-linked glycans from Taenia solium metacestodes. Mol.Biochem.Parasitol. 2003 Jan; 126(1):103-7

3. **When I moved back to the US (fled Colombia due to violence) I switched to TB studies due to its public health relevance in the Texas-Mexico border**. **I began doing my first epidemiology studies**.
   a. **Epidemiology of TB and DM2**. We pioneered the discovery of DM2 as a re-emerging risk factor for TB at a time when this was considered an event of the 1950s. Some of our most significant findings that have since been reproduced by other research groups include: **1**) TB-DM (versus TB-no DM) patients are more likely to have multi-drug resistant TB and to present TB characteristics associated with higher infectiousness [cavitary TB, smear positive, delays in smear culture conversion and pulmonary (vs extrapulmonary) TB]. Thus, TB-DM2 patients may be important contributors to the spread of TB in their community. **2**) Nearly 40% of our TB patients in the Texas-Mexico border had DM2 co-morbidity, and development of TB due to DM2 is 28% among adults and nearly 50% among those 35-60 years old (population-attributable fraction). **3**) TB patients with DM2 had been aware of their DM2 status for about 7 years. This highlights missed opportunities for TB prevention among DM2 patients. **4**) DM2 patients not previously aware of their DM2 status are more likely to be males. This points to the importance of TB clinics for screening and early detection of DM2 before DM2 complications, particularly among males.
      - Perez A, Brown HS, III, **Restrepo** BI. Association between tuberculosis and diabetes in the Mexican border and non-border regions of Texas. Am J Trop.Med.Hyg. 2006 Apr; 74(4):604-11.

- **Restrepo** BI, Fisher-Hoch SP, Crespo JG, Whitney E**, Perez A, Smith B, McCormick JB, Nuevo Santander TT. Type 2 diabetes and tuberculosis in a dynamic bi-national border population. Epidemiol.Infect. 2007 Apr; 135(3):483-91 PMCID: PMC2870584
- **Restrepo** BI, Fisher-Hoch SP, Smith B, Jeon S, Rahbar MH, McCormick JB, Nuevo Santander TT. Mycobacterial clearance from sputum is delayed during the first phase of treatment in patients with diabetes. Am J Trop.Med.Hyg. 2008 Oct; 79(4):541-4
- **Restrepo** BI, Camerlin AJ, Mohammad H. Rahbar, Weiwei Wang, Mary A. Restrepo, Izelda Zarate, Richard Wing, Francisco Mora-Guzman, J. Gonzalo Crespo-Solis, Jessica Briggs*, Joseph B. McCormick, Susan P. Fisher-Hoch. Cross-sectional assessment reveals high diabetes prevalence among newly-diagnosed tuberculosis cases. Bull World Health Organ. 2011 89:352-359.

b. **Immunology of TB and DM2**. In TB patients we have pioneered studies showing dysfunctional immunity in TB-DM2. They are characterized by heightened Th1 responses (contrary to what was expected) and probably a reflection of higher bacillary burden. We showed that their higher IFN-γ production did not associated with reduced sensitivity in IGRA assays (in contrast to HIV who are immune-suppressed). In TB-naïve individuals we have found defects in the innate response of DM2 patients to *M. tuberculosis* in vitro, specifically in monocytes and their serum. In recent contacts of TB patients were are finding similar defects in their innate immunity (unpublished). Together, our findings indicate an under-performing innate immune response when a DM2 patient has an initial encounter with *M. tuberculosis*, but an exaggerated and perhaps inefficient cell-mediated immunity once active TB has developed. This dynamic and complex interaction at different stages of TB deserved further elucidation and provides the foundation for the current grant proposal.
   - **Restrepo** BI, Fisher-Hoch SP, Pino PA, Salinas A, Rahbar MH, Mora F, Cortes-Penfield N*, McCormick JB. Tuberculosis in poorly controlled type 2 diabetes: altered cytokine expression in peripheral white blood cells. Clin.Infect.Dis. 2008 Sep 1; 47(5):634-41
   - Mary C. Walsh, Aulasa Camerlin, Reyna Miles, Francisco Mora, J. Gonzalo Crespo, Joseph B. McCormick, Susan P. Fisher-Hoch, Blanca I. **Restrepo**. Sensitivity of Interferon-γ release assays is not compromised in tuberculosis patients with diabetes. Int J Tub Lung Dis. 2011 15(2):179-184.
   - Diana I. Gomez, Marcel Twahirwa, Larry S. Schlesinger, Blanca I. **Restrepo**. (2013) Reduced *Mycobacterium tuberculosis* association with monocytes from diabetes patients that have poor glucose control. Tuberculosis. Mar;93(2):192-7.
   - Blanca I. **Restrepo**, Marcel Twahirwa, Mohammad H. Larry S. Schlesinger. Phagocytosis via complement or Fc-gamma receptors is compromised in monocytes from type 2 diabetes patients with chronic hyperglycemia. (2014) PlosONE 9(3): e92977.

c. **Molecular approaches to TB diagnosis**. We designed novel molecular methods for DNA extraction and mycobacterial quantification in sputum, and adapted to our community existing molecular approaches for detection of *M. tuberculosis* mutations associated with rifampicin resistance (prior to Xpert).
   - Gomez DI, Fisher-Hoch SP, Bordt AS, Quitugua TN, Robledo J, Alvarez N, Correa N, McCormick JB, Restrepo BI. Systematic interpretation of molecular beacon polymerase chain reaction for identifying rpoB mutations in Mycobacterium tuberculosis isolates with mixed resistant and susceptible bacteria. Diagn.Microbiol.Infect.Dis. 2010; 67:37-46.
   - D.I. Gomez**, C.S. Mullin, F. Mora, J. Gonzalo Crespo, S. P. Fisher-Hoch, J.B. McCormick, B.I. Restrepo. (2011) Rapid DNA extraction for specific detection and quantitation of Mycobacterium tuberculosis DNA in sputum specimens using taqman assays. Tuberculosis. 91: S43-S48;.

d. **Field studies for a variety of proof-of-concept studies**. Our access to more TB patients than many other TB study sites in the US and our field experience provides a resource to scientists across the US who want to test new diagnostics, immunoassays, basic science principles, field testing or other novel products in TB patients. I have been the local PI for a variety of studies. A recent and exciting collaboration is with Drs. Sarah Fortune and Galit Alter on the characterization of humoral immunity where we are demonstrating differential Fc receptor effector functions and glycosylation patterns in sera from TB versus LTBI patients.
   - Nira R. Pollock, Lilia Macovei, Kelly Kanunfre, Rakesh Dhiman, Blanca I. **Restrepo**, Izelda Zarate, Paula A. Pino, Francisco Mora-Guzman, Gerd Michel, Suely S. Kashino, Antonio Campos-Neto.

(2013) Validation of *Mycobacterium tuberculosis* Rv1681 protein as a diagnostic marker of active pulmonary tuberculosis. J Clin Microbiol. 51(5); 1367-1373 (2013 Feb 6.

- Lenette L. Lu, Amy W. Chung, Tracy Rosebrock, Musie Ghebremichael, Wen Han Yu, Matthew K. Schoen, Fikadu Tafesse, Constance Martin, Vivian Leung, Alison E. Mahan, Magdalena Sips, Jacquelynne Tedesco, Hannah Robinson, Elizabeth Tkachenko, Katherine J. Freedberg, Hendrik Streeck, Todd J. Suscovich, Monia Draghi, Douglas Lauffenburger, Blanca I. **Restrepo**, Cheryl Day, Sarah Fortune, Galit Alter. A functional role for antibodies in tuberculosis. Cell. (*submitted*)

D. Research Support in past 3 years

Ongoing Research Support

[redacted]

Research Support completed in past three years

Pilot-CCTS Restrepo (PI) Sept 2013-2014

Impact of the glucose-lowering medication Metformin on blood monocyte immune responses to tuberculosis in type 2 diabetes patients

The goal is to conduct pilot studies in-vitro and in-vivo to determine whether metformin enhances the phagocytosis and containment of Mycobacterium tuberculosis within monocytes from diabetes patients

Role: PI of Pilot study

P20 MD000170-04 McCormick (PI) Feb 2008-2013

Training core

The goal is to identify local students from this community and provide them with the scientific environment (Journal Clubs, Seminars) and the opportunity to participate in research projects. This will provide them with the opportunity to understand the process and hopefully motivate them to pursue a higher degree in the health sciences.

Role: PI of Training Core

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: Eric J. Rubin

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Irene Heinz Given Professor of Immunology and Infectious Diseases

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
Harvard College	AB	06/1980	Biochemical Science
Tufts University School of Medicine	MD	05/1990	
Tufts University	PhD	05/1990	Microbiology & Genetics

A. Personal Statement

My research focuses on tuberculosis, its pathogenesis and interventions that can help treat and prevent disease. My lab has developed many of the genetic tools that are used to study the causative organism, *Mycobacterium tuberculosis* and employed these to understand the molecular mechanisms underlying virulence and susceptibility and resistance to antibiotics. I have trained a number of graduate students and postdoctoral fellows, most of whom have gone on to academic careers. I continue to interact extensively with former trainees and to help with grants and career advice.

B. Positions and Honors

Positions and Employment

1990-1992 Intern and Resident in Medicine, Massachusetts General Hospital
1992-1997 Clinical and Research Fellow, Infectious Disease Unit, Massachusetts General Hospital
1993-1997 Research Fellow, Department of Microbiology and Molecular Genetics, Harvard Medical School
1997-2004 Instructor in Medicine, Harvard Medical School
1997 Associate Physician, Brigham and Women's Hospital
1999-2005 Assistant Professor, Harvard School of Public Health
2004 Assistant Professor of Medicine (Microbiology and Molecular Genetics), Harvard Medical School
2005-2008 Associate Professor, Harvard School of Public Health
2008 Professor, Harvard School of Public Health
2011 Senior Associate Member, Broad Institute
2014 Irene Heinz Given Professor of Immunology and Infectious Diseases

Other Experience and Professional Memberships

1989 Alpha Omega Alpha
1993-1994 Howard Hughes Medical Institute Physician Postdoctoral Fellow
2003-now Member, NIH AIDS Discovery and Development of Therapeutics (ADDT) study section, Chair, 2007-2008
2003-2008 Burroughs Wellcome Investigator in Pathogenesis of Infectious Disease
2009 Chair, TB Drug Development Gordon Research Conference
2009-2014, Scientific Advisory Committee, Global Alliance for TB Drug Development, Chair, 2012-2014

&2016-now
2012 Fellow, American Academy of Microbiology
2012-now Associate Editor, *New England Journal of Medicine*
2013 Organizer, TB Keystone Symposia
2013-now Interview Panel, Wellcome Trust
2016-now Member, NIH Microbiology and Infectious Diseases Study Section
Other Scientific Advisory Committees: NIAID Systems Biology Program (Co-Chair), Singapore-MIT Alliance for Research and Training, Structure-based Drug Design Consortium, TB Research Unit – Weill-Cornell Medical College, TB Research Unit – Boston University, SANTHE – University of KwaZulu-Natal (Chair)

C. Contribution to Science

1. *Defining key determinants of bacterial survival and growth*. Using methods described below we have found the key determinants of *Mycobacterium tuberculosis* survival and growth, both *in vitro* and during infection. This has been the jumping off point for in-depth study of key bacterial proteins and processes with important applications in early efforts to define potential antibiotic targets and for creating attenuated strains for vaccines and for challenge studies.

a. Sassetti CM, Boyd DH, Rubin EJ. Genes required for mycobacterial growth defined by high density mutagenesis. Mol Microbiol. 2003. 48(1):77-84. PMID: 12657046.
b. Sassetti CM, Rubin EJ. Genetic requirements for mycobacterial survival during infection. Proc Natl Acad Sci USA. 2003. 100(22):12989-94, PMCID: PMC240732.
c. Rengarajan J, Bloom BR, Rubin EJ. Genome-wide requirements for *Mycobacterium tuberculosis* adaptation and survival in macrophages. Proc Natl Acad Sci. 2005. 102(23):8327-32, PMCID: PMC1142121.
d. Zhang YJ, Ioerger TR, Huttenhower C, Long JE, Sassetti CM, Sacchettini JC, Rubin EJ. Global assessment of genomic regions required for growth in *Mycobacterium tuberculosis*. PLoS Pathog. 2012. 8(9):e1002946, PMCID: PMC3460630.

2. Understanding the physiology of bacterial growth. Mycobacteria are quite distinct from heavily studied model organisms. We have studied the cell biology of these bacteria concentrating on their unique aspects. Our contributions include studying the unusual mode of cell division (which, we have found, requires the formation of distinctive protein complexes), translational control systems (involving an error-prone system and numerous unusual ribosome-interacting proteins) and protein turnover (with a central role for the uniquely-arranged Clp protease). All of these represent attractive targets for therapeutic approaches.

a. Raju RM, Unnikrishnan M, Rubin DH, Krishnamoorthy V, Kandror O, Akopian TN, Goldberg AL, Rubin EJ. Mycobacterium tuberculosis ClpP1 and ClpP2 function together in protein degradation and are required for viability in vitro and during infection. PLoS Pathog. 2012. 8(2):e1002511. PMCID: PMC3280978.
b. Chao MC, Kieser KJ, Minami S, Mavrici D, Aldridge BB, Fortune SM, Alber T, Rubin EJ. Protein complexes and proteolytic activation of the cell wall hydrolase RipA regulate septal resolution in mycobacteria. PLoS Pathog. 2013. 9(2):e1003197, PMCID: PMC3585148.
c. Raju RM, Jedrychowski MP, Wei JR, Pinkham JT, Park AS, O'Brien K, Rehren G, Schnappinger D, Gygi SP, Rubin EJ. Post-translational regulation via Clp protease is critical for survival of Mycobacterium tuberculosis. PLoS Pathog. 2014. 10(3):e1003994, PMCID: PMC3946367.
d. Kieser, KJ, Baranowski, C, Chao, MC, Long, JE, Sassetti, CM, Waldor, MK, Sacchettini, JC, Ioerger, TR, Rubin EJ. Peptidoglycan synthesis in *Mycobacterium tuberculosis* is organized into networks with varying drug susceptibility. Proc Natl Acad Sci. 2015. 112(42):13087-92, PMCID: PMC4620856.

3. Understanding mechanisms underlying host-pathogen interactions. Mycobacterium tuberculosis has evolved along with its human host to be an optimal pathogen. Thus, both bacterial and host factors contribute to the development of disease. We have investigated both sides of this relationship by using widespread screens and targeting candidate genes. These approaches have found key host receptors, intracellular modulators of growth, host metabolic contributions and complex bacterial secretory pathways.

a. Fortune SM, Jaeger A, Sarracino DA, Chase MR, Sassetti CM, Sherman DR, Bloom BR, Rubin EJ. Mutually dependent secretion of proteins required for mycobacterial virulence. Proc Natl Acad Sci. 2005. 102(30):10676-81, PubMed PMCID: PMC1176248.
b. Zhang YJ, Reddy MC, Ioerger TR, Rothchild AC, Dartois V, Schuster BM, Trauner A, Wallis D, Galaviz S, Huttenhower C, Sacchettini JC, Behar SM, Rubin EJ. Tryptophan biosynthesis protects mycobacteria from CD4 T-cell-mediated killing. Cell. 2013. 155(6):1296-308, PMCID: PMC3902092.
c. Martinot AJ, Farrow M, Bai L, Layre E, Cheng TY, Tsai JH, Iqbal J, Annand JW, Sullivan ZA, Hussain MM, Sacchettini J, Moody DB, Seeliger JC, Rubin EJ. Mycobacterial Metabolic Syndrome: LprG and Rv1410 Regulate Triacylglyceride Levels, Growth Rate and Virulence in Mycobacterium tuberculosis. PLoS Pathog. 2016.12:e1005351. PMCID: PMC4709180.
d. Marakalala MJ, Raju RM, Sharma K, Zhang YJ, Eugenin EA, Prideaux B, Daudelin IB, Chen PY, Booty MG, Kim JH, Eum SY, Via LE, Behar SM, Barry CE 3rd, Mann M, Dartois V, Rubin EJ. Inflammatory signaling in human tuberculosis granulomas is spatially organized. Nature Med. 2016. 22: 531-538, PMCID: PMC4860068.

