

APPLICATION FOR FEDERAL ASSISTANCE  
**SF 424 (R&R)**

|   |                               |   |
|---|-------------------------------|---|
| <b>3. DATE RECEIVED BY STATE</b>  |                               | <b>State Application Identifier</b>   |
| <b>1. TYPE OF SUBMISSION*</b>   |                               | <b>4.a. Federal Identifier</b>  |
| <input type="radio"/> Pre-application <input type="radio"/> Application <input checked="" type="radio"/> Changed/Corrected Application  |                               | <b>b. Agency Routing Number</b>   |
| <b>2. DATE SUBMITTED</b>  | <b>Application Identifier</b> | <b>c. Previous Grants.gov Tracking Number</b><br>GRANT11797566  |
| <b>5. APPLICANT INFORMATION</b>   |                               | <b>Organizational DUNS*: 063690705</b>  |
| Legal Name*: University of Alabama at Birmingham<br>Department: Office of Sponsored Programs<br>Division:<br>Street1*: 1720 2nd Avenue South<br>Street2: AB 1170<br>City*: Birmingham<br>County: Jefferson<br>State*: AL: Alabama<br>Province:<br>Country*: USA: UNITED STATES<br>ZIP / Postal Code*: 352940111   |                               |   |
| Person to be contacted on matters involving this application<br>Prefix:      First Name*: Stephanie      Middle Name:      Last Name*: May      Suffix:<br>Position/Title: Grants and Contracts Officer<br>Street1*: 1720 2nd Avenue South<br>Street2: AB 1170<br>City*: Birmingham<br>County: Jefferson<br>State*: AL: Alabama<br>Province:<br>Country*: USA: UNITED STATES<br>ZIP / Postal Code*: 352940111<br>Phone Number*: 2059345266      Fax Number: 2059755977      Email: stephmay@uab.edu |                               |   |
| <b>6. EMPLOYER IDENTIFICATION NUMBER (EIN) or (TIN)*</b>  |                               | 1636005396A6  |
| <b>7. TYPE OF APPLICANT*</b>  |                               | H: Public/State Controlled Institution of Higher Education  |
| Other (Specify):<br><input checked="" type="radio"/> <b>Small Business Organization Type</b> <input type="radio"/> Women Owned <input type="radio"/> Socially and Economically Disadvantaged  |                               |   |
| <b>8. TYPE OF APPLICATION*</b>  |                               | If Revision, mark appropriate box(es).  |
| <input checked="" type="radio"/> New <input type="radio"/> Resubmission<br><input type="radio"/> Renewal <input type="radio"/> Continuation <input type="radio"/> Revision  |                               | <input type="radio"/> A. Increase Award <input type="radio"/> B. Decrease Award <input type="radio"/> C. Increase Duration<br><input type="radio"/> D. Decrease Duration <input type="radio"/> E. Other (specify) : |
| <b>Is this application being submitted to other agencies?*</b> <input type="radio"/> Yes <input checked="" type="radio"/> No      What other Agencies?  |                               |   |
| <b>9. NAME OF FEDERAL AGENCY*</b>   |                               | <b>10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER</b>  |
| National Institutes of Health   |                               | TITLE:  |
| <b>11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*</b>  |                               |   |
| Establishment and metabolic control of influenza-specific lung-resident memory B cells  |                               |   |
| <b>12. PROPOSED PROJECT</b>   |                               | <b>13. CONGRESSIONAL DISTRICTS OF APPLICANT</b>   |
| Start Date*   | Ending Date*                  | AL-007  |
| 07/01/2015  | 06/30/2018                    |   |



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## Facilities and Other Resources

**Laboratory:** The sponsor, Dr. Randall, runs an integrated lab with Dr. Francis Lund (see Biosketch of Key Personnel). Together they have approximately 2500 square feet of laboratory space on the 5<sup>th</sup> floor of the Shelby Biomedical Research Building at UAB. This space also includes a separate and dedicated tissue culture room, a microscopy suite and a pathogen prep room located within the lab. In addition, the PI has a dedicated fully equipped ABSL-2 lab suite (~300 sq. ft.) within the animal facility located in the adjacent (connected) RSB facility. The labs are fully equipped for the work planned in this application. Equipment includes: Biosafety cabinets, analytical balances, centrifuges (benchtop, free-standing, refrigerated, microfuges), incubators (dry, humidified, CO<sub>2</sub>), freezers (-150°C, -70°C, -25°C), refrigerators, ELISA plate readers, Luminometer, PCR machines, pH meters, microscopes (brightfield and dissecting), and MACs cell enrichment tools. Larger equipment items in the lab include an 8-color FACS Canto, a Magpix Luminex, a Biorad FPLC (used for producing B cell tetramers), and a Nikon inverted 4-channel fluorescent microscope with live cell imaging facility.

**Animal:** The UAB animal program is fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC), has Animal Welfare Assurance approval and is in compliance with state law, federal statute and NIH policy. Experimental animal used for this project will be housed in the Research Support Building (RSB) animal facility staffed with full-time veterinarian. RSB contains an animal x-ray irradiator and is available to all researchers requiring animal or cell irradiation, which can be used to irradiate mice if large #s of cells are being adoptively transferred. Dr. Randall has a suite within the RSB that contains 2 large dedicated mouse breeding rooms (10 vent racks), an ABSL-2 level procedure room and several ABSL-2 rooms for housing experimentally infected mice (9 vent racks). Dr. Randall maintains ~70 strains of laboratory mice that are commonly used for studies of infectious and autoimmune disease, including the  $\mu$ MT, B6, B6 Pepboy, B6 Blimp-YFP strains. Drs. Randall and Lund employ two full-time breeding core staff who are responsible for managing mouse breeding, generating new mouse strains, performing animal genotyping, and delivering experimental animals requested by the PI and other lab members to experimental rooms. Dr. Randall's experimental suites also house a procedure room that includes 3 BSL-2 cabinets, anesthesia and euthanasia equipment, surgical equipment and various other small equipment items used for animal handling.

**Office & Computer:** The PI, Rameeza Allie, has dedicated office space within the laboratory with access to, two computer rooms with 11 PC and Macintosh computer workstations, a color printer, a scanner, and a fax machine, are located within the laboratory. A remotely accessible expandable server (current 20 terabytes storage) is dedicated to the Randall-Lund labs for long-term storage of data files.

**Intellectual resources:** Dr. Randall shares an integrated lab with Dr. Francis Lund, with whom he has collaborated for more than 20 years. This collaboration has resulted in over 30 co-authored manuscripts, including seminal papers describing the role of effector B cells in protection to pathogens such as *H. polygyrus* and *Pneumocystis carinii*, and the role of antibodies and B cells in protection to influenza virus. Both Drs. Randall and Lund will provide technical and conceptual guidance to the PI in the completion of this proposal. Furthermore, UAB is home to many investigators studying B cell immunology. Of note is one of the PI's referees, Dr. John Kearney, who has conducted extensive research on IgM<sup>+</sup> plasma cell and memory B cell development and function. There is also a well-established community of basic and clinical immunologists with expertise in infectious disease, mucosal immunology and B cell biology (C Weaver, J. Mestecky, J. Novak, H. Schroeder, R. Kimberly) at UAB, all of who can provide advice and suggestions.

**The Office of Postdoctoral Education (OPE):** The OPE works in combination with the UAB Postdoctoral Association (PDA) to improve the quality of postdoctoral training at UAB. They offer various courses in grant writing, lab management, translational science and job skills. They also provide courses and seminars in teaching. Additionally they provide awards for career enhancement, travel, grant incentives and internships. The OPE and PDA has made UAB an excellent environment for postdoctoral training making them rank #8 as "the best places to work as a postdoc compared to national and international institutions " and #1 amongst public universities (Published in *The Scientists* (2012))

### **Core/shared facilities:**

The UAB Comprehensive Flow Cytometry Core has two locations including the Shelby Building (same building as the Randall lab). The core is available to lab members on a fee-for-use basis either with or without the assistance of dedicated operators. The cores provide multiple multi-color flow cytometry instruments that can be used for analysis (18 color LSRII) and cell sorting (14 color FACSArial).

The Center for Clinical and Translational Science (CCTS) provides access to ELISPOT readers and in addition, the BERD Core of the CCTS provides biostatistical support for researchers at UAB both at the time of grant submission and during the design/analysis phase of experiments.



## Equipment

**Within the Randall lab:** BSL-2 level biosafety cabinets, analytical balances, centrifuges (benchtop, free-standing, refrigerated, microfuges), incubators (dry, humidified, CO<sub>2</sub>), freezers (-150°C, -70°C, -25°C), refrigerators, SpectroMax M2 ELISA plate readers, SpectroMax L Luminometer, 2 PCR machines (standard), pH meters, microscopes (brightfield and dissecting), and MACs cell enrichment tools. Larger equipment items in the lab include an 8-color FACS Canto, a Magpix Luminex, a Biorad FPLC (used for producing B cell tetramers), and a Nikon Eclipse Ti Inverted 4-channel fluorescent microscope system with Intensilight mercury-fiber illuminator, Andor Clara Interline CCD camera and NIS-Elements software. This microscope is equipped with a Tokai Hit stage top incubator for live cell imaging applications.

**Outside the Randall lab:** BSL-2 Biosafety cabinets (procedure room in Dr. Randall's animal suite), LSRII and Aria sorters (UAB Comprehensive Flow Cytometry Core), Quantitative PCR and RNAseq (Dept. Microbiology Shared Equipment Core and Heflin Genomics Core), high speed and ultracentrifuges with aerosol containment (Microbiology Department Shared Equipment Core), X-ray irradiator (Animal Resources Program), ELISPOT reader (CCTS), Two 3730xl DNA Analyzers, One Biomek NXp Automation System, and several dual 9700 PCR thermocyclers (DNA sequencing Shared Facility at UAB), and the ImageStreamX Mark II (AMC, RDCC Comprehensive Flow Cytometry Core and the AIC High Resolution Imaging Component) for combined flow cytometry and visual imaging analysis of single cell populations.





| PROFILE - Senior/Key Person                  |                                     |                |   |                        |
|--|-------------------------------------|----------------|---|------------------------|
| Prefix:                                      | First Name*: Frances                | Middle Name E. | Last Name*: Lund                        | Suffix: PhD            |
| Position/Title*:                             | Professor and Chair                 |                |   |                        |
| Organization Name*:                          | University of Alabama at Birmingham |                |   |                        |
| Department:                                  | Microbiology                        |                |   |                        |
| Division:                                    | Medicine                            |                |   |                        |
| Street1*:                                    | 1720 2nd Avenue South               |                |   |                        |
| Street2:                                     | BBRB 276                            |                |   |                        |
| City*:                                       | Birmingham                          |                |   |                        |
| County:                                      | Jefferson                           |                |   |                        |
| State*:                                      | AL: Alabama                         |                |   |                        |
| Province:                                    |                                     |                |   |                        |
| Country*:                                    | USA: UNITED STATES                  |                |   |                        |
| Zip / Postal Code*:                          | 352942170                           |                |   |                        |
| Phone Number*:                               | 2059349339                          | Fax Number:    | 2059964008                              | E-Mail*: flund@uab.edu |
| Credential, e.g., agency login: FELund       |                                     |                |   |                        |
| Project Role*:                               | Other Professional                  |                | Other Project Role Category: Co-Sponsor |                        |
| Degree Type:                                 | PhD                                 |                | Degree Year: 1992                       |                        |
| <b>Attach Biographical Sketch*:</b>          |                                     |                | File Name                               |                        |
| <b>Attach Current &amp; Pending Support:</b> |                                     |                | 1236-Lund Biosketch.pdf                 |                        |

| PROFILE - Senior/Key Person                  |                                     |               |                                      |                         |
|--|-------------------------------------|---------------|--------------------------------------|-------------------------|
| Prefix:                                      | First Name*: Rodney                 | Middle Name G | Last Name*: King                     | Suffix: PhD             |
| Position/Title*:                             | Assistant Professor                 |               |                                      |                         |
| Organization Name*:                          | University of Alabama at Birmingham |               |                                      |                         |
| Department:                                  | Microbiology                        |               |                                      |                         |
| Division:                                    | Medicine                            |               |                                      |                         |
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| City*:                                       | Birmingham                          |               |                                      |                         |
| County:                                      | Jefferson                           |               |                                      |                         |
| State*:                                      | AL: Alabama                         |               |                                      |                         |
| Province:                                    |                                     |               |                                      |                         |
| Country*:                                    | USA: UNITED STATES                  |               |                                      |                         |
| Zip / Postal Code*:                          | 352942180                           |               |                                      |                         |
| Phone Number*:                               | 2059757150                          | Fax Number:   | 2059341875                           | E-Mail*: rgking@uab.edu |
| Credential, e.g., agency login: RGKING       |                                     |               |                                      |                         |
| Project Role*:                               | Other Professional                  |               | Other Project Role Category: Advisor |                         |
| Degree Type:                                 | PhD                                 |               | Degree Year: 2007                    |                         |
| <b>Attach Biographical Sketch*:</b>          |                                     |               | File Name                            |                         |
| <b>Attach Current &amp; Pending Support:</b> |                                     |               | 1237-King Biosketch.pdf              |                         |









8. Kaplin A, Carroll KA, Cheng J, **Allie R**, Lyketsos CG, Calabresi P, Rosenberg PB. 2008. IL-6 release by LPS-stimulated peripheral blood mononuclear cells as a potential biomarker in Alzheimer's disease. *Int Psychogeriatr.* 21(2):413-4. PMID: PMC2664968
9. Jerome J. Graber\*, **S. Rameeza Allie\***, Katherine M. Mullen, Melina V. Jones, Tongguang Wang, Chitra Krishnan, Avi Nath, Douglas A. Kerr, Peter A. Calabresi. 2008. Interleukin 17 Pathway in CNS Autoimmune Inflammation. *J Neuroimmunol.* 6(1-2):124-32. (\* **Co-first Author**) PMID: 18417225. Accepted for publication prior to April, 2008.
10. Wang T, **Allie R**, Conant K, Haughey N, Turchan-Chelowo J, Hahn K, Rosen A, Steiner J, Keswani S, Jones M, Calabresi PA, Nath A. 2006. Granzyme B mediates neurotoxicity through a G-protein-coupled receptor. *FASEB J.* 20(8):1209-11. PMID: 16636104
11. Rus H, Pardo CA, Hu L, Darrah E, Cudrici C, Niculescu T, Niculescu F, Mullen KM, **Allie R**, Guo L, Wulff H, Beeton C, Judge SI, Kerr DA, Knaus HG, Chandy KG, Calabresi PA. 2005. The voltage-gated potassium channel Kv1.3 is highly expressed on inflammatory infiltrates in multiple sclerosis brain. *Proc Natl Acad Sci U S A.* 102(31): 11094-9. PMID: PMC1182417
12. **Allie R**, Hu L, Mullen KM, Dhib-Jalbut S, Calabresi PA. 2005. Bystander modulation of chemokine receptor expression on peripheral blood T lymphocytes mediated by glatiramer therapy. *Arch Neurol.* 62(6):889-94. PMID: 15956159
13. Calabresi PA, **Allie R**, Mullen KM, Yun SH, Georgantas RW 3rd, Whartenby KA. 2003. Kinetics of CCR7 expression differ between primary activation and effector memory states of T(H)1 and T(H)2 cells. *J Neuroimmunol.* 139(1-2):58-65. PMID: 12799021
14. Wulff H, Calabresi PA, **Allie R**, Yun S, Pennington M, Beeton C, Chandy KG. 2003. The voltage-gated Kv1.3 K(+) channel in effector memory T cells as new target for MS. *J Clin Invest.* 111(11):1703-13. PMID: PMC156104
15. Calabresi PA, Yun SH, **Allie R**, Whartenby KA. 2002. Chemokine receptor expression on MBP-reactive T cells: CXCR6 is a marker of IFN $\gamma$ -producing effector cells. *J Neuroimmunol.* 127(1-2):96-105. PMID: 12044980

#### D. Scholastic Performance

| YEAR  | SCIENCE COURSE TITLE              | GRADE | YEAR                                   | OTHER COURSE TITLE                 | GRADE |
|---|-----------------------------------|-------|--|------------------------------------|-------|
| INSTITUTE OF TECH. STUDIES (ITS)– SRI LANKA |                                   |       | INSTITUTE OF TECH. STUDIES – SRI LANKA |                                    |       |
| 1994  | General Biology I & lab           |       | 1994                                   | Composition & Rhetoric I           |       |
| 1994  | General Chemistry I & lab         |       | 1994                                   | Analytical Geometry                |       |
| 1995  | General Biology II & lab          |       | 1995                                   | The US History to 1877             |       |
| 1995  | General Chemistry II & lab        |       | 1995                                   | Differential & Integral Calculus I |       |
| 1995  | General Psychology                |       | 1995                                   | American National & State Gov. I   |       |
| 1995  | Anatomy & Physiology I            |       | 1995                                   | Microcomputer Applications         |       |
| 1995  | Organic Chemistry                 |       | 1995                                   | Technical Communications           |       |
| YEAR  | SCIENCE COURSE TITLE              | GRADE | YEAR                                   | OTHER COURSE TITLE                 | GRADE |
| UNIVERSITY OF KENTUCKY – USA                |                                   |       | UNIVERSITY OF KENTUCKY – USA           |                                    |       |
| 1996  | Principles of Microbiology        |       | 1996                                   | English Writing II                 |       |
| 1996  | Intro. Cell Biology               |       | 1996                                   | Human Sexuality                    |       |
| 1997  | Organic Chemistry I               |       | 1997                                   | The Arab Awakening                 |       |
| 1997  | Organic Chemistry I lab           |       | 1997                                   | Introductory Sociology             |       |
| 1997  | Introductory Ecology              |       | 1997                                   | Mental Hygiene                     |       |
| 1997  | Food Microbiology                 |       | 1997                                   | Islamic Civilization I             |       |
| 1998  | Independent Work in Biology I     |       | 1998                                   | Visual Exploration I               |       |
| 1998  | Independent Work in Biology II    |       | 1998                                   | Basic Public Speaking              |       |
| 1998  | Organic Chemistry II              |       | 1998                                   | Aerobics: Aerobics Dance           |       |
| 1998  | Intro. Microbiology lab           |       | 1998                                   | SP. Prob. Foods/Nutrition          |       |
| 1998  | Principles of Genetics            |       | 1998                                   | History of US since 1865           |       |
| 1998  | Animal Physiology                 |       |  |                                    |       |
| 1998  | Bio Seminar: Environmental Issues |       |  |                                    |       |

| YEAR                                       | SCIENCE COURSE TITLE                  | GRADE | YEAR                           | OTHER COURSE TITLE          | GRADE |
|--|---------------------------------------|-------|--------------------------------|-----------------------------|-------|
| UNIVERSITY OF MARYLAND BALTIMORE CO. – USA |                                       |       | JOHNS HOPKINS UNIVERSITY – USA |                             |       |
| 2000                                       | Biological Chemistry                  |       | 2004                           | Statistical Methods I       |       |
| 2000                                       | Approaches to Molecular Biology       |       | 2004                           | Statistical Methods II      |       |
| 2000                                       | Advanced Molecular Biology Lab        |       | 2004                           | Statistical Methods III     |       |
| 2000                                       | RSRCH. Applied Molecular Biology      |       | 2004                           | Statistical Models IV       |       |
| 2000                                       | Microbial Molecular Genetics          |       | 2005                           | Principles of Epidemiology  |       |
| 2000                                       | Gen. & Eukaryotic Molecular Biology   |       |                                |                             |       |
| 2000                                       | Advanced Molecular Biology Lab II     |       |                                |                             |       |
| YEAR                                       | SCIENCE COURSE TITLE                  | GRADE | YEAR                           | SCIENCE COURSE TITLE        | GRADE |
| DARTMOUTH COLLEGE – USA                    |                                       |       | DARTMOUTH COLLEGE – USA        |                             |       |
| 2007                                       | Biochem. Cell & Molecular Biology     |       | 2009                           | Graduate Research II: Lev   |       |
| 2007                                       | Graduate Research: Level              |       | 2010                           | Cancer Biology Journal Club |       |
| 2007                                       | Immunology Journal Club               |       | 2010                           | Supervised Teaching         |       |
| 2008                                       | Biochem. Cell & Molecular Biology II  |       | 2010                           | Graduate Research II: Lev   |       |
| 2008                                       | Graduate Research: Level              |       | 2010                           | Immunology Journal Club     |       |
| 2008                                       | Immunology Journal Club               |       | 2010                           | Graduate Research II: Lev   |       |
| 2008                                       | Ethical Conduct of Research           |       | 2010                           | Graduate Research II: Lev   |       |
| 2008                                       | Biochem. Cell & Molecular Biology III |       | 2010                           | Immunology Journal Club     |       |
| 2008                                       | Res. Progress Colloquium              |       | 2010                           | Graduate Research II: Lev   |       |
| 2008                                       | Immunotherapy                         |       | 2011                           | Immunology Journal Club     |       |
| 2008                                       | Graduate Research: Level              |       | 2011                           | Graduate Research II: Lev   |       |
| 2008                                       | Immunology Journal Club               |       | 2011                           | Immunology Journal Club     |       |
| 2008                                       | Graduate Research I: Lev              |       | 2011                           | Graduate Research II: Lev   |       |
| 2008                                       | Graduate Research: Level              |       | 2011                           | Graduate Research II: Lev   |       |
| 2008                                       | Immunology Journal Club               |       | 2011                           | Immunology Journal Club     |       |
| 2008                                       | The Neurobiology of Disease           |       | 2011                           | Graduate Research II: Lev   |       |
| 2009                                       | Advanced Cell & Mol. Immunology       |       | 2012                           | Immunology Journal Club     |       |
| 2009                                       | Graduate Research I: Lev              |       | 2012                           | Graduate Research II: Lev   |       |
| 2009                                       | Immunology Journal Club               |       | 2012                           | Immunology Journal Club     |       |
| 2009                                       | Graduate Research I: Lev              |       | 2012                           | Graduate Research II: Lev   |       |
| 2009                                       | Immunology Journal Club               |       | 2012                           | Graduate Research II: Lev   |       |
| 2009                                       | Graduate Research I: Lev              |       | 2012                           | Graduate Research II: Lev   |       |
| 2009                                       | Immunology Journal Club               |       |                                |                             |       |

High Pass (HP) Superior Quality, Pass (P) Good Quality, Low Pass (LP) Acceptable Quality, No Credit (NC) Work that is not acceptable for graduate credit, Credit (CT). CT is the only passing grade in a course in which it is used.

## **E. Research Support**

### **ACTIVE**

R01 AI097357 (Randall)

5/11/12-4/30/17

NIH/NIAID

Central and effector memory B cells in the lung

The major goals of this project are to characterize the phenotype and function of central and effector memory B cells responding to influenza and to determine how the recirculation and homing of central and effector memory B cells is controlled, particularly in the lung.

Role: Postdoctoral fellow

## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.  
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME<br>Randall, Troy D.   | POSITION TITLE<br>Professor of Medicine and Microbiology and Immunology |       |                      |
|--|---|-------|----------------------|
| eRA COMMONS USER NAME (credential, e.g., agency login)<br>trandall   |   |       |                      |
| EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)</i> |   |       |                      |
| INSTITUTION AND LOCATION   | DEGREE<br><i>(if applicable)</i>  | MM/YY | FIELD OF STUDY       |
| University of Denver, Denver, CO   | B.S.  | 1987  | Chemistry            |
| Duke University, Durham, NC  | Ph.D.   | 1992  | Microbiology/Immunol |
| Stanford University, Stanford, CA  | postdoc   | 1997  | Immunology           |

### A. Personal Statement

My laboratory studies all aspects of the immune response to influenza virus using the mouse model of influenza infection. We have funded (past and present) projects concerning how functional memory CD8+ T cell responses to influenza are generated and maintained, how CD4+ effector and Tfh cell responses to influenza are controlled, how B cell responses to influenza are generated and how local lymphoid tissues like inducible Bronchus Associated Lymphoid Tissue (iBALT) control local immune responses to influenza in the lung. Thus, my lab has worked on the immune response to influenza for over 15 years. Most recently, we developed reagents to identify and characterize antigen-specific B cell responses to influenza. Taking a cue from the highly successful MHC class I and class II tetramers, we generated B cell tetramers that will bind to the BCRs of influenza-specific B cells. As a result, personnel in my lab can now identify and characterize B cells specific for influenza nucleoprotein, non-structural protein 1 and several hemagglutinin subtypes, including H1(A/PR8), H3(A/X31), H1(A/CA/7/09), H3(A/Texas/50/12) and influenza B/Massachusetts/2/12. When conjugated to fluorochromes, these reagents can be used in flow cytometry and immunohistology assays and, in their unconjugated form, used in traditional ELISPOT and ELISA assays. Thus, we are uniquely poised to make significant advances in understanding how and where influenza-specific effector and memory B cells are generated and maintained.