4. Defining mechanisms of antibiotics and antibiotic resistance. One of the primary goals of our work has been to develop novel approaches to developing new antibiotics and to understand the action of existing drugs. These efforts remain ongoing within two program project grants aimed at defining drug resistance mechanisms and developing new antibiotic screening methods.

a. La Rosa V, Poce G, Canseco JO, Buroni S, Pasca MR, Biava M, Raju RM, Porretta GC, Alfonso S, Battilocchio C, Javid B, Sorrentino F, Ioerger TR, Sacchettini JC, Manetti F, Botta M, De Logu A, Rubin EJ, De Rossi E. MmpL3 is the cellular target of the antitubercular pyrrole derivative BM212. Antimicrob Agents Chemother. 2012. 56(1):324-31, PMCID: PMC3256021.
b. Javid B, Sorrentino F, Toosky M, Zheng W, Pinkham JT, Jain N, Pan M, Deighan P, Rubin EJ. Mycobacterial mistranslation is necessary and sufficient for rifampicin phenotypic resistance. Proc Natl Acad Sci. 2014. 111(3):1132-7, PMCID: PMC3903211.
c. Dragset MS, Poce G, Alfonso S, Padilla-Benavides T, Ioerger TR, Kaneko T, Sacchettini JC, Biava M, Parish T, Argüello JM, Steigedal M, Rubin EJ. A novel antimycobacterial compound acts as an intracellular iron chelator. Antimicrob Agents Chemother. 2015. 59(4):2256-64, PubMed PMCID: PMC4356758.
d. Akopian T, Kandror O, Tsu C, Lai JH, Wu W, Liu Y, Zhao P, Park A, Wolf L, Dick LR, Rubin EJ, Bachovchin W, Goldberg AL. Cleavage specificity of Mycobacterium tuberculosis ClpP1P2 protease and identification of novel peptide substrates and boronate inhibitors with anti-bacterial activity. J Biol Chem. 2015. 290(17):11008-20, PMCID: PMC4409261.

5. Creating tools broadly useful for bacterial genetics. We constructed one of the most widely used transposons in bacterial mutagenesis and an efficient delivery system for many species of bacteria. We used this to develop an approach, Transposon Site Hybridization (TraSH) to perform high-density whole genome screening for selectable trait. With the advent of high throughput sequencing, we have continued to innovate, using similar methods for studying genetic interactions and developing new protein-silencing methods to study gene function.

a. Sassetti CM, Boyd DH, Rubin EJ. Comprehensive identification of conditionally essential genes in mycobacteria. Proc Natl Acad Sci. 2001. 98(22):12712-7, PMCID: PMC60119.
b. Joshi SM, Pandey AK, Capite N, Fortune SM, Rubin EJ, Sassetti CM. Characterization of mycobacterial virulence genes through genetic interaction mapping. Proc Natl Acad Sci. 2006. 103(31):11760-5, PMCID: PMC1544243.

c. Wei JR, Krishnamoorthy V, Murphy K, Kim JH, Schnappinger D, Alber T, Sassetti CM, Rhee KY, Rubin EJ. Depletion of antibiotic targets has widely varying effects on growth. Proc Natl Acad Sci. 2011. 108(10):4176-81, PMCID: PMC3053961.
d. Pritchard JR, Chao MC, Abel S, Davis BM, Baranowski C, Zhang YJ, Rubin EJ, Waldor MK. ARTIST: high-resolution genome-wide assessment of fitness using transposon-insertion sequencing. PLoS Genet. 2014. 10(11):e1004782, PMCID: PMC4222735.

URL to complete bibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/eric.rubin.1/bibliography/41166769/public/?sort=date&direction=ascending.

D. Research Support

Ongoing Research Support

[redacted]

5P01AI095208-03 J. Sacchettini (PI) 09/01/12 – 08/31/16
NIH/NIAID
Structure-based Discovery of Critical Vulnerabilities of Mycobacteria
The goal of this project is to perform as the Chemical and Genetics Core for the Tuberculosis Structural Genomics Center, including generation of conditional mycobacterial mutants, identification of protein interactions, protein localization and identification of small molecule inhibitors of targeted proteins.
Role: Subcontract Principal Investigator

1R01AI116604-01 B. Moody (PI) 02/01/15 – 01/31/17
NIH/NIAID
Role of tuberculosinyl metabolites in M. tuberculosis virulence
The major goals of this project are to study the biogenesis of TbAd and its effect on virulence in *Mycobacterium tuberculosis.*
Role: Subcontract Principal Investigator

1R21AI116142-01 A. Pym (PI) 02/25/15 – 01/31/17
NIH/NIAID
Fate of M. tuberculosis Antibiotic Survivors
The major goal of this project is to study genetic determinants of persistence.
Role: Subcontract Principal Investigator

5R01AI097191-04 K. Derbyshire (PI) 05/15/12 – 04/30/17
NIH/NIAID
A Community Mycobacterial Systems Resource
The major goal of this project is to study the phenotypes of *Mycobacterium smegmatis* mutants.
Role: Subcontract Principal Investigator

U19 AI107774-03 E. Rubin (PI) 07/02/13-06/30/18
NIH/NIAID
Decoding the roles of critical genes of unknown function in M. tuberculosis
The major goal of this project is to experimentally define the roles of genes. We will concentrate on a particularly important class: those that are required for the optimal growth of M. tuberculosis.
Role: Principal Investigator

[redacted]

U19 AI109755-02 M. Murray (PI) 03/01/14-02/28/19
NIH/NIAID

CETR: Discovery and validation of drug resistance mutations
The goal of this project is to identify and validate which of the resistance-associated mutations are causally connected to either high-level drug resistance or an increased risk of developing resistance.
Role: Subcontract Principal Investigator

P30 AI060354-12 T. Maira-Litran (PI) 08/01/15-07/31/16
NIH/NIAID - HU CFAR (Center for AIDS Research)
Vaccination against Mycobacterium tuberculosis
The goal of this project is to test the efficacy and safety of a vaccine candidate in the mouse model of TB.
Role: Subcontract Principal Investigator

Completed Research Support

[redacted]

P01 AI074805- T. Cross (PI) 08/20/09 – 07/31/14
NIH/NIAID
M tuberculosis Membrane Protein Pharmaceutical Targets
The major goal of this project was to construct knockout and knockdown strains for targeted genes in *M. tuberculosis*.
Role: Subcontract Principal Investigator

P30 AI060354- B. Walker (PI) 08/01/11 – 07/31/14
NIH/NIAID HU CFAR
Harvard University Center for AIDS Research: A Small Animal Model for HIV/TB
This project was to study HIV/TB co-infection in a humanized mouse animal model.
Role: Subcontract Principal Investigator

[redacted]

FORTUNE, SARAH M.

ACTIVE

[redacted]

R01 AI097191-05 (K. Derbyshire) 05/15/12-04/30/17
NIH/NIAID $[redacted]
A Community Mycobacterial Systems Resource
The major goal of this project is to study the phenotypes of Mycobacterium smegmatis mutants.

No Award No. (B. Walker) 07/01/15-06/30/17
Ragon Institute of MGH, MIT, and Harvard $[redacted]
Engineering a model of transmitted M. tuberculosis
We propose to develop a droplet based system to reproducibly capture individual Mycobacterium tuberculosis for infection for delivery as an inocula in nonhuman primate (NHP) infections.

[redacted]

U19 AI107774-03 (E. Rubin) 07/02/13-06/30/18
NIH/NIAID $[redacted]
Decoding the roles of critical genes of unknown function in M. tuberculosis
The major goal of this project is to experimentally define the roles of genes. We will concentrate on a particularly important class: those that are required for the optimal growth of M. tuberculosis.

U19 AI109755-03 (M. Murray) 03/01/14-02/28/19
NIH/NIAID $[redacted]
CETR: Discovery and validation of drug resistance mutations
The goal of this project is to identify and validate which of the resistance-associated mutations are causally connected to either high-level drug resistance or an increased risk of developing resistance.

R01 AI11871-03 (P. Lin) 03/01/14-02/28/19
NIH/NIAID $[redacted]
HIV-TB Co-infection: Tracking TB emergence after asymptomatic (latent) infection
The goal of this project is to use new molecular tools in the nonhuman primate model of tuberculosis to establish infection dynamics in the presence and absence of SIV infection. The Fortune lab is responsible for molecular analysis of viable and cumulative bacterial burdens in macaque lesions.

P30 AI060354-12 (B. Walker) 08/01/13-07/31/19
NIH/NIAID $[redacted]
HU CFAR (Center for AIDS Research)

Harvard University Center for AIDS Research: Core B Developmental Core
The primary goal of the Developmental Core of the Harvard University (HU) CFAR is to promote basic, clinical, behavioral and translational HIV/AIDS research activities at Harvard University.

R01 AI114674-02 (S. Fortune and J. Flynn) 07/01/15-12/31/19
NIH/NIAID $[redacted]
The Consequences of Reinfection with M. tuberculosis
The Fortune lab will be responsible for the proposed studies to generate tagged libraries of various M. tuberculosis strains as proposed in Aims 1 and 3.

[redacted]

R01 AI123286-01 (S. Behar) 02/15/16-01/31/21
NIH/NIAID $[redacted]
Tuberculosis and T cell recognition
The goal of this project is to determine the relative importance of quantitative differences in bacterial antigen abundance to T cell recognition of Mtb infected cells.

PENDING

None.

ALTER, GALIT

ACTIVE

5 P30 AI060354-11 (Walker) 08/01/14 – 07/31/19
NIH $[redacted] [Alter]
Harvard Medical School Center for AIDS Research
The major goals of this project are to 1) to consolidate and expand existing collaborations among the members of two existing, highly successful HMS-affiliated CFARs and 2) to promote new interactions and research initiatives capable of more effectively addressing key AIDS research questions. Dr. Alter will serve as the Head of the CFAR Immunology Core at MGH.

2U19 A166345-09 (Lauer) 08/01/10-07/31/16
Project Leader $[redacted]
Immune Control and Evasion during Acute HIV Infection – Project 2
This project aims to determine the role of NK cells in acute HCV infection, providing new potential therapeutic avenues to enhance the activity of particular NK cell populations to drive HCV clearance.

[redacted]

R01 AI 102660-04 (Alter) 07/01/12-06/30/16
NIH/NIAID $350,000
Tuning Fc-effector functions of HIV-specific antibodies
This proposal aims to define the mechanim(s) regulating antibody glycosylation in B cells. These studies may identify new strategies to specifically tune B cell responses during vaccination to induce antibodies with specific antiviral activity.

[redacted]

[redacted]

Ragon Institute of MGH, MIT & Harvard (Alter) 01/01/14-12/31/16
Ragon Clinical Trials Initiative Lab Studies Alter $[redacted]
The goal of these studies is to evaluate the non-neutralizing antibody responses elicited following Ad26, Ad35, and Ad5HVR48 vaccination of humans.

4R33AI110165-03 (Alter) 12/01/2015 – 01/31/2019
NIH $[redacted]
Killing the Reservoir with Antibodies
The goal of this award is to develop a monoclonal therapeutic approach, using Fc-enhanced anitbodies, to kill reactivated latently infected cells.

[redacted]

2R37AI080289-06A1 (Alter) 09/01/14 – 08/31/19
NIH/NIAID $[redacted]
Demystifying the antiviral activity of the IgG3+ antibody response
The goal of this project is to explore and define the specificity/functionality of IgG3+ B cell responses and the mechanism, by which the immune system programs such potent antiviral humoral immunity.

[redacted]

5R01AI102660-04 (Alter) 07/01/15 – 06/30/16
NIH $[redacted]
Tuning Fc-effector functions of HIV-specific antibodies: Administrative Supplement
This study supports vaccine development efforts, and specifically the characterization of the protective IgG3 signature identfied in RV144, through the development of a robust set of assays that probe and profile the non-neutralizing antibody response in remarkable depth, but in a highly objective manner, likely required for the idetnification of unexpected correlates of protective immunity against HIV.

(Walker/Scully) 08/01/2015 – 07/31/16
NIH/CFAR $[redacted]
HU FAR Supplement
Harvard University Center for AIDS Research: Host pathogen glycans in HIV-TB
These studies will provide the first critical insights into the specific humoral immune profiles that if induced via vaccination may provide enhanced control and potential clearance of MTB, as well as define novel and critical therapeutic strategies to enhance MTB control among HIV infected subjects globally.

[redacted]

[redacted]

[redacted]

(Saphire) 03/01/2016 – 02/28/2017
NIH 5U19AI109762 $[redacted]
The Consortium for Immunotherapeutics Against Viral Hemorrhagic Fevers/Supplement
The goal of this project is to define the extra-neutralizing functions of VIC-derived monoclonal antibodies and the contribution these functions play in providing protection and/or clearance of infection.

PENDING:

[redacted]

NIHU19 (Lauer) 09/01/2015 – 08/31/2020
NIH $[redacted]
The Role of NK Cells during Acute HCV Infection and Antiviral Therapy
The goal of this project is to determine the the role of NK cells in the resolution of acute HCV infection.

(Barouch) 07/01/2016 – 06/30/2021
NIH 1UM1AI124377 - 01 $[redacted]
Combined Immunologic Approaches to Cure HIV-1 - Focus 2
The goal of this project is to develop next-generation “killer” monoclonal antibodies to aggressively purge the HIV reservoir following reactivation.

(Barouch) 07/01/2016 – 06/30/2021
NIH 1UM1AI124377 - 01 $[redacted]
Combined Immunologic Approaches to Cure HIV-1 - Focus 2
The goal of this project is to profile the vaccine-induced humoral immune responses induced by therapeutic vaccination in both non-human primates and humans to define the the reservoir-depleting activity of these responses.

(Alter) 09/01/2016 – 08/31/2021
NIH 1 R01 AI127765-01 $[redacted]
Defining protective humoral immune responses against Ebola virus
The goal of this project is to define the specific antibody response profiles, both with respect to specificity and function, that may provide the broadest level of protective immunity against filoviruses at a global level.

(Coler) 12/01/17 – 11/30/2021
NIH U19 $[redacted]
A platform for profiling molecular signatures predictive of TRL4 vaccine response in clinical cohorts- Alter P1

The goal of this project is perform serological profiling of vaccine induced antibodies linked to anti-viral/microbial functions of these antibodies to define the immunological correlates of vaccine induced protective immunity.

(El Haddad) 12/01/16 – 11/30/2021
NIH U19 $[redacted]
Defining the Impact of Parasitic Co-Infection on Vaccine Induced Humoral Immunity-P1
The goal of this project is to define the impact of parasitic infection on modulating vaccine induced humoral immunity against hepatitis B virus.

(Lauer) 12/01/16 – 11/30/2021
NIH U19 $[redacted]
Systems glyco-immunology in viral infections of the liver - P2
The goal of this project is define the impact of changes in antibody glycosylation following hepatic viral infection on regulation of humoral immunity.

(Sekaly) 12/01/16 – 11/30/2021
NIHU19 $[redacted]
SIMBHAD (Systems Immunology Basis of HIV Acquisition and Dissemination)
The goal of this project is to define the antiviral profiles of natural and HIV-specific antibodies that may contribute to antiviral control or protection from HIV acquisition.

(Sekaly) 12/01/16 – 11/30/2021
NIHR01 $[redacted]
An unbiased OMICs approach to identify mechanisms of Cocaine regulation of the HIV reservoir
The goal of this project is to determine the impact of cocaine use on altering antibody effector functions that may disrupt humoral immune control of the HIV viral reservoir.

(Fouts) 12/01/17 – 11/30/2021
NIHR01 $[redacted]
Optimizing DNA prime/subunit boost regimen to enhance Fc-mediated effector functions
The goal of this project is examine the antibody-effector functional activity of vaccine induced antibodies following DNA prime/boosting.

(Juelg) 12/01/16-11/30/21
NIH R01 $[redacted]
Eradicating the HIV reservoir with glycosylation-optimized broadly functional antibodies (bFAbs)
This proposal aims to develop a set of glyco-engineered antibodies that are able to kill reactivated latently infected cells to elicit highly specialized anti-viral functions able to eradicate the reservoir in vivo in NHP following reactivation.

(Barouch) 12/01/16-11/30/21
NIH R01 $[redacted]
Optimization of Broadly Neutralizing Antibodies for HIV Eradication
The goal of this project is to develop a bi- or tetra-specific Fc-engineered single monoclonal antibody the covers global viral diversity and drive the rapid and highly effective clearance of reactivated latently infected cells to test in vivo in NHP following viral reactivation.

(Saphire) 06/01/2016-02/28/2018
NIH5U19AI109762 $[redacted]
Innovative tools to facilitate therapeutic/vaccine discovery
The goal of this project is to downselect and develop an optimized cocktail of monoclonal antibodies that are able to control/eradicate ebola infection most effectively.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **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 .		Lenette		Lu		PD/PI	[redacted]	9.0			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

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						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **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	[redacted]

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	[redacted]

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**	[redacted]

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

Tracking Number: GRANT12189025 Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
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	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	8.0	186,340.00	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) Louis Martillotti Department of Health & Human Services Telephone: 212-264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: FINAL_Budget_Justification.pdf
(Only attach one file.)