Our studies on influenza and pulmonary immunity began at the Trudeau Institute, where I supervised a handful of remarkable postdocs who defined the initial immunobiology of lymphoid tissues in the lung and determined the role of lymphoid chemokines in their development, organization and function. Many of these postdocs have now moved on to faculty positions at other universities or to similar positions in industry. Since Trudeau Institute was not a degree-granting institution, I did not have any graduate students in my laboratory for the first 12 years of my career as an independent investigator. However, upon moving to the University of Rochester and subsequently moving to the University of Alabama at Birmingham, I began to accept students into my lab and I am now mentoring two graduate students and an MSTP student. For each of my trainees, regardless of their level, I teach them how to design and perform experiments that test, in a physiological way, how immune responses work in vivo. These experiments are not always easy nor can they be performed in a short amount of time. Moreover, I also strive to have trainees complete an entire series of experiments and get the entire story before publishing. Thus, my trainees often require a year or two before they publish their first papers. However, we try (and often succeed!) in publishing in high impact journals. As a result, our papers are often cited in Faculty of 1000 reviews as well as the commentary sections of the journals. Thus, I believe that I am training my students and postdoctoral fellows how to perform and publish insightful experiments that answer important questions and will allow them to succeed in their careers as independent investigators.

## B. Positions and Honors

### Positions and Employment

|               |   |
|---------------|---|
| 09/87-04/92   | Predoctoral Fellow with Dr. Ronald Corley, Duke University        |
| 04/92-11/92   | Postdoctoral Fellow with Dr. Ronald Corley, Duke University       |
| 11/92-06/96   | Postdoctoral Fellow with Dr. Irving Weissman, Stanford University |
| 06/96-03/97   | Visiting Scientist, DNAX Research Institute, Palo Alto, CA        |
| 04/97-03/01   | Assistant Member, Trudeau Institute, Saranac Lake, NY             |
| 03/01-04/07   | Associate Member, Trudeau Institute, Saranac Lake, NY             |
| 04/07-06/08   | Member, Trudeau Institute, Saranac Lake, NY                       |
| 06/08-02/12   | Professor, Department of Medicine, University of Rochester        |
| 03/12-present | Professor of Medicine, University of Alabama at Birmingham        |

### Other Experience and Professional Memberships

|               |  |
|---------------|--|
| 08/97-06/08   | Supervisor, Flow Cytometry Facility, Trudeau Institute, Saranac Lake, NY |
| 06/98-12/08   | Member of American Society of Hematology                                 |
| 04/98-present | Member of American Association of Immunologists                          |
| 04/98-present | Member of American Association for the Advancement of Science            |
| 01/10-present | Faculty Member, Faculty of 1000  |
| 05/10-present | Academic Editor, PLOS ONE  |
| 03/13-present | Associate Editor, Mucosal Immunology                                     |

### Honors

National Merit Scholar, University of Denver, 1982-1886  
 NIH predoctoral training fellowship in Genetics, Duke University, 1987-1990  
 NIH Postdoctoral Fellowship in Immunology, Stanford University, 1992-1993  
 Helen Hay Whitney Postdoctoral Fellowship, Stanford University, 1993-1996  
 J Claude Bennett Professorship in Medicine, University of Alabama at Birmingham, 2012-present

## C. Selected Peer-reviewed Publications (Selected from 104)

1. **Randall, T.D.**, A.W. Heath, L. Santos-Argumedo, M.C. Howard, I.L. Weissman and and F.E. Lund. 1998. Arrest of B lymphocyte terminal differentiation by CD40 signaling: Mechanism for lack of antibody secreting cells in germinal centers. *Immunity* 8: 733-742.
2. Partida-Sanchez, S., Cockayne, D., Monard, S., Jacobson, E.L., Oppenheimer, N., Garvy, B.A., Kusser, K., Goodrich, S, Howard, M.C., Harmsen, A., **Randall, T.D.** and Lund, F.E. 2001. Cyclic ADP-ribose production by CD38 regulates intracellular calcium release, extracellular calcium influx and chemotaxis in neutrophils and is required for bacterial clearance in vivo. *Nat. Med* 7: 1209-1216.
3. Moyron-Quiroz, JE, J Rangel-Moreno, K Kusser, L. Hartson, F. Sprague, S. Goodrich, D.L. Woodland, FE Lund and **T.D. Randall**. 2004. Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. *Nat. Med.* 10: 927-934.
4. Moyron-Quiroz, JE, J. Rangel-Moreno, L. Hartson, K Kusser, M.P. Tighe, KD Klonowski, L Lefrancois, LS Cauley, A.G. Harmsen, F.E. Lund and **TD Randall**. 2006. Persistence and responsiveness of immunologic memory in the absence of secondary lymphoid organs. *Immunity* 25: 643-654
5. Wojciechowski, W, DP Harris, F Sprague, B Mousseau, M Makris, K Kusser, T Honjo, K Mohrs, M Mohrs, **TD Randall** and FE Lund. 2009. Regulation of type 2 immunity to H polygyrus by effector B cells: requirement for cytokine-producing B cells. *Immunity* 30: 421-433. PMC2745290
6. J Rangel-Moreno, Moyron-Quiroz, JE, DM Carragher, K Kusser, L Hartson, A Moquin, and **TD Randall**. 2009. Milky spots in the omentum develop independently of lymphoid tissue inducer cells and support T-dependent responses to peritoneal antigens. *Immunity* 30: 731-743. PMC2754314
7. Lin Y, Ritchea S, Logar A, Slight S, Messmer M, Rangel-Moreno J, Guglani L, Alcorn JF, Strawbridge H, Park SM, Onishi R, Nyugen N, Walter MJ, Pociask D, **Randall TD**, Gaffen SL, Iwakura Y, Kolls JK, Khader SA. 2009. Interleukin-17 is required for T helper 1 cell immunity and host resistance to the intracellular pathogen Francisella tularensis. *Immunity* 31: 799-810. PMC2789998
8. Ballesteros-Tato, A, B León, FE. Lund and **TD. Randall**. 2010. Temporal changes in dendritic cell subsets, cross-priming and costimulation via CD70 control CD8<sup>+</sup> T cells responses to influenza. *Nat. Immunol.* 11: 216-224. PMC2822886

9. Lund, FE and **TD Randall**. 2010 Regulatory and effector B cells: modulators of CD4+ T cell immunity. *Nat. Rev. Immunol.* 10: 236-247. PMC3038334
10. Rangel-Moreno, J, DM Carragher, JY Hwang, M de la L Garcia-Hernandez, K Kusser, L Hartson, J Kolls, SA. Khader and **TD Randall**. 2011. The development of inducible Bronchus Associated Lymphoid Tissue (iBALT) is dependent on IL-17. *Nat Immunol.* 12: 639-647. PMC3520063
11. Leon, B, A Ballesteros-Tato, **TD Randall** and FE Lund. 2012. Parasite-induced T<sub>FH</sub> and Th2 development relies on B cell dependent positioning of CXCR5+ dendritic cells in the lymph node. *Nat Immunol.* 13: 681-690. PMC3548431
12. Ballesteros-Tato, A, B León, BA. Graf, A Moquin, PS Adams, FE. Lund and **TD Randall**. 2012. IL-2 inhibits germinal center formation by limiting T follicular helper differentiation. *Immunity* 36: 847-846. PMC3361521
13. León, B, FE Lund, **TD Randall** and A. Ballesteros-Tato. 2014. FoxP3+ regulatory T cells promote influenza-specific Tfh responses by controlling IL-2 availability. *Nat. Comm.* 5: 3495. PMC4013682
14. Ballesteros-Tato, A. B León, BO Lee, FE Lund and **TD Randall**. 2014. Epitope-specific regulation of memory programming by differential duration of antigen presentation to influenza-specific CD8+ T cells. *Immunity* 41: 127-410. PMC4233138
15. León, B, A. Ballesteros-Tato, **TD Randall**, and FE Lund. 2014. Prolonged antigen presentation by immune complex-binding dendritic cells programs the proliferative capacity of memory CD8 T cells. *J. Exp. Med.* 211: 1637-1655. PMC4113940

#### D. Research Support

##### ACTIVE

R01 HL069409 (Randall) 9/01-3/17

NIH/NHLBI

“Unique aspects of respiratory immunity”

R01 AI100127 (Randall) 3/12-2/17

NIH/NIAID

“Pulmonary immunity to pathogens in neonates”

R01 AI097357 (Randall) 7/12-6/17

NIH/NIAID

“Central and effector memory B cells in the lung”

P01 AI078907 (Sanz) 08/10-07/15

NIH/NIAID

"B Cells in Health and Disease" Project 3. Evaluation of IFN $\gamma$  producing effector B cells in infectious and autoimmune disease (Project leader Lund)

Role: Co-Investigator

P30 AR048311 (Mountz) 09/01 – 08/17

NIH / NIAMS

Rheumatic Disease Core Center

Role: Co-Director Comprehensive Flow Cytometry Core

U19 AI109962 (Randall) 05/14-04/19

NIH/NIAID

Virus-induced cell fate decisions in anti-viral immunity

The goal of this project is to determine how cell fate decisions in CD4 T cells, CD8 T cells, B1 B cells and B2 B cells are regulated during antiviral immunity

Role: Overall PD/PII, Leader of Project 1, Leader of Core A

## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.  
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

|  |   |       |                      |
|--|---|-------|----------------------|
| NAME<br>Lund, Frances E.   | POSITION TITLE<br>Professor and Chair of Microbiology |       |                      |
| eRA COMMONS USER NAME (credential, e.g., agency login)<br>FELund   |   |       |                      |
| EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)</i> |   |       |                      |
| INSTITUTION AND LOCATION   | DEGREE<br><i>(if applicable)</i>                      | MM/YY | FIELD OF STUDY       |
| University of Notre Dame   | B.S.  | 05/87 | Microbiology         |
| Duke University  | Ph.D.   | 05/92 | Immunology           |
| DNAX Research Institute  | Postdoctoral  | 04/97 | Molecular Immunology |

### A. Personal Statement

The goal of this F32 application is to characterize the different memory B cell populations that seed effector sites, like the lung, and secondary lymphoid tissues. Dr. Allie proposes to determine the origin and repertoire of the different memory B cell populations, to functionally characterize each population and to determine whether the cells can enter the plasma cell pool. In addition, she plans to define the metabolic pathways that are used by the different populations of memory B cells to respond to antigen and differentiate into plasma cells. I believe that I am well-positioned to provide Dr. Allie with advice and mentoring as she develops this project as I have more than 20 yrs experience studying B cell responses to pathogens, autoantigens allergens and tumors. My laboratory currently has two major research focus areas. First, we are addressing the mechanisms that control the migration of immune cells to sites of inflammation and infection. In the course of these studies, we showed that the migration of neutrophils, monocytes and dendritic cells to sites of inflammation is regulated by the ecto-enzyme CD38. We demonstrated that CD38 catabolizes NAD and catalyzes the formation of metabolites that regulate calcium signaling and that are involved in NAD biosynthesis. We found that one of the metabolites produced by CD38 is required for intracellular calcium release and extracellular calcium influx in granulocytes, monocytes and dendritic cells that are responding to particular chemokines and chemoattractants. In addition, we found that NAD homeostasis is altered in CD38 deficient cells, which affects the ability of these cells to respond to oxidative stress. More recently, we focused on whether we can manipulate NAD metabolism in cancer cells to alter their growth properties and sensitivity to chemotherapeutics. Specifically, we are addressing whether we can alter NAD(P)(H)-dependent processes such as glycolysis, oxidative phosphorylation and the pentose phosphate pathway by altering CD38 expression or enzyme activity. We are currently funded for a high throughput screen to identify CD38 inhibitors to test in models of B cell leukemias, lymphomas and multiple myeloma. These studies fit very well with the second focus of my lab, which is on the function of B lymphocytes. Importantly, in the course of these studies we measured many of the relevant glycolytic/PPP/Ox-Phos metabolites in B cells using a combination of mass spec and NMR approaches and will be able to advise Dr. Allie when she begins her metabolic analyses of the memory B cell populations.

In addition to our NAD metabolism studies, my lab has an extensive track record in studying B cell biology, particularly in the areas of B cell selection and differentiation in the germinal center, B cell signaling, plasma cell differentiation and cytokine-producing B cell effector development and function. My lab has studied immune responses to numerous pathogens including viruses, bacteria, and parasites. We have been particularly interested in anti-viral immune responses and have used the influenza A virus infection model to study antibody and B cell responses to influenza and to study both CD4 and CD8 T cell responses to this virus. We are currently funded to identify the molecular signals that control influenza virus-induced long-lived plasma cell development with the long-term goal of developing anti-viral vaccines that activate this pathway and induce effective and enduring anti-viral humoral immunity. We are also funded to elucidate the role of T-box dependent transcription factors during B cell commitment to the plasma cell or memory B cell lineages within the germinal center. Finally, we are funded to phenotypically and functionally characterize human effector B cell subsets in healthy individuals, in patients with respiratory infections and in autoimmune individual. Thus, I



believe that we can assist Dr. Allie with many of her experiments examining the various memory B and plasma cell subsets.

Much of my scientific career was spent at Trudeau Institute, a basic science immunology and infectious disease research organization. Trudeau was not a degree granting institution so my lab has primarily trained post-doctoral fellows. Most of these fellows have gone on to independent scientific careers in academia and industry. Thus, given my expertise in immunology and my success with training post-doctoral fellows, I believe that I am qualified to act as an effective mentor to Ms. Stone as she pursues her graduate work in immunology.

## **B. Positions and Honors**

### **Positions and Employment**

04/97-04/01 Assistant Member, Trudeau Institute  
 04/01-06/06 Associate Member, Trudeau Institute  
 06/06-06/08 Member, Trudeau Institute  
 05/98-06/08 Adjunct Assoc. Prof. of Microbiol. Immunol. and Mol. Genetics, Albany Med. College  
 07/02-06/08 Adjunct Associate Professor of Medicine, University of Vermont  
 06/08-03/12 Professor, Dept. of Medicine and Dept. of Micro. and Immunol., University of Rochester, Rochester NY  
 03/12-present Professor and Chair, Dept. of Microbiology, University of Alabama at Birmingham (UAB), Birmingham AL  
 08/12-present Professor, Div. Clinical Immunology and Rheumatology (secondary appointment) UAB  
 03/12-present Senior Scientist, Comprehensive Cancer Center, UAB  
 03/12-present Senior Scientist, Comprehensive Arthritis Musculoskeletal Autoimmunity Center, UAB  
 03/12-present Senior Scientist, Center for AIDS Research, UAB  
 03/12-present Senior Scientist, Comprehensive Diabetes Center  
 01/13-present Co-Director UAB Immunology, Autoimmunity and Transplantation Steering Committee  
 01/13-present Member, Executive Advisory Board, RDC  
 11/13-present Member, Executive Advisory Board, CAMBAC

### **Other Experience and Professional Memberships**

1998-present Member of American Association of Immunologists  
 1998-present Member of American Association for the Advancement of Science  
 2007-present Associate Editor, The Journal of Immunology  
 2004-2007 AAI Education Advisory Committee  
 1998-2008 Faculty Supervisor of Trudeau Institute Animal Facilities

### **Honors**

1987 Recipient, Senior Scientist Service Award; Department of Biological Sciences/Microbiology, University of Notre Dame  
 1991 Recipient, The Norman Francis Conant Research award; Department of Microbiology and Immunology, Duke University  
 2011 Organizer, FASEB summer Conference: NAD Metabolism and Disease  
 2011 Organizer, Keystone Symposia "New Insights into Normal vs. Dysregulated B Cell Function"  
 2012 Charles H. McCauley Endowed Chair in Microbiology

## **C. Selected Publications – in chronological order (from 104 publications)**

- Howard, M, Grimaldi, J.C., Bazan, J.F., Lund, F.E., Santos-Argumedo, L., Parkhouse, R.M.E., Walseth, T.F., Lee, H.C. 1993. Formation and hydrolysis of cyclic ADP-ribose catalyzed by lymphocyte antigen CD38. *Science* 262:1056-1059.
- Randall T.D., A.W. Heath, L. Santos-Argumedo, M.C. Howard, I.L. Weissman, **Lund, F.E.** 1998. Arrest of B lymphocyte terminal differentiation by CD40 signaling: Mechanism for lack of antibody secreting cells in germinal centers. *Immunity* 8:733-742.

3. Harris, D.P., Haynes, L., Sayles, P.C., Duso, D.K., Eaton, S.M. Lepak, L.M., Johnson, L.L., Swain, S.L., **Lund, F.E.** 2000. Reciprocal regulation of polarized cytokine production by effector B and T cells. *Nature Immunology* 1: 475-482.
4. Partida-Sanchez, S., Cockayne, D., Monard, S., Jacobson, E.L., Oppenheimer, N., Garvy, B.A., Kusser, K., Goodrich, S., Howard, M.C., Harmsen, A., Randall, T.D. and **Lund, F.E.** 2001. Cyclic ADP-ribose production by CD38 regulates intracellular calcium release, extracellular calcium influx and chemotaxis in neutrophils and is required for bacterial clearance in vivo. *Nature Medicine* 7:1209-1216.
5. Partida-Sanchez, S., Goodrich, S., Kusser, K., Randall, T.D., **Lund, F.E.** 2004. Regulation of dendritic cell trafficking by the ADP-ribosyl cyclase CD38: Impact on the development of humoral immunity. *Immunity* 20:279-291.
6. Moyron-Quiroz, J.E., Rangel-Moreno, J., Kusser, K., Hartson, L., Sprague, F., Goodrich, S., Woodland, D.L., **Lund, F.E.**, Randall, T.D. 2004. Role of inducible Bronchus Associated Lymphoid Tissue (iBALT) in respiratory immunity. *Nature Med.* 10:927-934.
7. Moyron-Quiroz, J.E., Rangel-Moreno, J., Hartson, L., Kusser, K., Tighe, M., Klonowski, K.D., LeFrancois, L., Cauley, L.S., Harmsen, A.G., **Lund, F.E.**, Randall, T.D. 2006. Persistence and responsiveness of immunologic memory in the absence of secondary lymphoid organs. *Immunity* 25:643-654.
8. Shi, S., Partida-Sánchez, S., Misra, R.S., Tighe, M., Borchers, M., Lee, J.J. Simon, M., **Lund F.E.** 2007. Identification of an alternative G $\alpha$ q-dependent chemokine receptor signal transduction pathway in dendritic cells and granulocytes. *J. Exp. Med.* 204:2706-2718.
9. Wojciechowski, W., Harris, D.P., Sprague, F., Mousseau, B., Makris, M., Kusser, K., Honjo, T., Mohrs, K., Mohrs, M., Randall, T.D., and **Lund, F.E.** 2009. Regulation of type 2 immunity to *H. polygyrus* by effector B cells: Requirement for cytokine-producing B cells. *Immunity* 30:421-433. PMC2745290.
10. Ballesteros-Tato, A., León, B., **Lund, F.E.**, Randall, T.D. 2010. Temporal changes in dendritic cell subsets, cross-priming and costimulation via CD70 control CD8<sup>+</sup> T cells responses to influenza. *Nature Immunol.* 11:216-224. PMC2822886.
11. Misra, R.S., Shi, G., Moreno-Garcia, M.E., Thankappan, A., Tighe, M., Kusser, K., Becker-Herman, S., Hudkins Loya, K.L., Dunn, R., Kehry, M.R., Migone, T-S., Marshak-Rothstein, A., Simon, M., Randall, T.D., Alpers, C.E., Liggitt, D. Rawlings, D.J., **Lund, F.E.** 2010. G $\alpha$ q-containing G proteins regulate B cell selection and survival and are required to prevent B cell dependent autoimmunity. *J. Exp. Med.* 207:1775-1789. PMC2916136.
12. Ballesteros-Tato, A., León, B., Graf, B.A., Moquin, A., Adams, P.S., **Lund, F.E.**, Randall T.D. 2012. Interleukin-2 inhibits germinal center formation by limiting T follicular helper cell differentiation. *Immunity* 36:847-856. PMC3361521.
13. León, B., Ballesteros-Tato, A., Browning, J.L., Dunn, R., Randall, T.D., **Lund, F.E.** 2012. Regulation of T<sub>H</sub>2 development by CXCR5<sup>+</sup> dendritic cells and lymphotoxin-expressing B cells. *Nat. Immunol.* 13:681-690. PMC3548431.
14. Zeng, Q., Ng, Y.H., Singh, T., Jiang, K., Sheriff, K.A., Ippolito, R., Zahalka, S., Li, Q., Randhawa, P., Hoffman, R., Ramaswami, B., **Lund, F.E.**, Chalasani, G. 2014. B cells mediate chronic allograft rejection independent of antibody production. *J. Clin. Inv.* 124:1052-1056. PMC3934170.
15. León, B., Ballesteros-Tato, A., Randall, T.D., **Lund, F.E.** 2014. Prolonged antigen presentation by immune complex-binding dendritic cells programs the proliferative capacity of memory CD8 T cells. *J. Exp. Med.* 211:1637-1655. PMC4113940.

## D. Research Support

### Ongoing Research Support

P01 AI078907 (Sanz)

08/01/10-07/30/15

NIH/NIAID

B cells in Health and Disease - Lund (P.L.); *Project 3: Role of cytokine-producing effector B cells in autoimmunity*

The major goal of project 3 is to better understand how cytokine-producing B cells contribute to autoimmune disease and to facilitate the functional and phenotypic identification of protective and pathologic effector B cells.

Role: Project Leader, Project 3

R01 AI097357 (Randall)

05/11/12-04/30/17

NIH/NIAID

Central and Effector B Cells in the Lung

The major goal of this project is the identification and characterization of central and effector memory B cells that reside in lymphoid organs or peripheral non-lymphoid tissues.

Role: Co-Investigator

R01 AI104725 (Lund)

03/15/13-02/28/18

NIH/NIAID

Controlling Th2 immunity by tuning CXCL13-dependent DC migration in lymph nodes

The major goals of this project are to identify the stimuli that program BALB/c DCs to upregulate CXCR5 following *L. major* infection, to identify the signals in the perifollicular microenvironment that condition DCs to support Th2 priming and to determine whether susceptibility to cutaneous leishmaniasis can be reversed by modulating CXCL13 or lymphotoxin signaling.

R01 AI110508 (Lund)

03/01/14-02/28/19

NIH/NIAID

Control of anti-viral B cell responses by IFN $\gamma$ , T-bet and Eomes

To determine whether the T-box transcription factors, T-bet and Eomes, are required for the development of memory B cells following viral infection and vaccination, to identify the innate and adaptive signals which initiate and maintain the T-bet/Eomes pathway of B cell differentiation following virus infection and vaccination and to determine whether the virus-induced T-bet and IFN $\gamma$ -dependent B cell differentiation pathway is engaged in humans and mice following vaccination.

U19 AI109962 (Randall)

05/012/14-04/30/19

NIH/NIAID

Virus-induced cell fate decisions in anti-viral immunity - Lund (P.L.); *Core B: Viral Stocks and Reagents*

To produce, titrate and characterize viral stocks, to produce and purify viral proteins and to produce and validate fluorochrome-labeled recombinant virus proteins (B cell tetramers) for use in flow cytometry.