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

Tracking Number: GRANT12189025 Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2018 **End Date*:** 03-31-2019 **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 .		Lenette		Lu		PD/PI		9.0					

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

Additional Senior Key Persons: File Name: **Total Senior/Key Person**

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						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2018 **End Date*:** 03-31-2019 **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	[redacted]

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	[redacted]

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**	[redacted]

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

Tracking Number: GRANT12189025 Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2018 **End Date*:** 03-31-2019 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
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	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	8.0	183,265.00	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Louis Martillotti Department of Health & Human Services Telephone: 212-264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: FINAL_Budget_Justification.pdf
(Only attach one file.)

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

Tracking Number: GRANT12189025 Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **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 .		Lenette		Lu		PD/PI		9.0					

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

Additional Senior Key Persons: File Name: **Total Senior/Key Person**

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						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **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	[redacted]

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	[redacted]

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**	[redacted]

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

Tracking Number: GRANT12189025 Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
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	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	8.0	187,000.00	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) Louis Martillotti Department of Health & Human Services Telephone: 212-264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: FINAL_Budget_Justification.pdf
(Only attach one file.)

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

Tracking Number: GRANT12189025 Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **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 .		Lenette		Lu		PD/PI		9.0					

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

Additional Senior Key Persons: File Name: **Total Senior/Key Person**

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						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **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	[redacted]

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	[redacted]

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**	[redacted]

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

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
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	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	8.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Louis Martillotti Department of Health & Human Services Telephone: 212-264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: FINAL_Budget_Justification.pdf
(Only attach one file.)

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

Tracking Number: GRANT12189025 Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **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 .		Lenette		Lu		PD/PI		9.0					

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

Additional Senior Key Persons: File Name: **Total Senior/Key Person**

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						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **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	[redacted]

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	[redacted]

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**	[redacted]

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

Tracking Number: GRANT12189025 Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: THE GENERAL HOSPITAL CORP DBA MASSACHUSETTS GENERAL HOSPITAL

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
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	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	8.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Louis Martillotti Department of Health & Human Services Telephone: 212-264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: FINAL_Budget_Justification.pdf
(Only attach one file.)

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

Tracking Number: GRANT12189025 Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

BUDGET JUSTIFICATION

Senior/Key Person

Lenette Lu, MD, PhD, an instructor of Medicine at Harvard Medical School and Assistant in Medicine at Massachusetts General Hospital, is the Principal Investigator of this Career Development Award. Support in the amount of $100,000 plus fringe, at the DHHS negotiated rate of 37%, is requested for Dr. Lu in years 1-5. This salary is calculated using an annual institutional base salary commensurate with others of comparable training and responsibility at Massachusetts General Hospital. Dr. Lu will commit a minimum of 9 calendar months (75% effort) for the duration of this project.

Mentors:
Sarah Fortune, MD, Professor of Immunology and Infectious Diseases at the Harvard T.H. Chan School of Public Health, Associate Member and TB Program Director at the Ragon Institute of MGH, MIT and Harvard will serve as Dr. Lu's primary mentor on this Career Development Award. Dr Fortune has a well established NIH supported laboratory both at the Harvard School of Public Health and at the Ragon Institute of MGH, MIT and Harvard with a focus on determinants of the variability in clinical outcomes of tuberculosis infection and treatment. No salary support is requested.
Galit Alter, PhD, Associate Professor of Medicine at MGH, member of the Ragon Institute of MGH, Harvard and MIT, faculty of the Harvard Immunology and Virology Programs and an executive committee member of the Harvard Center for AIDS Research will serve as a co-mentor for Dr. Lu for this Career Development Award. Dr Alter has a well established, NIH funded laboratory in innate effector mechanisms of antibodies. No salary support is requested.

Other Significant Contributors:
Eric Rubin, MD, PhD, the Irene Heinz Given Professor of Immunology and Infectious Diseases at the Harvard School of Public Health. Dr Rubin is a prominent senior physician-scientist in tuberculosis research, infectious diseases and medicine, and will serve as a member of the Advisory Committee for this Career Development award. No salary support is requested.
Akiko Iwasaki, PhD, Howard Hughes Medical Institute Investigator and the Waldemar von Zedtwitz Professor of Immunobiology and of Molecular, Cellular and Developmental Biology at Yale University School of Medicine. Dr Iwasaki is a leader in innate immunity in viral infections and the link to adaptive immunity, and will serve as a member of the Advisory Committee for this Career Development award. No salary support is requested.
Falk Nimmerjahn, PhD, head of the Division of Genetics at University of Erlangen. Dr Nimmerjahn is an internationally recognized expert in understanding mechanisms of IgG effector activity via Fc receptors, and will serve as a member of the Advisory Committee for this Career Development award. No salary support is requested.
Facundo Batista, PhD, the Phillip and Susan Ragon Professor at Harvard Medical School and the Ragon Institute, member and Associate Director of the Ragon Institute of MGH, MIT and Harvard. Dr Batista is a renowned leader in combining state of the art imaging technology the cellular and molecular events activating B cells, and will serve as a member of the Advisory Committee for this Career Development award. No salary support is requested.

Collaborators:
Blanca Restrepo, PhD, Associate Professor, Division of Epidemiology at University of Texas Health Houston. Dr Restrepo is the principal investigator enrolling a cohort of latent and active TB patients in South Texas for immunological profiling. She will provide study samples. No salary or reagent support is requested.
Douglas Hayden, PhD, Assistant Professor of Medicine Harvard Medical School. Dr Hayden is a Biostatistician at the MGH Biostatistics Center with expertise in statistical methods associated with modern high dimensional datasets. He will provide statistical support. No salary support is requested.

Other Direct Costs

Materials and Supplies

Per the Career Development Instructions for NIH SF424 Applications, the total research development support being requested was entered as (F) Other Direct Costs (1) Materials and Supplies. $[redacted] has been requested annually in Year 1-5, for anticipated costs such as:

Tuition and Fees: Formal coursework to be completed in Years 1 and 2, as outlined in the Career Development Plan. The registration fee for Seeing is Believing EMBL in Year 1 is [redacted] ($[redacted] The tuition for Quantitative Imaging at Cold Spring Harbor Laboratory in Year 2 is $[redacted] Harvard Catalyst classes are free. Courses such as these are critical to becoming an independent in microscopy and cell biology.
Travel: $[redacted] for roundtrip economy airfare (Approximately $[redacted] accommodations, (Approximately [redacted] $[redacted] for 5 nights) and other travel related expenses, to complete the Seeing is Believing EMBL course in Heidelberg, Germany in Year 1; remaining funds will also be used for travel and accommodations to attend a scientific conference such as: Keystone and European Molecular Biology Organization. $[redacted] is also expected in Years 2-5, for continued annual attendance of a scientific conference.
Publications: $[redacted] for publication fees and the cost of preparations in Years 2-5 of the award.
Reagent/Supplies: For Year 1, $[redacted] Year 2, $[redacted] and Years 3-5, $[redacted] is requested for reagents/ supplies and core services including Microarray, Flow cytometry, Microscopy and BSL3 core charges. Reagents and supplies will include items such as tissue culture supplies, MTB proteome array slides, MTB antigens, monocyte selection kits, antibody purification kits and ultrafiltration units, PNGase, Ides, antibody quantification ELISA kits, siRNA and electroporation kits.

Indirect Costs

Indirect Costs of were calculated based on a Modified Total Direct Costs rate of 8% consistent with Massachusetts General Hospital's negotiated DHHS rate for Career Development Awards. The tuition and fees costs, identified above, are excluded from the MTDC base.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
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		[redacted]
1. Materials and Supplies	[redacted]	
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)		[redacted]
Section H, Indirect Costs		[redacted]
Section I, Total Direct and Indirect Costs (G + H)		[redacted]
Section J, Fee		0.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ○ Yes ● No

*Agency-Defined Phase III Clinical Trial? ○ Yes ○ No

2. 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 proved scientific justification

3. *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

4. 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, please 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):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ○ Yes ○ No

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

*Previously Reported: ○ Yes ○ No

6. 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:

Tracking Number: GRANT12189025 Funding Opportunity Number: PA-16-191 . Received Date: 2016-06-10T09:57:43.000-04:00

PHS 398 Career Development Award Supplemental Form

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (RESUBMISSION)	
Candidate Section	
2. Candidate Information and Goals for Career Development	FINAL_Candidate_Information.pdf
Research Plan Section	
3. Specific Aims	FINAL_Specific_Aims.pdf
4. Research Strategy*	06102016_Research_Plan.pdf
5. Progress Report Publication List (for RENEWAL applications only)	
6. Training in the Responsible Conduct of Research	FINAL_Training_in_the_Responsible_Conduct_of_Research.pdf
Other Candidate Information Section	
7. Candidate's Plan to Provide Mentoring	
Mentor, Co-Mentor, Consultant, Collaborators Section	
8. Plans and Statements of Mentor and Co-Mentor(s)	FINAL_Mentor_Letter.pdf
9. Letters of Support from Collaborators, Contributors, and Consultants	FINAL_Letters_of_support.pdf
Environment and Institutional Commitment to Candidate Section	
10. Description of Institutional Environment	FINAL_DescriptionoftheInstitutionalEnvironment.pdf
11. Institutional Commitment to Candidate's Research Career Development	Final_Insitutional_Commitment.pdf
Human Subject Section	
12. Protection of Human Subjects	06072016_FINAL_Protection_of_Human_Subjects.pdf
13. Data Safety Monitoring Plan	
14. Inclusion of Women and Minorities	06072016_Inclusion_of_women_and_minorities.pdf
15. Inclusion of Children	06072016_Inclusion_of_Children.pdf
Other Research Plan Section	
16. Vertebrate Animals	
17. Select Agent Research	
19. Consortium/Contractual Arrangements	
19. Resource Sharing	FINAL_Resource_Sharing_Plan.pdf
20. Authentication of Key Biological and/or Chemical Resources	FINAL_Authentication_of_Key_Biological.pdf
Appendix	
21. Appendix	

Funding Opportunity Number: PA-16-191. Received Date: 2016-06-10T09:57:43.000-04:00

PHS 398 Career Development Award Supplemental Form

Citizenship*:

U.S. Citizen or Non-Citizen National?* ☑ Yes ❑ No

If no, select most appropriate Non-U.S. Citizen option

- ❑ With a Permanent U.S. Resident Visa
- ❑ With a Temporary U.S. Visa
- ❑ Not Residing in the U.S.

If with a temporary U.S. visa who has applied for permanent resident status and expect to hold a permanent resident visa by the earliest possible start date of the award, also check here: ❑

Tracking Number: GRANT12189025 Funding Opportunity Number: PA-16-191. Received Date: 2016-06-10T09:57:43.000-04:00

Candidate Information

My career goal is to combine my basic science foundation, focused on mechanistic understanding of pathogenesis of infectious diseases, with my clinical skills to drive scientific questions informed by clinical insight and generate data with potential clinical implications. My research based on a breadth of experiences has evolved to become more in depth on the immune system and interactions with microorganisms. My clinical interests are in infectious diseases particularly relevant to resource limited areas. These pursuits have converged on the field of host pathogen interactions, a focus that has developed from my prior experiences.

My exposure to lab science began early as both my parents were lab technicians. As a high school student, I worked more formally in the lab of Kevin Shannon at UCSF for two summers on genetic mechanisms underlying leukemogenesis. This was the first dedicated time towards specific research projects and gave a glimpse into the potential clinical impacts of basic mechanistic work. As an undergraduate at Swarthmore College, I worked on yeast cell cycle regulation with Elizabeth Vallen that culminated into my Honors Program Thesis, characterizing *SID2*, a novel gene in *Saccharomyces cerevisae* involved in DNA replication. This experience specifically taught me classic techniques to dissect fundamental biological processes. These experiences directed me towards a combined MD PhD program with the intent of continuing mechanistic work at the bench with potential clinical implications. In the Medical Scientist Training Program at Case Western Reserve University my scientific focus was on host pathogen interactions in the context of innate immune response to viral infections in the laboratory of Ganes Sen at the Cleveland Clinic where I moved forward from yeast in college to tissue culture models addressing the biochemical steps involved in dsRNA signaling and how *Paramyxoviridiae* proteins in particular inhibit these first innate immune steps during infection. Clinically, I developed an interest in infectious diseases and global health. I spent one of my rotations in Thailand where I first appreciated the differences in diseases and management between the developing and developed worlds.

This interest I extended into my internal medicine residency through the global health relationships established between New York Presbyterian- Weill Cornell and Groupe Haitien d'Etude du Sarcome de Kaposi et des Infections Opportunistes in Haiti and Bugando Medical Centre in Tanzania. Haiti in particular is where I had my first significant exposure to tuberculosis, specifically multidrug resistant, with the diagnostic and treatment challenges of an extremely resource limited setting. Tanzania expanded my awareness of diseases and clinical skills as I both taught medical students and provided care in a slightly more developed world. These experiences solidified my interest to pursue infectious diseases as a subspecialty and showed me an area in which I believe the applicability of my basic research skills may have significant impact.

With these motivations, I moved to Boston for my Infectious Diseases fellowship, combining training at both the Massachusetts General Hospital and Brigham and Women’s Hospital. The program specifically aims to provide clinical training and in depth research mentorship to facilitate careers in academic research and clinical care. A project involving the functional role of antibodies in tuberculosis specifically interested me because of the understudied yet wide potential implications from an antibody perspective, the uniquely relevant human immunology approach and the vibrant Boston tuberculosis research community with the potential to drive innovative research. Sarah Fortune and Galit Alter provided a unique opportunity to study from both the host and pathogen sides. Supported for the past two years by the NIH T32 training award, I delineated differences in humoral immune responses in individuals with controlled bacterial burden in latent as opposed to uncontrolled bacterial burden in active tuberculosis. I went on to show in an *in vitro* macrophage model of tuberculosis infection, that antibodies from individuals with latent tuberculosis were able to mediate bacterial restriction more than antibodies from individuals with active disease, suggesting that antibodies might be leveraged for diagnostics and therapeutics. This involved learning new techniques in handling primary human immune cells, *M. tuberculosis*, microscopy and analysis of complex multiparametric data from human subjects and has been complemented by coursework which I have already or am in the process of completing: Models of disease (Harvard Catalyst), Advanced Course in Immunology, (American Association of Immunologists), Certificate in Biostatistics, (Harvard Catalyst), Programming in R (Harvard Catalyst), Introduction to Matlab, (Matlab), Fluorescence Microscopy (Harvard Medical School) and Using CellProfiler for Biological Image Analysis (Broad). The work thus far has led to multiple presentations by myself at local and national meetings such as Keystone. Moreover, a manuscript of this work for which I am co- first author has been submitted and is currently under review at Cell.

How exactly antibodies might function in the context of tuberculosis and its physiological relevance remain questions which I will begin to address in this proposal. More specifically, my goals for the next five years are to focus on elucidating antibody Fc effector mechanisms of action within a macrophage and identifying the *M. tuberculosis* antigens that elicit the most functional antibody responses. This will require further training in antibody effector function analysis, cell biology, microscopy and data analysis with both coursework and lab work to build upon the immunology, microbiology, molecular biology and biochemistry techniques I have already attained.

At this point in my career, with one first author publication in review at a high impact journal, a network of relationships within tuberculosis and human immunology and a project poised for success, the K08 funding to complete the experiments and training is a critical element to support my transition to independence from a both scientific and leadership aspects. This proposal specifically arises from my position as a highly trained infectious disease clinician with a strong background in basic science, enabling me to develop techniques and tools to translate potential correlates of protection from human studies into tractable models to probe mechanisms of disease to generate hypotheses that ultimately inform the direction and design of subsequent human studies. My unique perspective will allow me to pursue innovative concepts with a strong grounding in clinical relevance and the realities of translating research back to patient care.

Specific Aims

One third of the world's population carries the burden of tuberculosis (TB). Efforts to reduce this burden have been hindered by the lack effective diagnostics[1] and a protective vaccine underpinned by gaps in the understanding of the immune response in TB disease[2]. While cellular immunity is important, the antibody (Ab) or humoral landscape is poorly understood[3]. Passive transfer studies inconsistently provide protection and Ab titres alone are poor correlates of protection[3]. However, Ab function is determined by the combination of antigen specificity via the Fab and ability to recruit functional responses via the Fc domain. Fc receptors (FcR) bind to the Fc to mediate processes such as cellular activation, antigen presentation, complement activation, phagocytosis and cytotoxicity[4]. In humans, FcRs have been associated with different TB disease states correlated with low (latent disease: LTB) and high (active disease: ATB) bacterial burden[5-8] and in mouse models the lack of activating FcR leads to increased bacterial burden and disease[9]. Together, these lines of evidence implicate a potential role for Ab mediated antimicrobial activity against *Mycobacterium tuberculosis (Mtb).* Thus, while there is no clear role for neutralizing Ab responses in TB disease, Ab Fc recruitment of cellular responses is a promising underexplored potential for immune control.