Role: Core Leader

U19 AI109962 (Randall)

05/012/14-04/30/19

NIH/NIAID

Virus-induced cell fate decisions in anti-viral immunity - Lund (P.L.); *Project 3: Control of anti-viral B cell responses by IFN $\gamma$ , T-bet and Eomes*

To determine whether the T-box transcription factors, T-bet and Eomes, are required for the development or maintenance of Bmem cells following viral infection, to identify the signals that initiate and maintain the T-bet/Eomes pathway of B cell differentiation following virus infection and to determine whether the virus-induced T-bet and IFN $\gamma$ -dependent B cell differentiation pathway is effectively engaged following vaccination in humans.

Role: Project Leader, Project 3

Alabama Drug Discovery Alliance (Lund (PI))

01/01/13-12/31/15

ADDA

Treating B cell-derived neoplasms by targeting the ectoenzyme CD38: a regulator of the NAD metabolic pathway

The specific goals of this proposal are to generate the reagents and cell lines necessary to develop a high throughput screen for CD38 inhibitors, to develop a HTS screen and counter-screens, to validate the screens and to perform proof of concept in vitro and in vivo studies in B cell neoplasm with the validated hits.

Role: Principal Investigator

## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.  
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

|   |   |             |                        |
|---|---|-------------|------------------------|
| NAME<br><b>R. Glenn King</b>  | POSITION TITLE<br><b>Assistant Professor</b><br><b>Department of Microbiology</b> |             |                        |
| eRA COMMONS USER NAME (credential, e.g., agency login)<br><b>RGKING</b>   |   |             |                        |
| 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<br>(if applicable)   | MM/YY       | FIELD OF STUDY         |
| University of Arkansas, Fayetteville, AR  | B.S.  | 05/00       | Chemistry/Biochemistry |
| University of Alabama at Birmingham, AL   | Ph.D.   | 08/07       | Microbiology           |
| University of Alabama at Birmingham, AL   | Postdoc.  | 09/07-09/11 | Microbiology           |

### A. Personal Statement

I have a continuing interest in B cell biology. I have been involved in multiple projects which involve the analysis of B cell function. These studies include the analysis of immune function in mice with altered expression of the adaptor protein HSH2, which is predominantly expressed in B lymphocytes and is upregulated following signals that drive B cell activation and differentiation. Using both transgenic and targeted murine models we have demonstrated HSH2 functions downstream of TNF family receptors and its expression rheostatically controls the differentiation of these cells into plasma cells. Additionally I have been involved in studies investigating the role of membrane lymphotoxin beta on B cells within the context of the germinal center environment. Using chimeric mouse models we have demonstrated the upregulation on LTb by activated B cells is required for the activation of follicular dendritic cells during the induction of the germinal center highlighting one aspect of the complex interplay among the various cell types involved in the generation of T-dependent humoral immunity. Collectively these projects involve the use of numerous state of the art approaches to analyze the B cell biology including the characterization of alterations in immune function within animal models, in vitro analysis of B cell differentiation, and experimental approaches to analyze relevant receptor signaling in B lymphocytes, as well as the extensive use of molecular biology techniques. Recently, I have begun studies to investigate the functional repertoire of polysaccharide specific B cells in humans and Mice and have developed high throughput methodology to allow for the expression of antigen receptors isolated from sorted B cells as recombinant antibody. Thus I have extensive experience in the technical aspects involved in AIM 1.1 of the studies proposed by Dr. Allie, including single cell sorts, antigen receptor amplification and sequencing, as well as bioinformatics analysis, and will provide as much assistance as necessary for the successful completion of these studies.

### B. Positions and Honors

#### Positions and Employment

2004 - 2007 Pre-Doctoral Trainee: T32 Immunologic Diseases and Basic Immunology  
 2011 - present Assistant Professor, Department of Microbiology, University of Alabama at Birmingham, AL.

### C. Selected Peer-reviewed Publications

1. **King, R.G.**, Herrin, B.R., and Justement, L.B. 2006. Trem-Like Transcript 2 (TLT2) is Expressed on Cells of the Myeloid/Granuloid and B Lymphoid Lineage and is Upregulated in Response to Inflammation. *J. Immunol.*176: 6012.
2. Halpert, M.M., Thomas, K.A., **King, R.G.** and Justement, L.B. 2011. TLT2 Potentiates Neutrophil Antibacterial Activity and Chemotaxis in Response to G Protein-Coupled Receptor-Mediated Signaling. *J. Immunol* 187: 2346. PMID: PMC3159717

3. **King, R.G.**, Herrin, B.R. and Justement, L.B. 2011. Differential Expression of the Adaptor Protein HSH2 Controls the Quantitative and Qualitative Nature of the Humoral Response. *J. Immunol.* 187:3565. PMID: PMC3178712
4. Myers, R.C., **King, R.G.**, Carter, R.H. and Justement, L.B. 2013. Lymphotoxin  $\alpha 1\beta 2$  Expression on B Cells is Required for Follicular Dendritic Cell Activation During the Germinal Center Response. *Eur. J. Immunol.* 43(2): 348-59. PMID: PMC3753018
5. Wade WF, **King RG**, Grandjean C, Wade TK, Justement LB. 2014. Murine marginal zone B cells play a role in *Vibrio cholerae* LPS antibody responses. *Pathog Dis.* 70(2): 153-7.

#### **D. Research Support**

##### Ongoing Research Support

King (PI) 6/1/14-5/30/15  
UAB Immunology, Autoimmunity, and Transplantation Strategic Planning  
The Development of Improved Methods for Cloning and Expression of Human Antibodies

King (PI) 9/1/14-8/31/15  
Comprehensive Arthritis, Musculoskeletal, Bone and Autoimmunity Center Pilot Grant  
The Development of Improved Methods for Cloning and Expression of Human Antibodies

P30 AR048311 (Mountz) 9/1/14-8/31/15  
NIH/NIAMS  
Rheumatic Diseases Core Center Pilot Grant (King)  
The Development of Improved Methods for Cloning and Expression of Human Antibodies

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.  
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME<br><b>Teresa W-M. Fan, PhD</b>   | POSITION TITLE<br>Professor, Department of Toxicology<br>Member, Markey Cancer Center<br>Edith D. Gardner Chair in Cancer Research |           |  |
|---|--|-----------|--|
| eRA COMMONS USER NAME<br>TWFAN001   |  |           |  |
| EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i> |  |           |  |
| INSTITUTION AND LOCATION  | DEGREE<br><i>(if applicable)</i>   | YEAR(s)   | FIELD OF STUDY                           |
| National Taiwan University, Taipei  | BS   | 1973-1977 | Public Health<br>(Environmental Science) |
| University of Hawaii, Manoa   | MS   | 1977-1978 | Food Science                             |
| University of California, Davis   | PhD  | 1978-1983 | Biochemistry                             |

### A. Personal Statement

My research focuses on applying novel Stable Isotope-Resolved Metabolomic (SIRM) and metabolomics-edited transcriptomic approaches (META) which we have developed 1) to investigate anti-cancer mechanism of natural products and novel therapeutic agents, 2) to explore the functional role(s) of tumor microenvironment (including immune cells) and extrinsic environmental factors in cancer development, progression, and therapy, and 3) to uncover novel therapeutic targets and distinct functional biochemical marker patterns for human cancer by interrogating the cancer metabolome. The central tool that I have used to achieve the goal is the integration of nuclear magnetic resonance (NMR) with mass spectrometry (MS) technologies, which enables a systematic interrogation of human metabolic networks and their perturbations by diseases. Since 2006, I established and directed a state-of-the-art MS facility under the auspices of the Center for Regulatory and Environmental Analytical Metabolomics (CREAM) while at the University of Louisville. The biological systems that I have employed range from cultured cells, xenograft/transgenic mouse model, *ex vivo* tissue cultures to human subjects in several cancers including lung, breast, liver and kidney. Under my direction, CREAM developed several novel techniques for analyzing pathways of central metabolism including amino acid, carbohydrate, lipid, nucleotide, and anti-oxidant metabolism in cancer. With the SIRM tool and the systems, I have uncovered a persistent activation of pyruvate carboxylation *in situ* in human lung cancer tissues in early developmental stages and demonstrate that inhibition of this mitochondrial anaplerotic pathway intoxicates lung cancer cells but not normal cells. I have also defined metabolic dysregulations induced by different anti-cancer selenium agents and unraveled the metabolic basis (e.g. perturbed mitochondrial metabolic networks and oxidative damages) for the varying efficacy of these agents in chemopreventive clinical trials.

My long-term goal is to continue integrated 'omics approach to translate mechanistic understanding of human diseases and influences of environmental factors on disease etiology into clinical benefits such as prevention, early detection, prognosis, and discovery of molecular targets leading to efficacious individualized therapy. With the recent move of CREAM to Univ. of Kentucky to establish a new Center for Environmental and Systems Biochemistry (CESB), the instrumental (both NMR and MS) and informatic capabilities are greatly expanded to facilitate the fulfillment of my research goals.

I had the great pleasure of interacting extensively with Dr. Sityh Rameeza Allie during a two-week long hands-on workshop on SIRM at University of Kentucky, which I hosted and served as a major instructor. This workshop is a part of the Outreach and Education effort sponsored by our NIH Common Funds (U24) funded Metabolomics Resource Center. I found Dr. Allie to be exceptionally focused and inquisitive in learning the various experimental and informatics skills associated with the SIRM approach. I also learned that she is very keen in combining these new skills with her immunological research. As immune metabolism is an emerging and exciting area of research, I believe this F32 training grant will be an outstanding opportunity for her to greatly expand her research horizon in many years to come. CESB has State-of-the-art facilities and expertise to foster Dr. Allie's interest and training. I have provided such training for external collaborators in the past 4 years that resulted in 12 peer-reviewed publications, including high-impact papers in Cancer Cell, Cell Metabolism, Journal of National Cancer Institute, Journal of Clinical Investigation (accepted). These papers demonstrate my keen interest and expertise in fulfilling the training effort for Dr. Allie in immune metabolism research.

### B. Positions and Honors

#### Positions and Employment

|           |   |
|-----------|---|
| 1986-1988 | Postdoctoral Affiliate, Stanford University                       |
| 1986-1986 | Visiting Postdoctoral Fellow, University of California, Davis     |
| 1986-1989 | Postgraduate Research Biochemist, University of California, Davis |
| 1989-1998 | Assistant Research Biochemist, University of California, Davis    |

|              |  |
|--------------|--|
| 1998-2005    | Associate Research Biochemist, University of California, Davis   |
| 2002-2007    | Associate Professor, Department of Chemistry, University of Louisville   |
| 2002-2013    | Scientist, James Graham Brown Cancer Center, University of Louisville  |
| 2003-2013    | Adjunct member, Department of Pharmacology and Toxicology, University of Louisville  |
| 2007-2013    | Director, Center for Regulatory and Environmental Analytical Metabolomics (CREAM), University of Louisville                    |
| 2008-2013    | Professor, Department of Chemistry, University of Louisville   |
| 2013-present | Professor, Department of Toxicology, Markey Cancer Center, University of Kentucky<br>Edith D. Gardner Chair in Cancer Research |