Using a systems serological approach[10] we characterized functional profiles by antigen specificity, antibody subclass and titres, FcR affinity, critical post translation modifications such as glycosylation that mediate effector function and Fc effector functions themselves. We showed that different humoral signatures are associated with different TB disease states, correlating with controlled bacterial burden in LTB individuals compared to loss of control in ATB individuals. This paralleled findings in an *in vitro* macrophage model of TB infection where enhanced antimicrobial processes and decreased bacterial burden was observed in the presence of Abs from LTB compared to ATB individuals.

We hypothesize that Abs play unique roles via their Fc-domains in mediating immune protection against Mtb.

Aim 1: Define the antigen specificity of functional *Mtb* specific antibodies.
Hypothesis: A subset of *Mtb* antigens elicit Ab that recruit immune effector functions against *Mtb*.
We have shown that Ab from individuals with low bacterial burden in LTB compared to high bacterial burden in ATB mediate differential Fc effector functions and macrophage restriction of *Mtb*. However the specific antigens that elicit functional Abs are unknown. This aim utilizes an *Mtb* proteome array coupled with Ab Fc functional assays to identify the subset of *Mtb* specific antigens that elicit protective Fc effector functions.

Aim 2: Dissect the role of Fc/FcR mediated intracellular *Mtb* restriction.
Hypothesis: Abs activate macrophages to restrict intracellular *Mtb* via the activating FcγRIIIa.
Ab facilitated macrophage restriction of *Mtb* in LTB is associated uniquely with increased affinity for the activating FcγRIIIa, expressed on an array of innate immune cells and mediate Fc effector functions. While attractive, a more direct and causal relationship has yet to be established. Via an Ab Fc domain structure function approach, targeted knockdown and specific localization, this aim addresses the potential for FcγRIIIa to be the receptor through which Ab restrict intracellular *Mtb*.

Aim 3: Identify the macrophage effector mechanisms through which Ab restrict intracellular *Mtb*.
Hypothesis: Abs enhance phagolysosomal fusion via IL1β to direct intracellular bacterial fate.
Ab from individuals with low bacterial burden in LTB compared to high bacterial burden in ATB enhance phagolysosomal fusion and inflammasome activation, secreting IL1β. How these processes are intertwined remain unclear with potential implications in models of disease and therapeutics. Kinetic analysis to delineate the temporal relationships and the identification of specific drivers within these processes will be the first step in parsing the nuances of Ab directed intracellular bacterial fate.

The scientific objective of this K08 is to begin to define the Ab Fab and Fc features associated with intracellular *Mtb* control with the vision that they may be harnessed to dissect host pathogen interactions in tractable models of disease and ultimately expand the repertoire for immune correlates/diagnostics and next generation vaccine design. This portfolio of research and training will enable me to develop techniques and tools to translate potential correlates of protection from human studies into tractable models to probe mechanisms of disease.

Significance

One in three people worldwide carry the burden of tuberculosis (TB)[11]. Unlike many infections, TB disease is predominantly asymptomatic in a stage of latency (LTB) that can span decades. In this state, there is no evidence of bacterial burden yet a T cell based immunological signature of exposure is present. At an unclearly defined point, a subset of individuals with LTB will transition to active disease (ATB), defined by evidence of microbiological burden and clinical symptoms, the majority of which are pulmonary. These disease states are poorly recapitulated in mice, which remain at this point the most tractable animal model available. Consequently, the initial interactions that occur during acquisition, the immune response in latency and the switch to active disease are not well understood[12]. Moreover, the promise of effective vaccines and correlates of protection based on these models has yet to be met[13-16]. Thus, the challenge in advancing the armamentarium against TB disease lies in coordinated targeted translations between humans and animal models[17] and the search for a new paradigm of protective immunity[2,18].

In many infections and almost all current vaccines, antibodies (Ab) are considered correlates of protection[19]. The exception is the Bacille Calmette-Guérin (BCG) vaccine against *Mycobacterium tuberculosis* (*Mtb*) which is based on cellular immunity and whose efficacy is insufficient to garner routine immunization in the United States, a non endemic area[20]. These humoral correlates are almost entirely based on pathogen specific titres, extrapolating that Abs function in the context of neutralizing extracellular pathogen. However, Ab can direct defense mechanisms against intracellular pathogens. These may be conducted through effector functions directed by the Fc[21-26], whose relevance is supported by the existence of elaborate mechanisms of targeted evasion developed by bacteria[27,28]. Hence both specificity, directed by the Fab, and function, directed by the Fc are critical properties of an Ab (Fig 1).

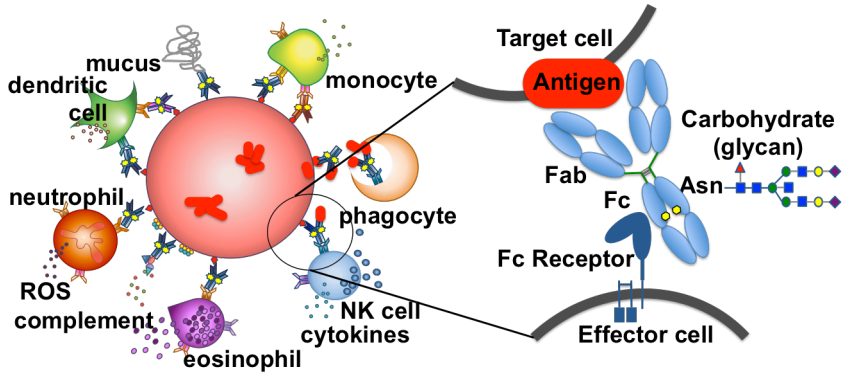


Fig 1. Ab function is determined by both antigen specificity via the Fab and ability to recruit functional responses via the Fc. Fc bind to Fc receptors (FcR) to mediate processes such as cellular activation, antigen presentation, complement activation, nitric oxide production, phagocytosis and cytotoxicity in diverse effector innate immune cells against infected target cells. This interaction is fine tuned by differential glycosylation on conserved asparagine (Asn) residues on the Fc domain and subclass specificity.

In TB disease, where the predominant portion of the bacterial lifespan is inside a cell, there is evidence both for and against the idea of protective Abs and the role of B cells[3,29]. Passive transfer of Abs inconsistently confer protection[30-35] and opsonization of extracellular *Mtb* by complement does not appear to affect *Mtb* survival[36]. Ab titers are poor correlates of protection and in fact are associated with more severe TB disease[37,38]. Additionally, in humans, B cell and Ab deficiencies are not clearly linked to increased susceptibility[34]. However, Abs and plasma cells are abundant in pulmonary TB granulomas, a quintessential immunological hallmark of infection[39]. In humans and non-human primates, they are tightly packed around the bacteria, surrounded by FcR laden innate immune effector cells[40,41]. Some Abs against *Mtb* arabinomannan can enhance bacterial opsonization and restrict intracellular growth[42]. Ab titres nadir in children with disseminated disease[43] and have recently been implicated as potential immune correlates in a post-hoc analysis of the MVA85 TB vaccine trial[44]. Finally, mice lacking B cells or the ability to secrete Abs have increased susceptibility to TB infection[45-47] and non human primates treated with the monoclonal CD20 Ab depleting B cells have increased bacterial burden thought to be due to the local granulomatous response [48].

In contrast, there is evidence supporting a role for Fc receptors in TB disease. Mice deficient in activating FcRs exhibit enhanced lung immunopathology and elevated bacterial burden while those lacking the inhibitory FcR have diminished pathology and lower bacterial burden[9]. In humans, higher expression of the activating high-affinity FcγRI[5,7,8] and loss of the activating low-affinity FcγRIIIb are associated with enhanced TB disease [6]. These lines of evidence suggest that Abs, via their Fc, contribute to immune modulation and potentially antimicrobial control. Thus, **while there is no clear role for neutralizing Ab responses in TB disease, Ab Fc recruitment of cellular responses is a promising underexplored potential for immune protection.**

Innovation

While Ab mediated immunity in infectious diseases has been centered on opsonizing and neutralizing extracellular pathogens, beyond these functions Abs direct innate immune anti-microbial activity via their Fc domains following engagement of Fc receptors (FcRs) [49,50]. Abs elicit distinct Fc effector functions such as Ab dependent cellular cytotoxicity (ADCC), Ab dependent phagocytosis (ADCP), upregulation of antigen presentation in dendritic cells (DC), activation of complement and induction of nitric oxide through differential interactions between their Fc domain and FcRs present on all innate immune cells. Alterations in Fc binding to FcRs is governed by isotype (n=5 in humans IgG, IgM, IgA, IgD and IgE), subclass (n=4 for IgG) and post translational modifications in N-linked glycosylation (n=35) [51,52] (Fig 1). While variation in Ab glycosylation is influenced by age and gender[53,54], both subclass and glycans are dynamically altered in the course of infection, treatment and vaccination[55-61]. In TB disease, IgG subclass distribution can be[62,63] but is not always[64-66] altered in the transition from LTB to ATB. Moreover, how the Fc is modified by glycosylation and how these changes may impact effector functions during the course of TB infection remain unclear. These Fc features, with the potential to recruit of innate and adaptive immunity, represent an underexplored path of protective immunity.

While Fc effector functions in humans parallel mice, the distinct repertoires of Fc and FcRs in each species presents a challenge in transiting between mouse models and humans[67]. Also unlike humans, the mouse model does not recapitulate latency as a disease state nor does it represent granulomas, a quintessential aspect of TB infection[68]. These differences may account for some of the inconsistent conclusions in the literature regarding the relevance of humoral immunity in TB disease. To isolate the most physiologically relevant interactions, this proposal focuses on human sera and human models of infection with the vision of targeted transition into an appropriate animal model to generate hypotheses that inform the direction and design of subsequent human studies.

Approach

In humans, latent TB (LTB) is defined by low (undetectable) bacterial burden while active TB (ATB) is defined by high bacterial burden, a natural dichotomy of states in which immune features can be measured to predict potential protective correlates. In collaboration with Cheryl Day, we used a systems serological approach and comprehensively characterized the Ab Fc signatures in individuals from South Africa with LTB (n=22) and ATB (n=20) by broad antigen specificity, Ab subclass and titres, FcR affinity, differential glycosylation and the Fc effector functions of phagocytosis, cellular cytotoxicity, upregulation of antigen presentation and nitric oxide production. We hypothesized that potential protective signatures of humoral immunity were associated with decreased bacterial burden and disease in LTB compared to ATB. A multivariate feature reduction approach[69] identified from the 69 initial Ab features potential immune correlates including Ab dependent cellular phagocytosis (ADCP), FcR affinity and differential Fc glycosylation. These differences were associated with enhanced human macrophage control of *Mtb* in Abs isolated from LTB as compared to ATB, measured by increased phagolysosomal fusion, inflammasome activation and lower bacterial burden (Fig 2). This proposal aims to leverage this preliminary data set to validate critical features identified and hypothesized to be protective in LTB vs ATB in an independent cohort of individuals from Texas that is gender and age matched to address any potential hidden confounders and comment on the generalizability of these findings. Specifically, it seeks to identify the subset of *Mtb* antigens that elicit potential protective Ab functions and the mechanism by which Ab direct intracellular bacterial fate.

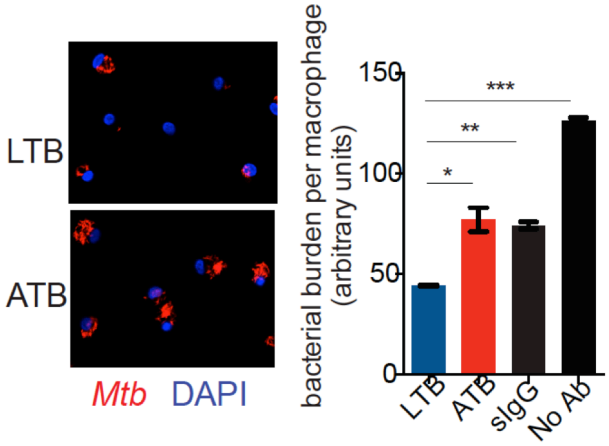


Fig 2. *Mtb* infected macrophages cultured with IgG from LTB compared to ATB have decreased bacterial burden. Primary human monocyte derived macrophages were infected at MOI 1 with a virulent strain of *Mtb* H37Rv-MCherry. Extracellular bacteria was washed away and IgG pooled from LTB, ATB, control healthy sigma IgG (sIgG) or no Ab were co-cultured with infected macrophages for 96 hrs. High throughput fluorescence microscopy was utilized for imaging. Via CellProfiler, bacterial burden was quantitated by pixel per macrophage as delineated by DAPI. Bars represent mean ± SEM. ***p<0.001, **p<0.01, *p<0.05 by student t test.

Aim 1: Define the antigen specificity of functional *Mtb* specific antibodies.

Rationale: Total polyclonal IgG from individuals with LTB with undetectable bacterial burden compared to ATB with high bacterial burden differentially mediate macrophage restriction of intracellular *Mtb* (Fig 2). Monoclonal Abs against *Mtb* LAM[42] also have this ability. Thus different *Mtb* antigens may elicit different Ab functionalities against intracellular *Mtb* with a subset that is protective. If Abs mediated protection in LTB than the subset of protective *Mtb* specific Abs would be predicted to be more dominant in this state compared to ATB. The attention paid to *Mtb* specific antigens in the literature has focused on recognition by CD4 T cells[70] but the identification of immunodominant antigens in this context has yet to be translated into protective immunity. Moreover the limitations of these approaches are driven by the size and complexity of the *Mtb* proteome in conjunction with the challenge of non tuberculous mycobacterial cross reactivity[70]. Only recently have larger screening capacities become more available with hints to the potential changing antigen spectrum recognized by serum from individuals with ATB compared to non TB diseases[71] and from individuals with ATB before and after antimicrobial treatment[72]. These dynamic changes suggest the existence of *Mtb* specific Abs that may be leveraged to modulate disease. Intriguingly both studies highlight multiple *Mtb* proteins yet to be characterized and suggest that only a small subset of pathogen specific antigens may elicit a measurable humoral response. No data is published distinguishing the *Mtb* antigen specificity between LTB and ATB. Comparing the spectrum of antigens in these two states- controlled versus uncontrolled bacterial burden- will help identify potentially novel *Mtb* candidates that may elicit functionally protective Ab responses.

Hypothesis: A subset of *Mtb* antigens elicit Ab that recruit immune effector functions against *Mtb*.

Preliminary Data: In the South African cohort, purified protein derivative (PPD)- a broad array of *Mtb* specific antigens- can mediate Fc effector functions such as Ab dependent cellular cytotoxicity (ADCC). LTB IgG has increased levels of ADCC compared to ATB IgG and depletion of PPD specific IgG abrogates this function (Fig 3A). This suggests that PPD specific LTB IgG drives ADCC. ADCC and affinity for FcγRIIIa are modulated by glycosylation, for example the presence of bisecting N-acetylglucosamine (GlcNAC) (Fig 3B-D). Comparison of bulk IgG between LTB and ATB in a second cohort of individuals from Texas in collaboration with Blanca Restrepo, show a difference consistent with increased affinity for FcγRIIIa with enriched B-GlcNAC structures (Fig 3B). These differences are not recapitulated in PPD specific IgG (Fig 3C) but are accentuated in a more granular analysis with Ag85A (Fig 3D), a major secretion product of *Mtb*, indicating that not all *Mtb* antigen reactive IgG are equivalent. These data support the hypothesis that different antigen specific Abs direct different functional capacities in LTB compared to ATB.

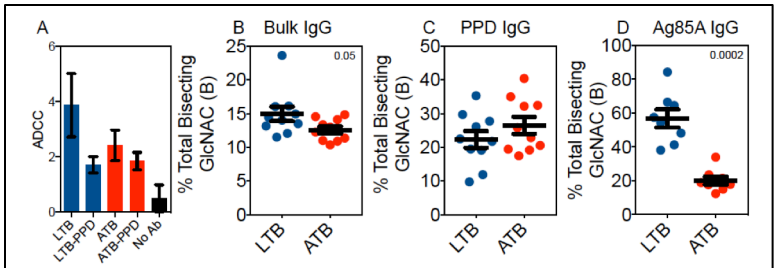


Fig 3. Antigen specificity is important in Fc effector function and glycosylation. A. Purified protein derivative (PPD) as *Mtb* specific antigen was used to deplete specific from bulk IgG pooled from LTB or ATB. ADCC was performed using PPD adsorbed CEM-NKr cells labeled with intracellular CFSE and membrane PKH26 dyes as target cells and primary NK cells as effector cells. Abs were added. The proportion of PKH26+ cells lacking intracellular CFSE (i.e., lysed target cells) was determined using flow cytometry. Bars represent mean ± SEM. Capillary electrophoresis was used to identify glycan structures purified from PNGase digested B. bulk, C. PPD specific and D. Ag85A specific IgG isolated on biotinylated antigen streptavidin columns. Bars represent mean ± SEM. P values are calculated by Mann Whitney.