### **Professional Activities and Honors**

|              |  |
|--------------|--|
| 1998         | Expert Panelist, "Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation", organized by US Environmental Protection Agency, Washington, DC.   |
| 2002         | Expert Panelist, EPA workshop on "Toxicological Review and Risk Characterization for Perchlorate", requested by US Environmental Protection Agency, Sacramento, CA.  |
| 2003         | Expert panelist for the NIEHS/DERT retreat entitled "Comparative Systems Biology Approaches as Applied to Environmental Health" for NIEHS program managers, Southern Pines, NC.  |
| 2004-present | NSF Review panelist, Molecular and Cellular Biosciences, Metabolic Biochemistry.   |
| 2004-present | Editorial Board <i>Metabolomics</i>  |
| 2004-2006    | Editorial Board <i>Environmental Health Perspectives</i>   |
| 2009-2013    | Editorial Board <i>AoB Plants</i>  |
| 2010-present | Editorial Board <i>Metabolites</i>   |
| 2012-present | Editorial Board <i>Cancer and Metabolism</i>   |
| 2004-present | Ad hoc reviewer for <i>Analytical Biochemistry</i> , <i>Analytical Chemistry</i> , <i>Environ. Sci. Technol.</i> , <i>Journal of Magnetic Resonance</i> , <i>Magnetic Resonance in Chemistry</i> , <i>Phytochemistry</i> , <i>Journal of American Chemical Society</i> , <i>Metabolomics</i> , <i>Metabolites</i> , <i>Journal of Biological Chemistry</i> , <i>PLoS One</i> , <i>Proceedings of the National Academy of Sciences USA</i> , <i>Nature Chemical Biology</i> , <i>Environmental Health Perspectives</i> , <i>Cancer Research</i> |
| 2005-2007    | Organizing Committee, Annual meeting of the Metabolomics Society   |
| 2007-2010    | Advisory Board member, National Library of Medicine-sponsored Biological Magnetic Resonance Data Bank (BMRB), University of Wisconsin, Madison.  |
| 2009         | Expert panelist, SETAC Pellston workshop on "Ecological Assessment of Selenium in the Aquatic Environment", Pensacola, Florida, 2009.  |
| 2010-present | Permanent member for NIH Special Emphasis Panel/Scientific Review Group/Enabling Bioanalytical and Biophysical Technologies Study Section, ad hoc Review Panelist for Small Business Innovation Research IMST, IMST/K01, NCI/Omnibus.  |

### **C. Selected peer-reviewed publications (from 110 peer-reviewed publications)**

#### **Most relevant to the current application**

1. Le A, Lane AN, Hamaker M, Bose S, Gouw A, Barbi J, Tsukamoto T, Rojas CJ, Slusher BS, Zhang H, Zimmerman LJ, Liebler DC, Slebos RJC, Lorkiewicz PK, Higashi RM, **Fan TW-M**, Dang CV. Glucose-independent glutamine metabolism via TCA cycling for proliferation and survival in B cells. *Cell Metab* 15:110-121, 2012. PMID: PMC3345194
2. Yuneva MO, **Fan TW-M**, Higashi RM, Allen TA, Ferraris DV, Tsukamoto T, Mates JM, Alonso FJ, Wang C, Seo Y, Chen X, Bishop JM. The metabolic profile of tumors depends on both the responsible genetic lesion and tissue type. *Cell Metab* 15:157-170, 2012. PMID: PMC3282107
3. Dong C, Yuan T, Wu Y, Wang Y, **Fan TW-M**, Miriyala S, Lin Y, Yao J, Shi J, Lorkiewicz PK, St Clair D, Hung M-C, Evers BM, Zhou BP. Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. *Cancer Cell* 23:316-331, 2013. PMID: PMC3703516
4. Xie, H., Hanai, J.-i., Ren, J.-G., Kats, L., Burgess, K., Bhargava, P., Signoretti, S., Billiard, J., Duffy, K.J., Grant, A., Wang, X., Lorkiewicz, P.K., Schatzman, S., Bousamra, M. II, Lane, A.N., Higashi, R.M., **Fan, T. W-M.**, Pandolfi, P.P., Sukhatme, V.P., and Seth, P. (2014) Targeting lactate dehydrogenase-A (LDH-A) inhibits tumorigenesis and tumor progression in mouse models of lung cancer and impacts tumor initiating cells. *Cell Metabolism*, **19**, 795–809.
5. "Role in Tumor Growth of a Glycogen Debranching Enzyme Lost in Glycogen Storage Disease", Guin, S., Pollard, C., Ru, Y., Ritterson Lew, C., Duex, J.E., Dancik, G., Owens, C., Spencer, A., Knight, S., Holemon, H., et al. (2014) *J Natl Cancer Inst.*, dju062 doi:10.1093/jnci/dju062.

#### **Additional publications relevant to the application (in chronological order)**

1. **Fan TW-M**, Lane AN, Higashi RM, Farag MA, Gao H, Bousamra M, Miller DM. Altered regulation of metabolic pathways in human lung cancer discerned by  $^{13}\text{C}$  stable isotope-resolved metabolomics (SIRM). *Mol Cancer* 8:41, 2009. PMID: PMC2717907
2. **Fan, T. W-M**. Metabolomics-Edited Transcriptomics Analysis (Meta) *Comprehensive Toxicology* vol 2 (McQueen, C.A., ed) pp 685–706, 2010. Academic Press.
3. **Fan TW-M**, Lane AN. Structure-based profiling of metabolites and isotopomers by NMR. *Progress in NMR Spectroscopy* 52:69-117, 2008 (invited review).
4. **Fan TW-M**, Lane AN. NMR-based stable isotope resolved metabolomics in systems biochemistry. *J Biomol NMR Sp. Edn.* 49:267–280, 2011. PMID: PMC3087304
5. Moseley HNB, Lane AN, Belshoff AC, Higashi RM, **Fan TW-M**. A novel deconvolution method for modeling UDP-N-acetyl-D-glucosamine biosynthetic pathways based on  $(^{13}\text{C})$  mass isotopologue profiles under non-steady-state conditions. *BMC Biol* 9:37, 2011. PMID: PMC3126751
6. **Fan TW-M**, Lorkiewicz P, Sellers K, Moseley HNB, Higashi RM, Lane AN. Stable isotope-resolved metabolomics and applications for drug development. *Pharmacol Ther* 133:366-391, 2012. (Invited review) PMID: PMC3471671
7. Liu W, Le A, Hancock C, Lane AN, Dang CV, **Fan TW-M**, Phang JM. Reprogramming of proline and glutamine metabolism contributes to the proliferative and metabolic responses regulated by oncogenic transcription factor c-MYC. *Proc Natl Acad Sci USA* 109:8983-8988, 2012. PMID: PMC3384197
8. Yang Y, Lane AN, Ricketts CJ, Sourbier C, Wei M-H, Shuch B, Pike L, Wu M, Roualt TA, Boros LG, **Fan TW-M**, Linehan WM. Metabolic reprogramming for producing energy and reducing power in fumarate hydratase null cells from hereditary leiomyomatosis renal cell carcinoma. *Plos One* 8:e72179, 2013. PMID: PMC3744468
9. Ren, J. G., Seth, P., Clish, C. B., Lorkiewicz, P. K., Higashi, R. M., Lane, A. N., **Fan, T. W.**, and Sukhatme, V. P., Knockdown of Malic Enzyme 2 Suppresses Lung Tumor Growth, Induces Differentiation and Impacts PI3K/AKT Signaling. *Scientific reports* 4, 5414 (2014). PMID: 4067620
10. Sellers, K., Fox, M.P., Bousamra, M., Slone, S., Lane, A.N., Higashi, R.M., Miller, D.M., Wang, Y., Yan, J., Yuneva, M.O., Lane, A.N., and **Fan, T. W-M**. Pyruvate carboxylase is critical in human non-small cell lung cancer *J. Clinical Investigation* (conditionally accepted).

#### D. Research Support

##### Ongoing Research Support

R01 ES022191 & R01 ES022191-04S1 (MPI: Fan, TWM) 09/25/12-05/31/17

NIH

“Integrated Chemoselective and Informatic Platform for Large-Scale Metabolomics”

This project will develop a variety of chemoselective probes for different functional groups for linking mass spectrometry to NMR with structural identification in metabolomics.

Role: Corresponding PI (MPI: Fan, TWM; Higashi, R; Moseley, H; Nantz, M)

P01 CA163223 (PD: Lane, AN) 03/01/13-02/28/18

NIH

“Systems Biochemistry in Lung Cancer: Toward a Mechanistic Understanding of NSCLC”

The program comprises three project areas utilizing stable isotope resolved metabolomics to gain a mechanistic understanding of NSCLC in situ. The projects combine cell culture, animal models and human subjects to define the influence of the tumor microenvironment on cancer progression.

Role: MPI (Project 1)

U24 DK097215 (PD: Higashi, RM) 09/11/13-08/31/18

NIH

“Resource Center for Stable Isotope-Resolved Metabolomics”

This regional center for metabolomics was established at UK to develop and support stable isotope resolved metabolomics.

Role: MPI, Sample Core

SBCR Pty Ltd. (PD: T.W-M. Fan) 4/1/14-11/1/14

“Plasma lipid biomarker(s) for breast cancer diagnosis”

This contract is to validate lipid biomarkers in plasma from women with early stage breast cancer



Role: PD

2 R01 DK054921-15 subcontract from U.Minnesota (J. Albrecht, PI) 5/1/14-4/30/16

Cyclin D1/CDK4 Complex in Hepatocyte Proliferation

This project will use SIRM methods to determine the metabolic changes controlled by Cyclin D1 dependent cell cycling in hepatocytes.

Role: PI (subcontract)

**Recently Completed Research Support**

R01 CA125330 (PI: Fan, TW-M) 06/01/12-02/28/14

NIH

"Pathway Specific Imaging in VHL Deficient Renal Cancer"

Subaward from Beth Israel (P. Seth)

R01 CA118434 (PI: Fan, TW-M) 09/01/08-08/30/13

Biochemical Mechanisms of Se Anticancer Activity in Lung

This project utilizes stable isotope-resolved metabolomics and metabolomics-edited transcriptomics approach to examine the biochemical toxic action of different selenium compounds in lung cancer cells.

No ID Number (PI: Fan, TW-M) 12/01/10-11/30/13

Commonwealth of Kentucky Lung Cancer Research Program

"Stable Isotope-resolved Metabolomics to Elucidate the Mechanism of a Tumor-associated Cytochrome in Lung Cancer Growth and Metabolism"

The goal is to use metabolomics to characterize Pgrmc1 function in lung cancer and Pgrmc1-associated exosomal lipid metabolism as a biomarker of therapeutic response.

RC2 GM092729 (PIs: Kaddurah-Daouk) 10/01/09-09/30/12

Metabolomics Network for Drug Response Phenotype

This grant is concerned with metabolomics readout of drug response in several diseases. My role is to consult on the metabolic biochemistry.

Role: Consultant

R21 CA133688 (PI: Lane, AN) 07/01/09-06/30/12

Stable Isotopomer analysis of anabolic metabolic pathways in breast cancer

This project aims to relate effects of nutrient supply and hypoxia on the metabolism of breast cancer cells in culture to the metabolism of the same cells in a mouse xenograft model in discovering the metabolic influence of the tumor microenvironment

Role: Co-I

Rounsavall Family Foundation (PI: Lane, AN) 01/01/10-12/31/11

Mechanism-based prognosis and diagnostic tools for early stage lung cancer from stable isotope resolved metabolomics

Goals: To use <sup>13</sup>C stable isotope-resolved metabolomic (SIRM) analysis to define mechanism in metabolic networks in NSCLC patients

Role: Co-PI

KY CTSPGP (PI: Fan, TW-M) 07/01/10-12/31/11

Biomarker Discovery by Interrogating Lung Cancer Metabolome

The goal is to use metabolomics to define biofluid biomarkers in lung cancer patients.

KY CTSPGP (PI: Lane, AN) 07/01/10-12/31/11

Identifying predictive biomarkers in breast cancer patients by metabolomics" PI

The goal of this grant is to use metabolomics to define biomarkers in breast cancer patients.

Role: Co-PI

## PHS Fellowship Supplemental Form

**A. Application Type:**

From SF424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated here for your reference, as you attach the sections that are appropriate for this Career Development Award.

New    Resubmission    Renewal    Continuation    Revision

**B. Research Training Plan**

## 1. Introduction to Application

(for RESUBMISSION applications only)

## 2. Specific Aims\*

1246-Specific Aims Final.pdf

## 3. Research Strategy\*

1247-Research Strategy Final.pdf

## 4. Progress Report Publication List

(for RENEWAL applications only)

**Human Subjects**

Please note. The following item is taken from the Research & Related Other Project Information form. The response provided on that page, regarding the involvement of human subjects, is repeated here for your reference as you provide related responses for this Fellowship application. If you wish to change the answer to the item shown below, please do so on the Research & Related Other Project Information form; you will not be able to edit the response here.

Are Human Subjects Involved?    Yes    No

## 5. Human Subjects Involvement Indefinite?

## 6. Clinical Trial?

## 7. Agency-Defined Phase III Clinical Trial?

## 8. Protection of Human Subjects

## 9. Inclusion of Women and Minorities

## 10. Inclusion of Children

**Other Research Training Plan Sections**

Please note. The following item is taken from the Research & Related Other Project Information form. The response provided on that page, regarding the use of vertebrate animals, is repeated here for your reference as you provide related responses for this Fellowship application. If you wish to change the answer to the item shown below, please do so on the Research & Related Other Project Information form; you will not be able to edit the response here.

Are Vertebrate Animals Used?    Yes    No

## 11. Vertebrate Animals Use Indefinite?

Yes    No

## 12. Vertebrate Animals

1248-Vertbrate Animals.pdf

## 13. Select Agent Research

1249-Select Agents Research.pdf

## 14. Resource Sharing Plan

1250-Resource Sharing Plan.pdf

## 17. Respective Contributions\*

1251-Respective Contributions.pdf

## 16. Selection of Sponsor and Institution\*

1252-Selection of Sponsor and Institution.pdf

## 17. Responsible Conduct of Research\*

1253-Training in the Responsible Conduct of Research.pdf

## PHS Fellowship Supplemental Form

### C. Additional Information

#### Human Embryonic Stem Cells

1. 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), using the registry information provided within the agency instructions. 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):

|  |  |  |  |
|--|--|--|--|
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

#### Fellowship Applicant

2. Alternate Phone Number:

3. Degree Sought During Proposed Award:

Degree: \_\_\_\_\_ If "other", please indicate degree type: \_\_\_\_\_ Expected Completion Date (month/year): \_\_\_\_\_

4. Field of Training for Current Proposal\*: 2200 IMMUNOLOGY

5. Current Or Prior Kirschstein-NRSA Support?\*  Yes  No

*If yes, please identify current and prior Kirschstein-NRSA support below:*

| Level* | Type* | Start Date (if known) | End Date (if known) | Grant Number (if known) |
|--------|-------|-----------------------|---------------------|-------------------------|
| .....  |       |                       |                     |                         |
| .....  |       |                       |                     |                         |
| .....  |       |                       |                     |                         |

6. Applications for Concurrent Support?\*  Yes  No

*If yes, please describe in an attached file:*

7. Goals for Fellowship Training and Career\* 1254-Goals for Fellowship Training and Career.pdf

8. Activities Planned Under This Award\* 1255-Activities Planned Under This Award.pdf

9. Doctoral Dissertation and Other Research Experience 1256-Doctoral Dissertation and Other Research Experience.pdf

10. Citizenship\*  U.S. Citizen or noncitizen national  Permanent Resident of U.S. Pending  
 Permanent Resident of U.S.  Non-U.S. Citizen with temporary U.S. visa  
(If a permanent resident of the U.S., a notarized statement must be provided by the time of award)

#### Institution

11.  Change of Sponsoring Institution Name of Former Institution:\*

## PHS Fellowship Supplemental Form

### D. Sponsor(s) and Co-Sponsor(s)

Sponsor(s) and Co-Sponsor(s) Information\*

1257-Sponsor and Co-Sponsor Information.pdf

### E. Budget

#### All Fellowship Applicants:

1. Tuition and Fees\*:

None Requested       Funds Requested

Year 1

Year 2

Year 3

Year 4

Year 5

Year 6 (when applicable)

**Total Funds Requested:**

#### Senior Fellowship Applicants Only:

|  | Amount | Academic Period                       | Number of Months |
|--|--------|---------------------------------------|------------------|
| 2. Present Institutional Base Salary:                        |        |                                       |                  |
| 3. Stipends/Salary During First Year of Proposed Fellowship: |        |                                       |                  |
| a. Federal Stipend Requested:                                | Amount |                                       | Number of Months |
| b. Supplementation from other sources:                       | Amount |                                       | Number of Months |
|  |        | Type (sabbatical leave, salary, etc.) |                  |
|  |        | Source                                |                  |

### F. Appendix

**Specific Aims:**

**Rationale:** As B cell memory ( $B_{MEM}$ ) is vital for the efficacy of an influenza vaccine, it is essential to determine the populations that should be targeted to establish a good memory population and to determine the formation and function of the memory cells at the site of entry of the virus during a challenge. When influenza infected mice were parabiotically joined, we identified a non-circulating resident memory (BRM) population as early as 30 days post infection, while GCs are ongoing. The  $B_{MEM}$  in the lymph node (LN) and the lung BRMs constituted isotype switched IgM-IgD- (swlg+) and IgM+IgD- (IgM+) antigen (Ag) specific populations. In immunization models, the swlg+ population forms antibody-secreting cells (ASCs) while the IgM+ seeds germinal centers (GCs) upon challenge. Further, the IgM+ compartment arises from the extrafollicular area (EFA), as seen by the absence of CD73 expression, which is a marker of GC emigrants. Our anti-B220 infusion studies show that the lung BRMs form four subsets: CD73+swlg+, CD73+IgM+, CD73-swlg+ and CD73-IgM+. Thus, it will be important to confirm if these BRMs that establish early constitute the founding population for the late BRMs in the lung. To benefit vaccine studies, it is important to identify the BRM subset with the greatest propensity to form protective ASCs upon challenge.

As the lung BRMs are located at the site of re-entry of the virus, they need to be in a state of “readiness” to robustly respond to a challenge unlike their lymphoid counterparts. Cells that need to rapidly proliferate or have higher metabolic demands are like cancer cells and exhibit the Warburg effect. Effector memory CD8 T cells have an *imprinted* glycolytic potential as seen by their increased glycolytic flux upon stimulation. This glycolytic flux has been attributed to mTORC1/HIF1a signaling pathway in T cells. mTORC1 pathway has also been implicated in protective B cell memory formation. Collectively these data posit effector memory cells to be in a different metabolic state, which may be a result of their differentiation state. As the BRMs in the lung need to be robust responders it will be essential to determine if they exploit glycolysis to meet these requirements. IgG1 memory B cells are predisposed to become ASCs due to the repression of a transcriptional regulator (Bach2)<sup>1</sup>, which suppresses effector memory genes in CD8 T cells, as well<sup>2</sup>. As the BRMs in the lung need to be rapid acting it will be important to determine if they are transcriptionally poised to meet this functional requirement by suppressing Bach2, as Bach2 suppresses even the antibody secreting function.

***In this proposal we hypothesize that the lung BRMs form protective constant populations, independent of GCs, which employs the mTORC1 signaling (i) to be poised in an effector memory state by Bach2 suppression and (ii) drive ASC formation post challenge by undergoing glycolytic metabolism.***

**AIM 1 To determine the origin and functional significance of lung resident memory B cells (BRMs).**

**Rationale:** GC B cells undergo somatic hypermutation (SHM) and affinity maturation (AM). Thus GC derived CD73+ B cells should accumulate more mutations than those that were primed in the EFA. Since we observe an early seeding of lung BRMs **we hypothesize that the BRMs in the lung should maintain their mutation pattern and proportion early and late during the response, due to early seeding and establishment. Further, the GC derived CD73+swlg+ subset should be the most protective, as they have gone through rigorous selection and differentiation through (SHM and AM) in the GC.** We will test these hypotheses by analyzing the repertoire of the B cell population in the lung, which should remain constant if our hypothesis is correct. To identify the lung BRM with the greatest inclination to form ASCs, we will image live BRMs post challenge from Blimp-YFP reporter mice (YFP+ will be ASCs) to determine ASC formation. (Model1)

**AIM 2 To test the metabolic pathways and transcriptional regulators employed by BRMs.**

**Rationale:** Activated B cells increase glucose uptake, Glut1 transporter expression and glycolysis. Inhibition of glycolysis by pyruvate dehydrogenase kinase inhibitor – dichloroacetate – shows significant reduction in antibody production, reconfirming the importance of the glycolytic shift for proper ASC function. Since immunization models show that GC emigrants (CD73+) form high quality memory due to SHM and that swlg+ cells form mostly ASCs upon challenge, **we hypothesize that the CD73+swlg+ (A) subset will have the greatest propensity to harness the glycolytic pathway.** We will test this hypothesis by using multiple techniques to quantify glycolytic shift in the four BRM populations at steady state and post stimulation. B cells increase glycolysis to carry out ASC functions and in effector CD8 T cells the glycolytic shift is mediated by mTORC1. mTORC1 blockade reduces the Bach2 suppression, which directly binds and reduces plasma cell inducing Prdm1/Blimp-1 expression. Collectively from this data we infer that mTORC1 promotes glycolysis by suppressing Bach2, therefore **we hypothesize that the CD73+swlg+ subset will remain poised in a state of readiness to form ASCs by suppressing Bach2; using the mTORC1 pathway to upregulate glycolysis upon challenge to form ASCs.** We will test this hypothesis by determining the impact of mTORC1 blockade on Bach2 levels and glycolytic shift at steady state and post stimulation. (Model2)

**Impact:** Sophisticated high-resolution imaging combined with metabolomics will be used to determine the function, metabolism and transcriptional control of Ag specific BRMs, which were identified using influenza specific tetramers in Infused parabiotic mice. Very little is known about BRMs and there is no information about the metabolic preferences of these cells, therefore the outcomes of this study will be extremely novel and informative for the field of  $B_{MEM}$  and for immune-metabolism. Further, as this study is being conducted in the context of an actual viral infection, which holds great public health relevance, we feel that the findings from these experiments will be important to improve vaccines to influenza and broadly applicable to other pathogens.

## Research Strategy:

**Significance:** The CDC reports that 3000-49,000 deaths per year in the US are associated with influenza infection and that many of these deaths can be attributed to the low efficacy of vaccination. Thus, it is essential that we develop more effective vaccines. Effective vaccination to influenza requires high-quality B cell responses, including the generation of long-lived memory B cells that, upon challenge, reseed germinal centers (GCs) or form protective antibody secreting cells (ASCs); two functions that are crucial for protective immunity against influenza. However, we have only a limited understanding of how or where memory B cells are formed or how they are reactivated, particularly influenza-specific memory B cells that are maintained in the lung. Therefore, the experiments in this application are significant because they will determine how resident memory B cells (BRMs) in the lung contribute to immune protection and how the metabolic pathways used by these cells affect their differentiation and function.

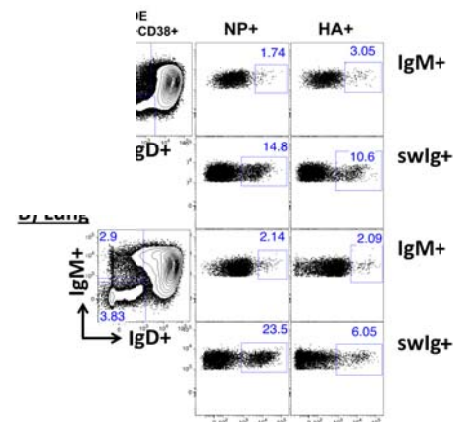
**Innovation:** Although memory B cells have been characterized in both mice and humans, most mouse studies use model antigens like PE<sup>3</sup> or follow adoptively transferred antigen-specific transgenic B cells<sup>4, 5</sup>, or simply follow bulk populations of memory B cells without regard to antigen specificity. Thus, these studies fail to provide information about pathogen-specific B cells in the context of an infection. In response to this deficit, we have developed recombinant whole protein tetramers that bind to the BCRs of antigen-specific B cells. These tetramers are derived from influenza hemagglutinin (HA), nucleoprotein (NP) and non-structural protein-1 (NS1) proteins and bind to HA-specific, NP-specific and NS1-specific B cells, respectively. This innovative technology will allow us to study antigen-specific cells in the context of infection (**Figure 1**)

In addition to innovative reagents, we will also use innovative techniques to study the function of influenza-specific memory B cells. For example, we use a combination of parabiosis (surgical joining of congenically mismatched animals) and intravenous labeling of circulating B cells to identify BRMs (**Figure 2**), similar to what has been done with memory T cells<sup>6, 7</sup>. We will also use live cell imaging to observe B cell differentiation and proliferation in real time. Additionally we will use imaging flow cytometry, which provides morphological information combined with a quantitative analysis of protein expression, to identify cells with high glycolytic shifts that also change the morphology of their cytoplasm and mitochondria.