Experimental Design:
Mtb antigens. To isolate specific *Mtb* antigens that elicit functional Abs we will utilize arrays[72] containing 4262 full-length recombinant proteins encompassing >95% of the *Mtb* proteome derived from the virulent H37Rv reference strain and the pathogenic strain CDC1551. To probe this set of antigens, we will use sera and specifically highlight the IgG class given that it is the most functional and also most abundant in blood[51]. These arrays will be read on a microarray scanner (Molecular Devices GenePix 4000B) and data processed by a microarray analysis program (GenePix Pro 6.1). The sera from LTB n=34 and ATB n=34 will be obtained in collaboration with Blanca Restrepo (University of Texas) to identify the differential spectrum of antigens recognized in LTB compared to ATB. This will generate antigens of interest and or provide the basis for further power and sample size calculations should it be necessary to conduct a larger study. Ultimately, three groups of antigens are expected: 1) antigens recognized by sera in both LTB and ATB 2) antigens recognized by LTB sera only and 3) antigens recognized by sera by ATB sera only (Fig 4). If the LTB state harbors Abs that are

protective, then antigens recognized by LTB sera specifically would be most likely to elicit a functional Fc signature. Once *Mtb* antigens are identified by array, functional validation of these antigen specific IgG will be performed by experiments involving depletion of antigen specific Ab, antigen specific Fc effector assays and antigen specific Ab glycosylation. This approach is directed by the fact that even the highest titres of antigen specific Abs are low in sera[73], rendering the isolation of antigen specific Abs in adequate amounts for many functional studies difficult. Purified antigens (CDI NextGen Proteomics, BEI Resources) will be biotinylated and bound to streptavidin columns through which antigen specific fractions may be separated from bulk total IgG from individual patients. As multiple as opposed to a single antigen will likely be highlighted, the first level of depletion will use all antigens identified by array analysis to observe the largest potential impact possible.

Functional validation. In the South African cohort Ab dependent cellular cytotoxicity (ADCC) is higher in LTB compared to ATB (Fig 3), suggesting one way in which innate immune cells may eliminate target cells infected with the pathogen. Thus, in the Texas cohort for this proposal, ADCC[74] specific to PPD will be a first validation for potential protective Ab mediated functions. This will be performed with IgG depleted of antigen specific Ab from LTB and ATB. In addition, the antigen of choice is flexible in the ADCC assay. Thus a second validation will be based on a side by side comparison of antigens identified in the array. This will be performed with bulk IgG from LTB and ATB. Finally, IgG glycosylation patterns impact affinity for FcR and thus Fc effector functionality. Therefore a third level of validation will be of the glycan forms on antigen specific IgG using high throughput capillary electropheresis[60,75]. Antigens of interest will be defined by the following characteristics: depletion of antigen specific IgG decreases ADCC, ADCC in response to one antigen is increased compared to others and antigen specific IgG have increased bisecting GlcNAC, decreased fucose and increased galactose compared to bulk as observed in the LTB and ATB IgG from the South African cohort.

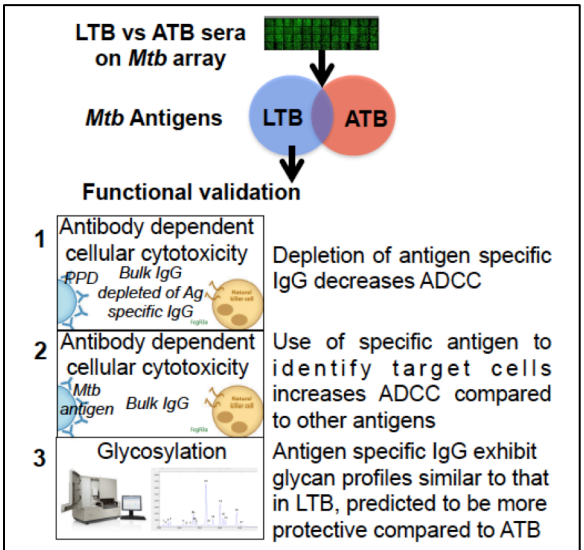


Fig 4. Identifying *Mtb* antigens of interest. *Mtb* protein array will identify potential antigens reactive to IgG from LTB and ATB. Antigens specifically recognized in LTB will be first screened for the ability to identify functional Abs. Functional validation will be performed 1. using purified IgG from LTB and ATB depleted of antigen specific fractions in ADCC specific to PPD 2. using bulk IgG from LTB and ATB in ADCC specific to different Mtb antigens 3. glycosylation analysis of antigen specific IgG using capillary electrophoresis. Combined results will identify *Mtb* antigens of interest.

Expected Results: If the LTB state restricting bacterial burden is protective compared to the ATB which is permissive to bacterial replication, then antigens recognized by LTB sera specifically would be predicted to elicit a functional Fc signature. This will likely be reflected by a small subset of *Mtb* specific antigens since only a small subset was significantly different between sera of ATB pre and post treatment[72] and non TB compared to LTB[71]. However, even if LTB and ATB recognize the same spectrum of antigen, the possibility exists that Fc modifications alone such as glycosylation are sufficient to change functionality. For this reason antigens reactive to both LTB and ATB sera will be considered as a second group of potential candidate antigens. We expect that multiple *Mtb* antigens elicit Abs and induce protection collaboratively. Thus depletion of all candidate antigen specific Ab together will abrogate Fc effector functions such as ADCC more than depletion of one single candidate. Alternatively, Fc effector functions may be recruited in an *Mtb* antigen non specific manner and the observed PPD dependence of functions may be explained by cross reactivity between mycobacterial and self epitopes. In this case, the more targeted depletion of *Mtb* protein specific population of total IgG would not abrogate Fc effector functions.

Potential pitfalls and alternative strategies: It is possible that *Mtb* proteome alone insufficiently represents the entire antigen spectrum as glycolipids represent a formidable portion of the bacterial cell wall [76] and can generate humoral immune reponses[77],[78],[42]. To address this potential pitfall, glycan arrays[79] may be utilized in parallel to the protein based approach here to more fully interrogate the gamut of pathogen specific epitopes.

Comparing the differential spectrum of antigens recognized by Ab in LTB and ATB will provide potentially specific novel candidates for the generation of monoclonal Abs for further mechanistic studies and, potentially, therapeutics and vaccine design.

Aim 2: Dissect the role of Fc/FcR mediated intracellular *Mtb* restriction.

Rationale: Activating and inhibitory FcRs regulate the immune system through the dynamic integration of negative and positive signals that trigger innate effector cell activation and impact adaptive immunity by directing immunogenic and tolerogenic responses[4]. These are dependent on the affinity between the Ab Fc-domain and the FcR[80] which is modulated by differential glycosylation[51]. The precedence of FcR mediated modulation in infectious diseases involving predominantly intracellular organisms ranges from *L. pneumophila*[23] and *M. bovis* bacillus Calmette-Guerin[23], *L. monocytogenes*[81], *L. donovani*[24,82], *C. burnetti*[21], *B. burgdorferi*[83], *S. enterica*[84] and *C. neoformans*[85,86]. The importance of FcRs is implicated by the development of bacterial enzymes that abrogate Fc function, PNGase[28] and Ides,[87] which can be utilized in structure function analysis of the Ab Fc domain (Fig 5).

In TB, FcR regulation is implicated by *in vivo* and *in vitro* data. Mice deficient in activating FcRs have enhanced disease and elevated bacterial burden and mice lacking the inhibitory FcR are the opposite[9]. In humans, higher expression of the activating high-affinity FcγRI[5,7,8] and loss of the activating low-affinity FcγRIIIb are associated with enhanced TB disease[6]. In tissue culture, FcR are associated with phagolysosomal maturation in opsonization of extracellular *Mtb*[42,88]. Our data suggest that FcR may impact intracellular in addition to extracellular *Mtb* (Fig 2). In this model, extracellular Ab, specifically LTB as compared to ATB, recognize Ags presented or excreted by infected macrophages to mediate Fc effector functions (Fig 5A). FcγRIIIa is a low affinity activating FcR important in mediating cellular cytotoxicity. In the lower bacterial burden LTB state, Abs have higher affinity for FcγRIIIa as compared to the higher burden ATB state (Fig 6A). This difference is not observed for FcγRIIa or FcγRIIb (data not shown). Thus, FcγRIIIa specifically is implicated in mediating Ab direction of intracellular bacterial fate. Since FcγRIIIa is expressed on the cell membrane of a variety of innate immune cells and has low to medium affinity for Ab that allow for dynamic and fined tuned activation of signaling that can be observed with FcR clustering, it is a compelling candidate for a receptor that can modulate *Mtb*. Alternatively cytosolic FcRs[22] (Fig 5B) or an FcR independent path via neutralization of important cytokines may regulate intracellular *Mtb*.

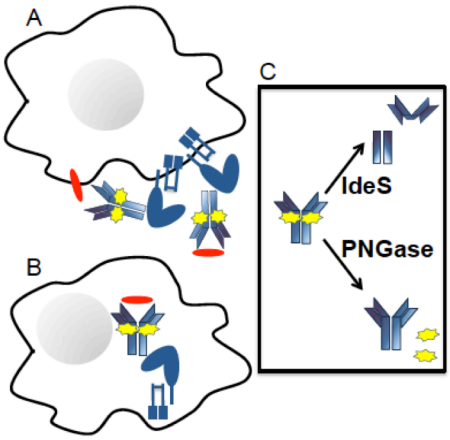


Fig 5. Potential models for Fc/FcR mediated modulation of intracellular *Mtb*. A. Membrane FcRs such as FcRγIIIa or B. intracellular FcRs such as TRIM21 may mediate Ab effector functions in response to antigens (red) escaping into the cytosol, presented or excreted by the cell. These Fc functions would be abrogated by C. Ab cleavage by IdeS or deglycosylation by PNGase.

Importantly, Fc effector functions can restrict pathogen or enhance disease[82,89] such as in Dengue. Thus differential *Mtb* restriction mediated by Abs in LTB and ATB may be a result of enhanced effector functions that clear the pathogen in LTB or a more permissive environment in ATB towards bacterial replication. Distinguishing if Fc/FcR do indeed impact intracellular *Mtb* and if that impact is restrictive or permissive will help define the underlying mechanisms at play.

Hypothesis: Abs activate macrophages to restrict intracellular *Mtb* via the activating FcγRIIIa.

Preliminary Data: Affinity for the activating FcγRIIIa is enhanced in serum IgG purified from LTB, a state of controlled bacterial burden, compared to ATB, a state of uncontrolled bacterial burden (Fig 6). Moreover, deglycosolation by the *F. meningosepticum* PNGase decreased the Fc effector function ADCP mediated by both LTB and ATB Abs (Fig 6B). These lines of evidence implicate a role for Ab Fc/FcR binding in determining intracellular bacterial fate.

Experimental Design:

Fc/FcR. To assess a potential causal link between FcR and intracellular *Mtb* restriction, we will inhibit Ab Fc and FcR binding to prevent function. Classically, Ab Fab have been used to block specific FcRs in short term experiments[90]. In the context of a four day *in vitro* human macrophage model of TB during which the bacterial replication cycle is 24 hours, FcRs and their bound complexes are continually recycled, thus generating

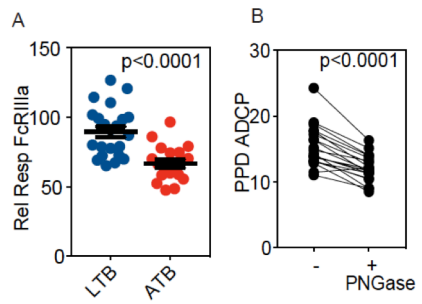


Fig 6. Fc/FcR is implicated in intracellular *Mtb* regulation. A. Surface plasmon resonance show enhanced affinity (reported as relative units) of LTB IgG for the activating FcγRIIIa compared to ATB. B. Deglycosylation of LTB and ATB Ab by PNGase abrogates PPD specific ADCP, where THP1 are exposed to Ab opsonized PPD adsorbed fluorescent beads and flow cytometry is performed to generate a phagocytic score calculated by MFI and percent frequency. Bars represent mean ± SEM. P values calculated by Mann Whitney in A and Wilcoxon matched-pairs signed rank test B.

inconsistent blocking effects (data not shown). However, Fc and FcR engagement and function may also be abrogated by cleavage of the Fc[91] from the Fab and or enzymatic removal of glycans[35,92]. The *S. pyogenes* enzyme IdeS[87] cleaves IgG specifically below the hinge region to generate $F(ab)_2$ and Fc (Fig 5). To obtain the Fc fraction, the $F(ab)_2$ fragments will be purified by protein A beads and IdeS via its commercial His tag. We will use these Ab fractions, generated from LTB and ATB, alone and in combination ($F(ab)_2$, Fc and ($F(ab)_2$ + Fc) in our *in vitro* macrophage model of infection (Fig 2) to assess the ability to restrict intracellular *Mtb*. In brief, this involves primary human monocyte derived macrophages infected at low multiplicity (1) with a virulent *H37Rv Mtb-MCherry*[93] to mimic the most physiologically relevant burden of infection after which extracellular bacteria are washed prior to the addition of Abs. After 96 hours of co-culture, the cells are fixed for high throughput microscopy and quantitative image analysis via CellProfiler to assess bacterial burden by fluorescence. In parallel, we will cleave the glycan structures from purified IgG from LTB and ATB by PNGase[28] (Fig 5, 6) and similarly assess the ability of these modified Abs to restrict intracellular *Mtb*. These two approaches will be more direct interrogations of a causal link between Fc/FcR and intracellular *Mtb* restriction.

FcγRIIIa. To more precisely address the role of FcγRIIIa, we will localize Ab and FcγRIIIa and employ targeted siRNA knockdown of FcγRIIIa. *Mtb* infected primary monocyte derived macrophages co cultured with fluorescently labeled IgG from LTB and ATB will be stained by standard Abs against FcγRIIIa to visualize clustering in response to activation[94] and co localization between IgG and FcγRIIIa, events that would be expected if Fc and FcR were engaged. Independently, siRNA in primary monocyte derived macrophages[95,96] against FcγRIIIa[97] and FcγRIIb[98] as a control will be employed to knockdown expression. These knockdown cells will subsequently be infected with *H37Rv Mtb-MCherry*[93], co cultured with Ab from LTB and ATB and bacterial burden assessed by fluorescence microscopy as described above. Together, these experiments more directly address the question of a causal link between FcγRIIIa and intracellular MTB restriction.

Expected Results: The engagement of Fc and FcR is dependent on an intact Ab (Fc and Fab connected by the hinge) and the presence of glycans. Thus, none of the Ab products generated by Ides and PNGase would be able to mediate an Fc effector effect. If Ab functionality is mainly in the context of LTB Abs directing increased bacterial restriction compared to ATB Abs through Fc/FcR, then when the Ab is enzymatically manipulated to prevent binding with FcRs, the bacterial burden for both LTB and ATB enzymatically manipulated Abs will be at the baseline level observed with intact Abs from ATB. Alternatively if Ab functionality is mainly in the context of ATB Abs enhancing bacterial replication, then this replication would be abrogated when the Ab is enzymatically manipulated to prevent FcR binding and bacterial burden for both enzymatically manipulated LTB and ATB Abs will be at the baseline level observed with intact Abs from LTB. If intracellular bacterial fate was not linked to Fc/FcR, then potentially $F(ab)_2$ and also deglycosylated IgG could function by neutralizing cytokines and mediating bacterial restriction or enhancement. If FcγRIIIa was involved directing intracellular *Mtb* fate, we would expect that knockdown of FcγRIIIa but not the inhibitory FcγRIIb would abrogate this ability. Moreover, we would expect enhanced FcγRIIIa clustering at the cell membrane in response to IgG from LTB compared to ATB individuals and enhanced co localization with fluorescently labeled LTB compared to ATB Ab.

Potential pitfalls and alternative strategies: It is possible that siRNA transfection of monocyte derived macrophages may activate macrophages indiscriminately. One alternative strategy to address this potential pitfall is to use the monocyte cell line THP1, a more malleable system to manipulate gene expression that can be matured into into macrophages.

Delineating the role for FcγRIIIa will help further elucidate the mechanism of action, informing the development of physiological relevant models to explore a role for Ab in TB disease.

Aim 3: Identify the macrophage effector mechanisms through which Ab restrict intracellular *Mtb*.

Rationale: Enhanced *Mtb* restriction in macrophages is associated with co-localization of *Mtb* with acidic lysosomal compartments (Fig 7A) and inflammasome activation (Fig 7B and C). However, the temporal and causal natures of these events are unclear.

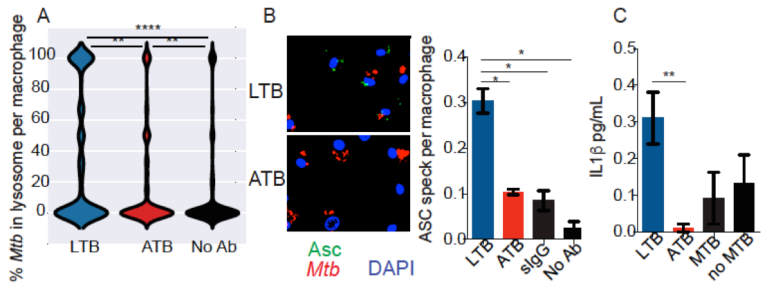


Fig 7: Enhanced *Mtb*-lysosome co localization, ASC speck formation and IL1β production with Abs from LTB vs ATB. Primary human monocyte derived macrophages were infected at MOI 1 with a virulent strain of *Mtb* H37Rv-MCherry. Extracellular bacteria was washed away and IgG pooled from LTB, ATB, control healthy sigma IgG (sIgG) or no Ab were co-cultured with infected macrophages for 96 hrs. High throughput fluorescence microscopy was utilized for imaging and quantitation via CellProfiler. A. Violin plots show the distribution of % co-localization of bacteria in lysosome (Lysotracker) in each cell along the y-axis with more clustering of macrophages containing all (100%) of *Mtb* within lysosomes in wells treated with LTB compared to ATB or no Ab. Statistical significance between the populations was calculated by Mann Whitney. B. ASC-specks (green) were stained (Adipogen) and quantified. Bars represent mean of two wells ± SEM. P values were calculated by student t test. C. To quantitate IL-1β levels an ELISA was performed on culture supernatants. Bars represent mean of two replicates ± SEM. P values were calculated by student t test.

Co localization of *Mtb* with the phagosome is critical yet alone insufficient for macrophages to kill intracellular *Mtb*[99]. Ab complexes can activate the inflammasome, an innate molecular platform inducing inflammation[100]. As a product of inflammasome activation, IL1β can restrict bacteria via pyroptosis and efferocytosis[101]. However, there were no differences in macrophage survival by DAPI or TUNEL (data not shown), making these two scenarios less likely. Alternatively, FcR signaling upon bacterial opsonization[23] and IL1β through the IL1 receptor can enhance phagolysosomal fusion[102], forming phagolysosomes that direct bacterial killing with hydrolases and antimicrobial peptides. In addition, autophagolysosomes, not clearly distinguished from phagolysosomes by Lysotracker dye can mediate bacterial killing by ubiquitination[103]. FcR signaling in Ab dependent enhancement in Dengue infection[104] is linked to autophagy[105], the process of creating autophagolysosomes, and the intracellular FcR TRIM21 can act as autophagic receptor regulators[106]. Moreover, autophagy, an intracellular degradation system that delivers cytoplasmic constituents to the lysosome, can restrict *Mtb*[107]. Finally, activation of the inflammasome can also activate autophagy and autophagy can in turn inhibit the inflammasome by ubiquitinating its components[108]. Thus, one model could involve Ab, via FcR, activation of the inflammasome to direct the development of phagolysosomes and autophagolysosomes that restrict *Mtb*, in which the autophagy feeds back to modulate innate immune inflammatory signaling. These specific processes including the inflammasome and autophagy are important distinctions given the availability of specific knockout mice for potential *in vivo* experiments and small molecule inducers and inhibitors already FDA approved or being developed for other clinical indications[109,110].

Hypothesis: <u>Abs enhance phagolysosomal fusion via IL1β to direct intracellular bacterial fate.</u>

Preliminary Data: Ab from LTB, a clinical state that restricts bacterial burden, compared to ATB, a clinical state permissive to bacterial replication, is able to mediate similar restriction and permission for bacterial replication *in vitro* (Fig 2). This is associated in macrophages with enhanced *Mtb*-lysosomal co localization (Fig 7A) and inflammasome activation as shown by speck formation of the inflammasome adaptor Apoptosis-Associated Speck-Like Protein Containing CARD (ASC) (Fig 7B) and IL1β production (Fig 7C).

Experimental Design:
Kinetics. To gain insight into the temporal relationship between inflammasome activation and phagolysosomal fusion, we will perform a time course at 6, 24, 48, 72 and 96 hours with IgG from individuals with LTB compared to ATB. In brief, primary human monocyte derived macrophages will be infected at low multiplicity (1) with a virulent *H37Rv Mtb-MCherry*[93] to mimic the most physiologically relevant burden of infection after which extracellular bacteria will be washed prior to the addition of Abs. At the specified timepoints, the supernatant will be removed and cells prepared for high throughput microscopy and quantitative image analysis via CellProfiler. To assess inflammasome activation, IL1β levels in the supernatant will be analyzed by ELISA (Fig 7C). ASC staining will be used as confirmation[108]. An inflammasome activator nigericin will be used

as a positive control[108]. To assess phagolysosomal fusion and bacterial burden, the cells will be stained with Lysotracker Red (Fig 7A), fixed and imaged.

IL1β as a product of the inflammasome. To ask if Ab mediated restriction of *Mtb* is through inflammasome mediated induction of IL1β, we will utilize the *in vitro* macrophage model of infection as described above but rather than co-culturing with IgG, use a dose curve of recombinant IL1β[101] and an activator of the inflammasome upstream of IL1β, nigericin[108]. To drive home specificity as to the most direct mediators, we will abrogate IL1β signaling by anti-human IL1β[101] and inflammasome activation by small molecule inhibitors MCC950[111], beta-hydroxybutyrate (BHB)[112] and glyburide[113].

Autophagy. Autophagy can be distinguished by the lipid binding proteins LC3, autophagy specific ubiquitin adaptors such as p62 and the autophagy related protein (ATG) family of proteins[107]. At the points surrounding maximal phagosomal-*Mtb* co localization as determined by the kinetic studies above, we will ask if these autophagy distinguishing markers may be observed. As a positive control, rapamycin will be used[114]. We will stain with antibody against LC3[107], the ubiquitin specific adaptor p62[104,115] and also use autophagocytic selective dyes[114]. For LC3 and p62 staining, we will simultaneously stain with Lysotracker to ascertain the proportion of acidic vesicles that may be related to autophagy. The contribution of any observed autophagolysosomes to bacterial burden will be subsequently validated by the appropriately timed addition of an autophagy blocker such as 3-methyladenine or wortmannin[107] or targeted siRNA[95] knockdown of autophagy components. Quantitative image analysis will be performed via CellProfiler.

Expected Result: If Abs from individuals with LTB with controlled bacterial burden enhance phagolysosomal fusion via IL1β to direct intracellular bacterial fate, we would expect that inflammasome activation and IL1β production occur prior to phagolysosomal fusion in our kinetic studies. We would also expect that IL1β and or the inflammasome activator nigericin would be sufficient to promote phagolysosomal fusion and decrease bacterial burden. This effect would be abrogated when inflammasome activation or IL1β signaling is antagonized. Alternatively, Abs may enhance phagolysosomal fusion independent of IL1β. In this scenario, neutralizing IL1β would have no effect on phagolysosomal fusion though the small molecule inflammasome inhibitors MCC950[111], BGH[112] and glyburide[113] whose targets are upstream of IL1β may still retain effect. If autophagolysosomes were important, we would expect its specific markers to co localize with *Mtb*. In addition, autophagy blockers or knockdown of components would abrogate any effect of Abs on bacterial burden.

Potential pitfalls and alternative strategies: It is possible that Ab driven macrophage effector functions are independent of the inflammasome, the phagolysosome or autophagy and that they instead act as markers of bacterial trafficking rather than direct mediators. A plethora of additional effector functions such as antimicrobial peptides, radical formation and sequestration of nutrients and metal may be at play. One strategy to widen the breadth of discovery is to utilize RNAseq, technology already developed in the Fortune and Alter labs, to more broadly assess gene regulation upon treatment with Ab from individuals with LTB vs ATB.

Identifying how the inflammasome and autophagy are tied to Ab direction of intracellular *Mtb* fate will give mechanistic insights that can be used to enhance models of disease and potentially therapeutics.

Future Studies

Together, these studies will provide mechanistic insight into Ab mediated modulation of the immune response in TB by comparing human disease states restricting bacterial burden in LTB and permissive to bacterial replication in ATB. Aim 1 will identify the differential spectrum of pathogen antigens recognized by Ab in the restrictive LTB compared to permissive ATB conditions. At minimum, data from this aim will highlight potentially important differences from the pathogen side that might mediate progression of disease but the immediate goal is to use these potential antigens that elicit functional Abs is the construction of monoclonal Abs for further and more specifically interrogate mechanisms of disease. This couples with Aim 2, which seeks to define specific cellular receptors that mediate Ab modulation of intracellular *Mtb*. More precisely, it addresses the relationship between FcγRIIIa and intracellular bacterial fate, which is suggested by our preliminary correlative evidence. Should the relationship hold, this point could be leveraged to help develop hypotheses of where and when Ab may exert their immunomodulatory effects and to direct more appropriate animal models such as the humanized FcγRIIIa mouse[116] to test Ab functionality *in vivo*. Even if FcγRIIIa did not appear to be directly

involved, these studies would then implicate the importance of studying other FcRs including the intracellular FcR TRIM21[22]. As critical as the receptor are the effector mechanisms that mediate bacterial fate in Aim 3. Phagolysosomal fusion is a well accepted part of macrophage *Mtb* processing yet how this is connected to the inflammasome is less clear. By interrogating more directly the relationship of these processes with small molecule inhibitors, not only will further insight into mechanism be gained but this *in vitro* data could also be leveraged to generate insight into what could be utilized *in vivo* in animal or even human studies to understand how these effector functions in the context of Abs might be physiologically relevant in TB disease. Negative studies would only help direct attention towards other cellular processes or hitherto undefined mechanisms of *Mtb* control. Collectively, the data from studies will help inform the direction and design of subsequent *in vivo* studies in animal models and in humans for a better appreciation of the functional role of Ab in TB disease.

Statistical Analysis

To estimate the sample size for our experiments, we used preliminary data generated from an initial cohort of patients with LTB (n=20) and ATB (n=22) from South Africa. For 75 antibody features linear regression analysis of the association between diagnosis and antibody feature, controlling for age and gender, was performed by Douglas Hayden at the MGH Biostatistics Center. For the thirteen features exceeding a false discovery rate threshold of 10%, the partial correlation between diagnosis and antibody feature was found to be 0,50 or greater. Using this estimate as the effect size of biologically active antibody features, 68 individuals in an independent cohort (34 LTB, 34 ATB) would provide a statistical power of 80% to observe significant differences in top Ab features between LTB and ATB at an alpha level of 0.0005. This alpha level is the required threshold for significance for the Bonferroni-Holm 0.05 alpha level when testing 100 antibody features[117]. We are confident that this sample size is feasible and aim to increase our n to 40 for LTB and 40 for ATB to increase our power. Statistical analysis will be performed with the support of the MGH Biostatistics Center through the Harvard Catalyst Biostatistical Consulting Program. P values will be calculated by linear regression controlling for age and gender. Adjustment for multiple comparisons will be performed by the Benjamini and Yekutieli method which controls for false discovery in multiple testing under dependency[118].

Timeline and feasibility

The experiments described in this proposal are feasible within the requested 5 year period of performance as summarized in the table below with the following manuscripts anticipated: 1. Define the antigen specificity of functional *Mtb* specific antibodies**.** 2. Dissect the role of Fc/FcR mediated intracellular *Mtb* restriction and 3. Identify the macrophage effector mechanisms through which Ab restrict intracellular *Mtb*

Research Approach	Year 1	Year 2	Year 3	Year 4	Year 5
Aim 1	**X**	**X**	**X** Manuscript 1		
Aim 2	**X**	**X** Manuscript 2			
Aim 3		**X**	**X**	**X** Manuscript 3	
Additional pilot studies				**X**	**X**
R21 preparation			**X**	**X**	
R01 preparation				**X**	**X**

Biohazards

All laboratory work with *Mycobacterium tuberculosis* will be conducted in the BSL3 facility at the Harvard School of Public Health or the Ragon Institute of MGH, MIT and Harvard per institutional protocols approved by the Committee on Microbiological Safety (Harvard University's IBC) and the Partners Institutional Biosafety Committee (Ragon Institute's IBC). All work will be conducted in compliance with local (Boston and Cambridge), state and federal regulations, as well as the NIH *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* and the CDC *Biosafety in Microbiological and Biomedical Laboratories* guidance document. All serum patient samples and cell culture will be performed in a BSL2 facility using selected BSL3 biosafety practices. All personnel have or will receive training covering bloodborne pathogens and biosafety, and will be required to demonstrate competency in BSL3 laboratory work before receiving approval for unescorted access. All BSL3 personnel will also be required to be medically cleared by the institution's occupational medicine department prior to being allowed to access the BSL3 laboratories.

Training in the Responsible Conduct of Research

As a T32 grant trainee and a researcher at the Ragon Institute, I maintain several Collaborative Institutional Training Initiative (CITI) certifications for Biomedical Research Investigators, renewed in the Fall of 2014. I have also completed the Partners HealthCare Responsible Conduct of Research Training Program 2014-2016 administered by the Partners Research Compliance Office. The Program consists of eight hours of face-to-face instruction and the CITI (Collaborative Institutional Training Initiative) online, basic RCR course. Trainees fulfill the face-to-face instruction requirement by attending a four-hour Partners RCR seminar and four additional hour-long hospital-based courses, lectures or discussion groups that are deemed “RCR eligible.” The four-hour Partners RCR seminar includes faculty presentations and discussions on authorship, data management, documentation and integrity; conflict of interest and interactions with industry; research misconduct; and post-award financial management. The seminar also includes an overview of basic regulatory and institutional requirements for research involving human subjects, stem cells, animals, and biohazards/recombinant DNA. The hospital-based courses/lectures/discussion group offerings also focus on these topics and more. For example, depending on the year and faculty availability, they include sessions on effective mentoring; ethical considerations in clinical research; safe lab practices; research subject privacy; peer review; humane and compliant use of animals in research; and collaborative science.

During this next phase, I will continue to update my RCR training. I will plan to participate in the RCR course sponsored by the Harvard Catalyst. This program is designed for trainees with career development awards and has 2 hours of class per week over an 8 week period in the spring semester at the Harvard School of Public Health. The curriculum covers issues relevant to research by incorporating discussion, assigned readings and critical thinking about many of the issues referenced above. As I transition to independence, topics regarding authorship, responsibility for data integrity generated by collaborators or mentees, collaboration with industry and the scientist will be particularly relevant.

To supplement the above program, I will discuss my training in Responsible Conduct of Research with members of my Training Advisory Group on a bi-annual basis. Dr Alter has developed and implemented protocols requiring the use of human samples. In my weekly meetings with my primary mentor Dr Fortune and co-mentor Dr Alter, I will also discuss RCR topics including data acquisition and management, complying with federal grant and contract requirements, peer review and collaborative research.

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Sarah M. Fortune | Professor

Ragon Institute of MGH, MIT and Harvard

Dr. Galit Alter, PhD
Associate Professor of Medicine
Kristine and Bob Higgins MGH Research Scholar
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To the K08 committee

We are please to be writing as primary mentors for Lenette Lu's application for the K08 Mentored Clinical Scientist Research Career Development Award. Lenette is an outstanding individual with great potential who is at a crucial stage of development in her physician scientist career. We both met Lenette two years ago and have been impressed by the progress she has made during this time under our mentorship and we are committed to her further scientific development.

Lenette received her MD and PhD degrees from Case Western Reserve University. Her degree in Molecular Virology under her mentor Ganes Sen was based on her thesis focused on dsRNA signaling in viral infections at the host pathogen interface. In particular, she identified the role of several paramyxovirus accessory proteins that act on the IRF-3 signaling pathway, published in the Journal of Biological Chemistry. Lenette went on to complete her internal medicine residency at New York Presbyterian/Weill Cornell Medical Center program and then moved to Boston for a fellowship in Infectious Disease at Massachusetts General Hospital/Brigham and Women's Hospital.

Here, Lenette approached both of us independently about potential post doctoral work that would further her host pathogen interest in infectious diseases with a global perspective. She talked to Galit about her expertise in antibody recruitment of Fc effector functions in innate immune cells and Sarah about her expertise in the molecular pathogenesis of tuberculosis. Lenette's interest in a collaborative project was something that she independently developed after speaking with us. She proposed this at an ideal time for our groups as we had been working together over the course of the prior 18 months to develop a study on the role of antibodies in tuberculosis infection. It was complete serendipity.

In the past two years, Lenette has flourished, managing a challenging project, mastering the fields of immunology and tuberculosis with almost no prior experience in either. Clearly she draws from the breadth of her prior experiences that encompassed the rigorousness developed in her graduate and even in her undergraduate years. However what really drives her success is her personality- her ability to work well and learn from others and her endeavor to do more. The original data from a cohort of patients from South Africa showed striking differences in the functional antibody signatures of individuals with latent compared to active TB. Lenette took this to the next level by assessing the impact of these differences on the actual pathogen Mycobacterium tuberculosis in the biologically relevant primary macrophage. Importantly, the experimental design she developed addressed

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specifically MTB as an intracellular pathogen within the macrophage as opposed to the more traditionally studied process of opsonization. She then went on to provide the first insights into mechanism by looking at phagolysosomal fusion and inflammasome activation. This series of work significantly elevated the original body of data and now is at the level of a manuscript currently under revision at Cell, surpassing our initial expectations.

To accomplish this required a significant amount of independence which both of us felt more than comfortable giving. Lenette took classes in immunology (Advanced Course in Immunology, American Association of Immunologists), statistics (Certificate in Biostatistics, Harvard Catalyst) and computer programming (Programming in R, Harvard Catalyst and Introduction to Matlab, Matlab). This is now reflected in her nuanced grasp of many of the aspects pertinent to understanding, analyzing and interpreting data from human studies in which she has had no prior experience. She actively sought out opportunities to submit abstracts and present at the Keystone conferences, which have garnered her much praise in terms of her work and also her presentation skills. She has actively sought out new collaborations and resources.

From a leadership and management perspective, she is the main point person for the collaborations we have on going for the TB project, managing relationships just next door to those all the way across the country and beyond. She is our representative at meetings to which we are unable to attend and speaks on our behalf. She has supervised successfully two summer students back to back (one in each of our labs) and is now managing a new post doctoral fellow.

She does this of course while also maintaining her clinical skills in her outpatient infectious disease clinic and brief one month time on the inpatient consults service per year at MGH, where she trained as an infectious disease fellow. She attends weekly clinical conferences and still actively engages with her clinical mentor Kimon Zachary and many others in the infectious disease department who are also particularly interested in tuberculosis. Especially since tuberculosis is such a clinical disease, the influence of Lenette's clinical experience is most keenly felt in the types of questions she asks and her persistent strive for physiological relevance in her research. Without this unique point of view, Lenette would not be able to develop and answer the same kinds of questions in the lab. Lenette is clearly at this point primed to transition into more independence with the support of a K08.

Lenette's proposal outlines an extremely important goal to begin to understand the key mechanisms by which antibodies may function in tuberculosis. Despite the burden of TB disease, very little is known about the role of humoral immunity- whether it be protective or not and how. The advances in the antibody field on understanding how antibodies might be able to impact intracellular pathogens via Fc receptors have only recently reached and been more fully appreciated in the TB field. Lenette herself is one of those people who have helped make this happen. Further insight into the host pathogen relationship in this context will not just elucidate pathogenesis of disease but also help to build more biologically relevant models to help inform the direction and design of future human studies with the goal of improved therapeutics, diagnostics and vaccine design.

We are both extremely committed to support Lenette every step of the way. The expertise and strength of the Fortune lab lies within its cross disciplinary approaches to understand the molecular basis of the populational heterogeneity that underlies clinical tuberculosis disease and treatment outcomes. In particular, the Fortune lab itself combines bacterial genetic approaches with high throughput methodologies. Specific focus is paid attention to high density whole genome sequencing, RNAseq and quantitative live cell imaging to define how diversity enables the bacterium to survive subsequent selective forces including antibiotics and immune selection. To accomplish this, a broad network of collaborators exists which includes experts in technologies to assess single cell behavior at MIT and MGH and experts in sequencing methodologies at the Broad Institute in addition to the human immunology experts at the Ragon. In the more recent years, the Fortune lab in collaboration with Joann Flynn at the University of Pittsburgh has published high impact papers on granuloma lesional heterogeneity in the non human primate model suggesting that critical responses at this level ultimately determine the clinical outcome of infection (Lin et al *Nature Medicine*, 2014 and Gideon et al *PLoS Pathogens.* 2015). Antibodies at the site of the granulomas might be one local factor that helps to dictate this outcome. Sarah's expertise and guidance in MTB and specifically MTB within a macrophage will be integral to Lenette's proposed studies on the mechanism of Ab mediated macrophage restriction of MTB.

In the past eight years, the Alter lab has focused on understanding the role of the innate immune response to chronic viral infections, defining the role of Natural Killer cells in antiviral control. Recently, these studies have broadened to define the mechanism by which innate immune effector cells may be harnessed through vaccination or immunotherapeutic strategies to gain more effective control over viral replication. In this capacity, a systems serological approach encompassing a suite of antibody profiling assays that aim at gaining a deeper appreciation of the correlates of humoral immune activity against HIV has been developed (Chung et al Sci Transl Med 2014 and Chung et al Cell 2015). Most recently, these antibody screening technologies have been adapted to identify potential antibody correlates of protective immunity in infections beyond HIV, which includes Mycobacterium tuberculosis. These antibody profiling techniques linked to antigen-specific B cell profiling efforts will help define the potential role of functional antibodies in the control/clearance of Mtb with the goal of developing more effective diagnostics, therapeutics as well as provide insights for the design of next generation Mtb vaccines. Galit's experience in antibodies and Fc recruitment of innate immune cell functions will be invaluable to Lenette's proposed studies providing mechanistic insight into how antibodies might provide protection in tuberculosis.

Lenette has outlined a clear path for career development during this phase of her training. She has chosen coursework to specifically gain more experience independently in microscopic techniques and computational analyses critical to the aims of her proposal. Moreover, she will take advantage of the multitude of resources and courses available at Harvard to make the transition from K to R in terms of grantsmanship and leadership. She has previously completed training requirements for human subjects research and the responsible conduct of research and will continue to update. She has outlined annual national conferences such as Keystone in which she has the opportunity to share her work and establish new collaborations. In addition, she has outlined a plethora of more frequent local conferences during which she can obtain feedback from the perspectives of human immunology at Ragon, TB at the Harvard School of Public Health, clinical relevance at MGH Infectious Disease and tuberculosis immunology, microbiology and epidemiology from the Boston TB community. In addition, Lenette will have all the benefits of the cutting edge scientific research hub- Ragon, Harvard School of Public Health, MIT and Massachusetts General Hospital (MGH). She will have access to high throughput fluorescence and confocal microscopy, high throughput image analysis, microarray cores, single cell RNAseq, statistical, computational and network analysis support, BL2 and BL3 FACS and sorting, multiplex luminex and mouse capabilities and experience with human samples and human immunology. Given that Lenette has an entirely new focus on human immunology and tuberculosis in her current work as compared to her dissertation in dsRNA signaling in response to viral infections, we believe a five year period of mentored career development is appropriate.

As mentor and co-mentor, we are both fully committed to Lenette's development as a physician scientist. As an MD and a PhD respectively, we recognize the challenges and rewards of a dual career. We both have co mentored other mentees and are acutely aware of those challenges and rewards. We have both mentored multiple graduate students and post doctoral fellows, several of whom have continued onto independent academic positions. We will provide the necessary support including resources, technician support, access to cutting-edge technology and career advice. We support Lenette's clinical efforts to complement her research work which will be less than 15% of time. Her teaching responsibilities per her Instructor appointment at Harvard is 50 hours per year to medical students and may be fulfilled by teaching on clinical service. Overall Lenette's dedicated portion of time to research is 75%. We will meet together with Lenette weekly to review her progress at the Ragon where Sarah spends at least one full day a week and is Director of the TB program and Galit is primarily based. As Lenette gains further experience, the frequency of these meetings will decrease but to at minimum once monthly. Of course, we will both be available on an as needed basis at all times. As a team, we will provide an annual evaluation of Lenette's progress as required in the annual progress report.

In addition, Lenette will have guidance from Eric Rubin, a senior physician scientist at the Harvard School of Public Health with a lab of his own focused on tuberculosis microbiology. Eric has a significant mentor track record with multiple fellows who have transitioned into academic independence and success including Sarah herself. The Fortune and Rubin laboratories have weekly group meetings where Lenette can gain further feedback. Eric will head Lenette's scientific advisory committee. In addition, she has assemble outstanding leaders in diverse fields to complement her proposal: Akiko Iwasaki, Falk Nimmerjahn and Facundo Batista. The

expertise of this group ranges from innate immunity to Fc receptors to cell biology and microbiology, complementing the antibody and tuberculosis backgrounds we provide. This group has committed to meeting on a biannual basis via Skype or in person to assess the progress of Lenette's project as well as be available on an as needed basis. Finally, Blanca Restrepo will extend her ongoing collaboration to provide serum samples from individuals with latent and active TB disease that are critical to the project's aims and Douglas Hayden at the MGH Biostatistics Center will provide statistical oversight and support. We anticipate multiple publications from the work described in Lenette's proposal with one annually in Years 2, 3 and 4. We expect that Lenette will prepare to submit an R21 in Year 4 and an R01 in Year 5 with the support of the Harvard GRASP grant writing and K to R transition courses. She will of course be encouraged to submit applications for other grant mechanisms to the NIH or other funding opportunities should they arise. Thus, Lenette will become more independent in her last two years of this project relying on us for mainly advice and guidance as opposed to direct oversight. Ultimately we expect Lenette to take her own mechanistic work described in this proposal with her upon transition to an independent physician scientist.

We anticipate that managing a collaborative project will be challenging, as it will require Lenette to master two disciplines. However, Lenette's accomplishments in the past two years support our confidence that with her communication, intellectual and bench skills, she will continue to endeavor and help shape the field of tuberculosis and antibodies. This award would come at a pivotal time in Lenette's career, as she begins to make her transition towards independence. With the full support of both labs, we are confident of Lenette's success.

Sincerely,

Sarah Fortune, MD
Professor of Immunology and Infectious Diseases
Director, TB Research Program at the Ragon Institute of MGH, MIT and Harvard

Galit Alter, PhD
Associate Professor of Medicine, Ragon Insittute of MGH, MIT, and Harvard
Director, Advanced Technologies Core at the Ragon Institute

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06/01/2016

Dear Lenette

Thanks for considering me to be on your scientific advisory committee for your K08 proposal about the role of antibodies in tuberculosis - I am delighted to be a member. I myself work both in vivo and in vitro by combining state-of-the-art imaging technology with biochemistry and genetic models to study how cellular and molecular events lead the activation of B cells and their ability to produce antibodies. I will be able to show you how to use some of these techniques and approaches for your intriguing questions in tuberculosis. I look forward to working with the other diverse set of people you have asked to be on your committee in meetings. I am also of course available as needed and this should be easy since I am just one floor below you at the Ragon.

Best wishes,

[redacted]

Prof. Facundo D. Batista, PhD,
Associate Director
Ragon Institute of MGH, MIT and Harvard

Terry and Susan Ragon Professor, Harvard Medical School

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

Yale University
School of Medicine

May 23, 2016

Dear Lenette,

I am happy to serve as a member of your K08 advisory committee. To your proposal concerning antibody-mediated mechanisms of immune modulation in tuberculosis, I bring my expertise in innate immunity and the path to adaptive responses, which antibodies naturally pave.

Specifically, how inflammation mediated by antibodies during the course of infections may lead to pathogen control or loss of control with a potential autoimmune component is something not limited to tuberculosis as you discuss in your proposal but may very well be applicable to viruses as well. This interest has as you know been a driver of exchange between Galit and myself, reflected by the recent visit of one of my post docs to Galit's lab to learn to profile Fc effector signatures and the recent interview for a post doc position in Galit's lab by one of my graduate students.

I look forward to providing advice for your exciting project.

Best,

[redacted]

Akiko Iwasaki, Ph.D.
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Waldemar Von Zedtwitz Professor of Immunobiology
and of Molecular Cellular and Developmental Biology
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Erlangen, May-8th, 2015

Dear Lenette,

I am writing to confirm my participation as part of your Scientific Advisory Committee for your K08: “Antibody mediated mechanisms of immune modulation in tuberculosis.” I believe that cellular Fcγ receptors are crucial for the activity of different IgG subclasses to direct the immune response in infections towards clearance of virus or bacteria or towards excessive inflammation in autoimmunity. This can be dramatically modulated by differential IgG glycovariants. Based on the recent data you and others have produced under Galit Alter and Sarah Fortune I believe tuberculosis represents an important infectious disease in which IgG glycosylation potentially impacting FcγR activity may play a role. I can offer you my expertise in FcγR function, specifically my ongoing work in cross species differences in expression and function of antibodies and Fc receptors, which would complement the expertise of your other mentors (Lux and Nimmerjahn *J Clin Immunol* 2013, Lux et al *Cell Rep* 2014, Schwab et al., *Cell Rep* 2015, Kao et al., *Cell Rep* 2015).

I anticipate interesting discussions with you and your advisory committee via Skype or email on this fascinating project.

Best regards,

[redacted]

Falk Nimmerjahn, PhD

Professor of Immunology and Genetics

Chairman of the Institute of Genetics at the Department of Biology

Friedrich-Alexander University Erlangen-Nürnberg

Eric J. Rubin, MD, PhD

Irene Heinz Given Professor of Immunology and Infectious Diseases

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May 28, 2016

Dr. Lenette Lu
via email

Dear Lenette,

I am pleased to serve as a member of your Scientific Advisory Committee. I have followed your scientific research progress these past few years under the mentorships of Sarah and Galit and enjoyed discussing with you personally your progress. Despite over a century of research, we still have a very poor understanding of tuberculous infections. Antibody immunomodulation of TB disease is a fascinating and promising area in much need of further research and while I may have been a skeptic at first I am now a complete convert. As a researcher, I am happy to offer you my expertise on the causative organism, *Mycobacterium tuberculosis*, and, as a practicing physician and associate editor at the New England Journal of Medicine, I can offer my unique clinical perspective on your findings and facilitate collaborations across the TB community. With Sarah and Galit as your primary mentors supported by Akiko Iwasaki and her work in innate immunity, Falk Nimmerjahn and his expertise in Fc receptors and antibody, Facundo Batista and his state of the art imaging technology, I am excited about contributing my TB viewpoint in your scientific pursuits and through mentorship as a senior clinician and researcher.

Good luck with the grant!

[redacted]

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Lenette Lu MD PhD
Instructor of Medicine at Harvard Medical School
Assistant in Medicine at the Massachusetts General Hospital
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Boston, MA 02114

05/26/2016

Dear Lenette,

I am pleased to contribute to your very interesting project "Antibody mediated mechanisms of immune modulation in tuberculosis." I have experience with and continuing interest in the statistical methods associated with modern high dimensional datasets. It has been a pleasure discussing your project with you so far and I look forward to working with you to analyze the data acquired in the course of your studies.

Sincerely yours,

[redacted]

Douglas Hayden

UTHealth
The University of Texas
Health Science Center at Houston

School of Public Health in Brownsville
Brownsville, TX

May 20, 2016

Lenette Lu, M.D., Ph.D.
Instructor of Medicine at Harvard Medical School
Assistant in Medicine at the Massachusetts General Hospital
Postdoctoral Research Fellow at Harvard School of Public Health

Dear Lenette,

It is my pleasure to extend our current collaborative efforts to the studies proposed in your K08 application. As you know, when Sarah and Galit approached me initially about working together on the TB antibody project I was very excited. I have been intrigued by the mechanisms that govern MTB entry into macrophages, specifically in the background of diabetes but in general as well (Restrepo et al., 2008; Restrepo et al., 2014). Very little is known about antibodies in TB disease and I am convinced that there is a lot more to learn. Of course, a clinically well characterized cohort (Restrepo et al., 2011; Restrepo et al., 2007) would provide the best resolution to address these questions and more. I am very pleased with the progress from the patient samples I have already shared with you and I look forward to the next phase of our collaboration as I continue to enroll patients in my field studies.

Sincerely,

[redacted]

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Description of the Institutional Environment

My research and training environment includes state-of-the-art laboratory resources and mentorship from experts in antibodies and tuberculosis. Situated at the Ragon Institute of MGH, MIT and Harvard are the offices and laboratories of Dr Sarah Fortune (primary mentor), Dr Galit Alter (co-mentor) and Dr Facundo Batista (SAC member). Situated at the Harvard School of Public Health area are the offices and laboratories of Dr Sarah Fortune's second site of work and Dr Eric Rubin (SAC member). These two areas will form the primary environment where my work will take place, supported by a greater community with the Massachusetts General Hospital and Partners with their associated resources and the Boston Area tuberculosis community.

The Phillip T and Susan M. Ragon Institute of MGH, MIT and Harvard officially established in February 2009 has the dual mission: to contribute to the accelerated discovery of an HIV/AIDS vaccine and subsequently to establish itself as a world leader in the collaborative study of immunology. Tuberculosis being the largest burden in terms of opportunistic infection in the HIV population is a prominent subject of research here. Specifically, the strength of the institute lies within the specialized resources supporting human immunology that enables translational research. Moreover, the Ragon facilities include the flow cytometry core, the microscopy core, a Biacore, and a BSL3 facility with the capacity for flow cytometry and sorting as well as live cell imaging. Weekly Monday Morning Conferences discuss ongoing research. In addition to other meetings, I will present preliminary data in this forum on an annual basis for feedback from the human immunology community, which includes Drs Fortune, Alter and Batista.

The Harvard School of Public Health is where the offices and laboratories of Drs Fortune and Rubin are situated. The two labs interact intimately with weekly group meetings together in addition to individual lab meetings. The larger community within this locale provides resources and expertise in terms of TB immunology and epidemiology. Moreover, situated next door is the Department of Systems Biology at Harvard Medical School, where the Physical Property Measure System (PPMS) for the Laboratory of Systems Pharmacology is located with live and fixed cell Operetta High-Content Imaging Systems available for use. In addition to lab meetings, I will present at Group meetings here for feedback from the TB community that includes Drs Fortune and Rubin.

The Boston TB research community is well nucleated with monthly work in progress meetings that draw from the 10 primary TB labs at Harvard, the Brigham and Women's Hospital, Children's Hospital, the Broad Institute, Tufts, University of Massachusetts Medical School and Boston University. I will present at these meetings as well for feedback from the broader TB community.

Beyond the Ragon and Harvard School of Public Health, Massachusetts General Hospital, the Partners Health System that also includes Brigham and Women's Hospital, Beth Israel Deaconess Hospital and the Dana Farber Cancer Center, MIT and the Harvard community that includes the Harvard Catalyst provides rich resources and support. This includes multiple microarray cores with associated support services (Harvard Partners Center for Genetics and Genomics/ Partners HealthCare Center for Personalized Genetic Medicine Microarray Facility, Microarray Core Facility at Dana Farber Cancer Center), the National Center for Functional Glycomics headed by Dr Richard Cummings at Beth Israel, the Imaging Platform at the Broad Institute of MIT from which the image analysis program Cell Profiler was developed and is actively being updated, the MIT Department of Biological Engineering headed by Dr Douglas Lauffenburger with close relationships in systems biology and beyond and the Massachusetts General Biostatistics Core supported also by Harvard Catalyst. In the wider Harvard Medical School and Harvard University community, there is an abundance of both scientific and computational resources that I will use in my training. These include weekly seminars, journal clubs and conferences on topics of tuberculosis, immunology, bioinformatics, computational biology, microbiology, molecular biology, bacterial pathogenesis and epidemiology. Additionally, there are a number of practical courses offered to young investigators, including grant and manuscript writing and career development guidance. The resources and expertise available at the Ragon, Harvard School of Public Health and the wider Harvard and Boston community are immense, and combined with the institutional commitment offered by the Infectious Diseases Division at MGH, and the guidance of my Scientific Advisory Group, this institutional environment will support me as I transition from mentored to independent research during the course of the K08 award.

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Statement of Institutional Commitment

I am very pleased to underscore the institutional commitment to the career development plan presented here by Dr. Lenette Lu in her application titled, "Antibody-Mediated Mechanisms of Immune Modulation in Tuberculosis." Dr. Lu's primary mentors are Drs. Sarah Fortune and Galit Alter at the Harvard School of Public Health and the Ragon Institute of the Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard University, respectively. These are both very well established and well respected investigators, who bring complementary expertise in the areas of tuberculosis and functional antibody-mediated responses to infection. Dr. Lu's progress will also be overseen by a scientific advisory committee that includes Drs. Eric Ruben, Akiko Iwasaki, Falk Nimmerjahn, and Facundo Batista, each of whom bring additional complementary expertise. The scientific advisory committee will meet with Dr. Lu in person, as well as her mentors, every six months for her to present work completed and her plans for future research and training.

Dr. Lu obtained her MD and PhD degrees at Case Western Reserve University in 2010. Her PhD thesis was focused on innate immune responses to dsRNA and viral immunity under the mentorship of Dr. Ganes Sen; one first-authored publication in *JBC* resulted. Dr. Lu then did Internal Medicine residency training at Cornell (2010-2013) and joined the Infectious Diseases Fellowship Training Program at Harvard Medical School, combined between the Massachusetts General Hospital and the Brigham and Women's Hospital in 2013. After her initial clinical fellowship year, Dr. Lu joined the laboratories of Drs. Fortune and Alter in 2014, supported by an institutional T32 grant. She largely conducts her BSL3 work at the Harvard School of Public Health and her immunology work at the Ragon Institute. Since beginning her research, Dr. Lu has focused on the functional role of antibodies with different Fc profiles in immune responses to tuberculosis, and how these differ between latent and active disease. She has used a systems serology approach to examine different antibody specificities and different Fc effector functions related to post-transcriptional glycosylation, and the subsequent effects on the interactions of these antibodies with Fc receptors on innate immune responses to TB. She has presented this work at both a talk and poster at a Keystone meeting this year, and a paper with her as first author describing the work is currently under review.

Dr. Lu was promoted to Instructor of Medicine at Harvard Medical School and Assistant in Medicine at the Massachusetts General Hospital in July 2015. Her position is not dependent on her obtaining this grant, and additional funds for her research are available from the many grants of her mentors. We will ensure that Dr. Lu has the protected time to effectively develop her research career (no less than 75%). In addition, we will ensure that she has the opportunity to maintain her clinical skills, appropriate to her role as a physician-scientist in an academic setting. During the period of support, however, her clinical activities will occupy no more than 10% of her time, in order to ensure her optimal development as an independent investigator. We will also ensure that she has the required space and other resources to successfully carry out the career development plan outlined in her application. Dr. Lu is an outstanding young physician-scientist, and we are pleased to underscore the institutional commitment to the career plan presented here.

[redacted]

Stephen B. Calderwood, MD

Human Subjects Research

Only coded specimens collected from subjects who have consented to participate in existing IRB approved protocols will be used. All specimens used for these studies will be coded with the subjects' unique research identification numbers and the PI will not have access to either the PHI or the key to the code linking the identity of the subjects with their unique research numbers. In addition to the existing protocols, a project specific protocol has been submitted to the local IRB to cover the use of coded samples obtained from our collaborator Cheryl Day at the University of Texas.

Protocol #: 2014P000437/MGH: Secondary Use of Plasma Specimens from the University of Texas. Expedited Review 3/12/2014. Action: Not Human Research.

Protocol #: 2014P000460/BWH: Research Use of Blood Products from Research Blood Components, LLC for Host Genetics, Immunology and Virology of HIV and Other Infectious Diseases (Fortune Lab). Expedited Review 3/11/2014. Action: Not Human Research.

Protocol #: 2006P000849/MGH: Cellular Immunology Specimen Repository and Database, Approved 8/7/2013.

1. Risks to the subjects

Human Subjects Involvement and Characteristics: The study enrolls individuals with latent or active TB who are willing and able to give informed consent and are adult males and females aged 18-75. Study sites take place in University of Texas Health Science Center at Houston-School of Public Health-Brownsville Regional Campus in association with Hidalgo County Health Department and Cameron County Health Department and or MGH Infectious Disease clinical sites as noted above. All consent forms have been reviewed and approved by the Partners IRB.

Sources of materials: Blood will be collected from all study participants. The specimen draw date, patient gender, year of birth and race, diagnosis, treatment and duration, will be collected. Blood draw limits are in accordance with institutional policy and safety insured by clinical laboratory monitoring. Data collected will be coded by research identification number. Each user has a unique username and password and level of access determined by assigned role. The PI of this grant will receive coded data/specimens and will never be given access to the key to the codes that contains identifiers that could be used to link the samples/data to individual subjects who provided the samples/data.

Potential risks: For standard blood draws, phlebotomy may cause a small amount of pain or bruising at the needle insertion site. Rarely, people faint during or after phlebotomy. Very rarely, an infection occurs at the site. Collection of blood samples by standard venipuncture from healthy appearing, nonpregnant adults who weigh at least 110 pounds generally poses minimal risk. Repeated blood donation can lead to anemia and iron efficiency. This can cause an individual to feel weak and/or tired and may require taking an iron supplement. The timing and frequency of blood collection as well as the quantity of blood collected will be recorded and closely monitored to avoid these problems. For whole blood procedures, there are some additional considerations. Both of these procedures are routinely performed on healthy volunteers who donate in Blood Donor Centers to contribute to the general blood supply. These procedures are also performed for therapeutic purposes as clinically indicated. In general approximately 2% of donors experience an adverse reaction due to the procedure, most of which are minor. Hypovolemic reactions can occur due to rapid change in blood pressure in both procedures. These include: nausea, vomiting, dizziness, fainting, dyspnea, lightheadedness, pallor, feeling of warmth, chills, excessive tiredness, or convulsions. There are similar risks to phlebotomy as indicated above. The less common risks include arterial puncture, delayed bleeding, nerve irritation, nerve injury, tendon injury, thrombophlebitis, or allergic reaction. There are additional known risks associated with leukapheresis such as adverse reactions to the anticoagulant (Acid Citrate Dextrose Type A) used during the procedure since the anticoagulant is returned to the donor with the blood components not being collected. The anticoagulant binds to calcium in the donor's blood, which can result in hypocalcemia and is associated with tingling in the lips but may cause convulsions. Donors are sometimes given a calcium supplement during the donation to prevent this reaction.

2. Adequacy of protection against risk

Recruitment and informed consent:
Potential participants may also contact the study team directly in response to flyers, Internet postings, and advertisements. Written informed consent will be obtained only after a potential subject has had an opportunity to read the consent, discuss study participation with a study team member, and ask questions. No study-related activity will take place prior to obtaining written informed consent. All informed consent documents have been reviewed and approved by the UT-H - SPH - Brownsville Regional Camp/RAHC and Partners IRB.

Protection against risk: Clinical laboratory data will be monitored to insure eligibility for blood donation and the frequency of sample collection will be directly monitored. Close clinical monitoring at the time of blood draw or leukapheresis is used to assess for any of the rare complications of phlebotomy. If the subject has an adverse event, he or she will be offered medical care as needed. Subject confidentiality is protected by storage of all data using a unique research identification number. Data is stored in a secure database with limited, monitored access. The PI of this grant will receive coded data/specimens and will never be given access to the key to the codes that contains identifiers that could be used to link the samples/data to individual subjects who provided the samples/data.

3. Potential benefits of the proposed research to human subjects and others

As clearly indicated in the informed consent, by taking part in the University of Texas study (Protocol #: 2014P000437/MGH), subjects may obtain the results from standard tests (ie TSPOT) that are not routinely performed as part of medical care. These test results should be discussed further with medical providers or health department staff for advice about the possible need to seek medical care. In addition, by participating in the study, the subjects are contributing to the understanding of TB pathogenesis and the host immune response to infection. By taking part in Protocol #: 2014P000460/BWH and Protocol #: 2006P000849/MGH, there are no immediate clinical benefits as is indicated in the informed consent form. However, by participating in the study, the subjects are contributing to the understanding of HIV pathogenesis and associated co morbidities such as tuberculosis as well as host immune response to infection.

4. Importance of the knowledge to be gained

The specific aims of these proposed studies seek to define a novel approach to tuberculosis biomarker and vaccine development. Understanding the mechanisms by which antibodies may modulate the immune response in tuberculosis disease is crucial, particularly given the large worldwide tuberculosis burden.

Targeted/Planned Enrollment Table: No subjects will be specifically recruited or enrolled for this project. Therefore, no Targeted/Planned Enrollment Table will be submitted with this proposal.

Data and Safety Monitoring for Clinical Trials: Not applicable (Category B: not a clinical trial).

Inclusion of women and minorities

Eligible adult males and females from all ethnic backgrounds are included in the cohorts utilized in this study.

PHS Inclusion Enrollment Report

This report format should NOT be used for collecting data from study participants.

OMB Number:0925-0001 and 0925-0002
Expiration Date: 10/31/2018

***Study Title:** Antibody Mediated Mechanisms of Immune Modulation in Tuberculosis

***Delayed Onset Study?** ❑ Yes ☑ No

If study is not delayed onset, the following selections are required:

Enrollment Type ❑ Planned ☑ Cumulative (Actual)

Using an Existing Dataset or Resource ☑ Yes ❑ No

Enrollment Location ☑ Domestic ❑ Foreign

Clinical Trial ❑ Yes ☑ No

NIH-Defined Phase III Clinical Trial ❑ Yes ☑ No

Comments:

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0	0	0	0	0	0	0	0	**0**
Asian	0	0	0	0	0	0	2	2	0	**4**
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	**0**
Black or African American	0	0	0	0	0	0	0	0	0	**0**
White	5	5	0	35	35	0	0	0	0	**80**
More than One Race	0	0	0	0	0	0	0	0	0	**0**
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	**0**
Total	**5**	**5**	**0**	**35**	**35**	**0**	**2**	**2**	**0**	**84**

Inclusion of Children

The samples used in the studies described in this proposal will be obtained from cohorts of individuals with latent or active tuberculosis. Only samples from adults and children aged 18 and over will be obtained.

Resource Sharing Plan

I acknowledge the importance of timely release of scientific information and resources to the broader community. This proposal does not involve animal research and will not generate model organisms and does not include plans for genome-wide association studies so the relevant policies for data sharing are not applicable. However, I am aware of the policies and guidelines established by the NIH with regard to release of research results. I plan to present the results generated by these studies at national and international conferences and publish the data in peer reviewed journals in a timely fashion. In addition, I am committed to the NIH Public Access policy. In keeping with general practices at Partners Healthcare, transfers of materials to academic and not-for-profit researchers for non-commercial research will be done in a timely fashion and at no cost, except for standard maintenance and transportation expenses, with terms as found in the institutional standard material transfer agreements.

Authentication of Key Biological and/or Chemical Resources

Key biological resources in this proposal include

1: Sera from human individuals with latent or active tuberculosis.
These are collected by our collaborator Dr Blanca Restrepo. Key control experiments performed in Dr Alter's lab have validated that the functional activity of IgG isolated via our methods of preparation and storage are maintained over time and freeze thaw cycles consistent with our manner of use. This includes the function of antibody dependent cellular phagocytosis. Multiple filtration steps by ultracentrifugation are employed in the isolation of IgG from the sera of these individuals to prevent indiscriminant contamination. This is confirmed by endotoxin testing.

2: Primary human monocyte derived macrophages.
There are multiple approaches to isolating primary human monocyte derived macrophages in the literature. In this study, they are isolated from peripheral blood from healthy human donors in the Boston, MA area which carries a low prevalence of tuberculosis. These donors are screened and negative for HIV, HCV and HBV. CD14 positive cells are isolated from whole blood with EasySep CD14 Selection Kit and matured for 7 days in RPMI with 10% fetal calf serum in low adherent flasks. Monocyte derived macrophages were characterized by flow cytometry as CD14-, CD40+, HLA ABC+, CD11b+, CD16+ (FcγRIIIa) and CD32+ (FcγRIIa). Alternative methods of maturation include the use of human sera instead of fetal calf sera and GM-CSF for the purposes of macrophage polarization. We have extensively tested these methods of maturation as well as duration of maturation (7, 14 and 21 days). Our method of maturation provides the most reliable and reproducible results for our *M. tuberculosis* macrophage restriction assay.

3. CEM-NKr CCR5+ T lymphoblast cell line.
This cell line is used in the Fc effector assay for Ab dependent cellular cytotoxicity in Aim 1. These cells were obtained from NIH AIDS reagents. These are maintained per suggested protocol by NIH AIDS reagents. This includes regular image cytometer analysis based on fluorescent dyes acridine orange and DAPI to stain the total and dead cell populations, respectively via Chemometec Nucleocounter. They are monitored by Internal controls to validate their functionality include the lack of antibody and the absence of antigen to assess indiscriminate cellular cytotoxicity. Further validation of the Ab dependent cellular cytotoxicity assays lies within validation of natural killer (NK) cells as described below.

4. Primary human natural killer (NK) cells.
Primary human natural killer (NK) cells are used in the Fc effector assay for Ab dependent cellular cytotoxicity in Aim 1. These cells are isolated from peripheral blood from healthy human donors in the Boston, MA area which carries a low prevalence of tuberculosis. These donors are screened and negative for HIV, HCV and HBV. NK cells were isolated from whole blood with RosetteSep (Stem Cell Technologies). They are used on the day of isolation and the purity of their isolation is confirmed by flow cytometry as CD3- and CD16 and/or CD56+. Wells containing serve as a positive control for maximal NK cell activation to ensure that they are functionally optimatlly.