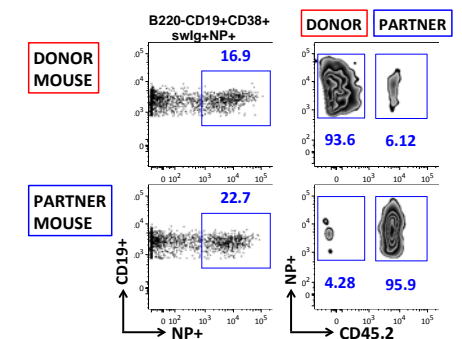
Finally, we believe that this proposal is conceptually innovative, as there is a scarcity of information on BRMs in any tissue, including the lung. Moreover, the relationship between metabolism and cellular function in these cells is completely unknown. Thus, we believe that using a combination of novel tools and techniques to determine the function and metabolic regulation of influenza-specific BRMs will provide valuable information to the field of influenza research and B cell biology.

**Background:** *Establishment of BRMs in the lung.* Memory B cells are formed in germinal centers (GCs), or in some cases, in the extrafollicular area (EFA)<sup>8</sup>. GC-dependent memory B cells express high levels of CD73, whereas EFA-dependent memory B cells do not<sup>8</sup>. Interestingly, our data show that influenza-specific memory B cells in lymphoid organs are mostly CD73+, indicative of a GC-derived memory population<sup>8,9</sup>, whereas many memory B cells in the lung are CD73- (**Figure 3**, day 71), suggesting that they are derived from the EFA. Moreover, CD73- memory B cells are more frequent in the lymph node and lung at earlier time points (**Figure 3**, day 34), suggesting a temporal shift in the supply of memory B cells. Thus, the origin of memory B cells in the lung following influenza infection is unclear

Following immunization with experimental antigens, EFA-derived memory B cells are enriched in the IgD-IgM+ (IgM+) compartment, whereas GC-dependent memory B cells are enriched in the isotype switched (swIg+) compartment<sup>3,5</sup>. Some data suggest that swIg+ memory B cells preferentially form antibody secreting cells (ASCs), whereas IgM+ memory B cells seed new GCs<sup>3,5</sup>. However, other data suggest that CD73+ memory B cells preferentially form ASCs compared to CD73- memory B cells<sup>10</sup>. Interestingly, we observe both CD73+



**Figure 1: Tetramer staining identifies Ag specific memory B cells.** Cells were obtained 30 days after infection. Events shown are live, doublet-excluded Ag-specific (NP or HA) CD19+CD38+ cells, which are either IgM+ or swIg



**Figure 2: Ag specific BRMs in the lung at day 30 post infection.** CD45.1 donor and CD45.2 partner mice were parabiotically joined at 30 days post PR8 infection for 2 weeks. Events in the right panels are gated on B220-CD19+CD38+swIg+NP+ cells and indicate donor (CD45.1) or partner (CD45.2) origins.

and CD73- influenza-specific memory B cells that express either IgM+ or swlg+ memory B cells in both the LN and lung (**Figure 3**). Given that there are 4 subsets of influenza-specific memory B cells (subset A: CD73+swlg+, subset B: CD73+IgM+, subset C: CD73-swlg+ and subset D: CD73-IgM+) in the lung and LN after infection, we propose experiments to determine their origins and functions.

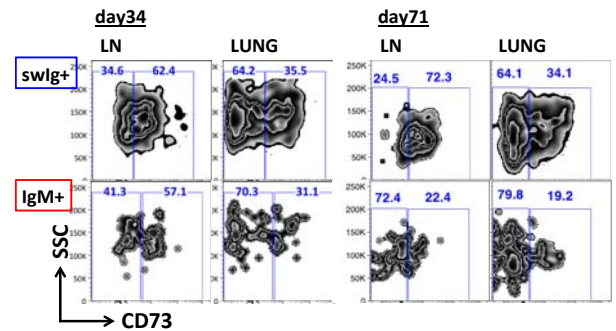
Interestingly, our data shows that the four memory subsets of influenza specific memory B cells are established in the lung as early as 34 days post infection (**Figure 3**), but following 2 weeks of parabiosis, we see very few *partner-derived* memory B cells in the lung (**Figure 2**), despite full equilibrium of host and partner cells in the blood and spleen. These data suggest that memory B cells in the lung are resident memory B cells (BRMs) and that they are established in the lung early after infection with little input from GC emigrants, despite the persistence of GCs in the LN and spleen for more than 60 days. Thus, one of the experiments proposed in this application is to test whether BRMs in the lung are low-affinity B cells that are established early and maintained independently of GCs in the lymph node.

**Protective functions of memory cells:** Memory B cells provide protection following challenge infection by rapidly differentiating into ASCs that provide a burst of protective antibodies<sup>11, 12</sup>, or by reseeding the GC and rapidly expanding the pool of antigen-specific B cells. The differentiation of ASCs is promoted by increased expression of the transcription factor, Blimp-1<sup>13, 14</sup>, and is inhibited by the transcription factor, Bach2<sup>15</sup>. Thus, the relative balance of these transcription factors likely controls the cell fates of BRMs in the lung that are responding to challenge infection. Given that we observe 4 subsets of BRMs in the lung, we propose to determine whether some of these subsets are more effective at differentiating into Blimp-1+ ASCs or turning on Bach2 and reseeding the GC.

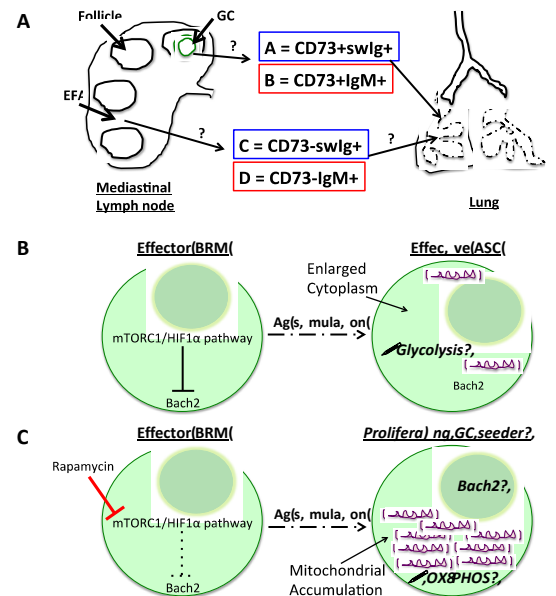
**Energetic demands of effector memory cells:** Following challenge infection, influenza-specific BRMs convert from resting memory cells to highly active effector cells that either secrete antibody or proliferate. The conversion from resting to active states in such a speedy manner may require the cells to be in a state of “readiness”. Thus, they may need to be transcriptionally and metabolically poised to differentiate. Cells that have higher metabolic demands are often compared to cancer cells, which exhibit the Warburg effect and use glycolysis to meet their energetic needs<sup>16</sup>. T cell studies show that, in addition to energetic requirements, the metabolic switch provides metabolites that enhance effector functions<sup>17</sup>. Similarly, B cells increase glycolysis upon activation to carry out ASC functions<sup>18</sup>. Conversely, the inhibition of glycolysis by a pyruvate dehydrogenase kinase inhibitor, dichloroacetate, reduces antibody production<sup>18</sup>. Thus, we propose to determine whether the 4 BRM subsets in the lung use Warburg metabolism following challenge.

**Signaling pathways used to meet metabolic demands:** Subsets of BRMs that are poised to rapidly differentiate into ASCs in the lung may be more differentiated<sup>19</sup>. For example, more differentiated memory B cells may have reduced expression of the Blimp-1 suppressor, Bach2, which makes them ready to form ASCs upon Ag exposure<sup>1</sup>. Bach2 heterodimerizes with small Maf proteins and binds Maf recognition elements on DNA to suppress genes. The Bach2/MafK heterodimer directly binds and represses the Prdm1 gene, leading to reduced Blimp-1 expression<sup>20</sup>. Thus, suppression of Bach2 in memory B cells drives them to become ASCs (**Model 1**).

Importantly, Bach2 activation requires mTORC1 signaling, as rapamycin mediated mTORC1 blockade reduced Bach2-mediated suppression<sup>1</sup>. Consistent with these data, mTORC1 signaling in B cells drives GC formation and class switching (presumably at the expense of ASC differentiation), however this aspect was not examined<sup>21</sup>. Although the role of mTORC1 signaling in the glycolytic shift has not been explored in B cells,



**Figure 3: CD73 expression on NP+ memory B cells.** Cells from LN and lung were obtained from anti-B220 infused C57Bl/6 mice at d34 and d71 after infection. Live doublet excluded Ag (NP) specific B220-CD19+CD38+ cells, which are either swlg+(top) or IgM+ (bottom).



**Model 1: Model figure of the questions that this study proposes to test.** A) Shows the seeding of GC and EFA derived cells in the lung post infection. The BRM subsets A and B originate in the GC while subsets C and E arise from the EFA. Question marks are on arrows that show mechanisms that we have not proved in the context of an infection. B) Shows the mTORC1 pathway mediated Bach2 suppression leading to Glycolysis (?) and the formation of ASCs. C) Shows mTORC1 blockade leading to nuclear localization of Bach2 (?), OX-PHOS (?), and a switch to Non-ASC or GC-seeders (?).

studies in CD8 T cells show that the glycolytic shift is mediated by mTORC1 signaling in a PI3K-independent, HIF1a-dependent manner<sup>22</sup>. Thus, we will determine whether mTORC1 promotes glycolysis and induces ASC differentiation by suppressing Bach2.

In summary, our preliminary data show that 4 subsets of BRMs are formed in the lung following influenza infection and based on published data, we suspect that these cells will be poised to differentiate into different effector B cell types and will use different transcriptional and metabolic pathways to achieve this process. As little is known about BRMs in the lung, our proposed studies will be extremely important to understand the immunology of the B cell response in the lung and to develop new vaccines that will effectively prevent pulmonary infections with viruses like influenza.

### **AIM 1 To determine the origin and functional significance of lung resident memory B cells (BRMs).**

**AIM 1.1 Does a stable BRM population become established in the lung as early as day 30 post infection?** *Rationale:* Our parabiosis data at 30 days post infection showed the establishment of non-circulating resident memory B cells (BRMs) in the lung (**Figure 2**). This was surprising, as we observe GCs as late as 60 days post infection and would expect GC-derived memory B cells to migrate into the lung until GCs are resolved. B cells in the GC undergo somatic hypermutation (SHM) and affinity maturation (AM). Thus GC-derived BRMs should accumulate mutations over time. Moreover, GC-derived BRMs should have more mutations than EFA-derived BRMs. Therefore, sequencing VH genes of BRM subsets in the lung and LN at various times after infection should reveal the origin of those subsets and whether the BRMs in the lung are exclusively derived early after infection or are seeded continuously. *We hypothesize that BRMs in the lung will have similar numbers of VH mutations early and later after infection, due to early seeding and establishment.*

*Approach:* We will sort the CD73+swIlg+ (A), CD73+IgM+ (B), CD73-swIlg+ (C) and CD73-IgM+ (D) subsets of memory B cells from the LN and lungs of influenza (PR8)-infected mice. These subsets will be sorted as single cells and the corresponding VH genes will be amplified as described<sup>23</sup>. The resulting amplicons will be directly sequenced at the UAB CFAR sequencing core. IGVH family identification, D and J gene usage, as well as the number and position of somatic mutations will be determined using the IMGT platform (online resource). This analysis will be conducted at day 15, 30 and 70-post infection.

*Results, interpretations & alternatives:* If we find that the number of mutations in the VH sequences of BRMs remains the same on days 15, 30 and 70, but that the number of mutations in GC B cells in the LN continue to accumulate over these times, then we will conclude that BRMs are established in the lung early after infection and, once established, are maintained without significant immigration of GC-derived memory B cells. This result would imply that the BRMs in the lung have relatively low affinity BCRs. Alternatively, we may find that VH sequences of BRMs in the lung continue to accumulate mutations through day 70. This result would contradict our parabiosis data, which shows that only about 5% of the BRMs in the lung are imported between 30 and 45 days after infection and may suggest the surprising possibility that BRMs accumulate mutations in situ in the lung, possibly in locations like bronchus associated lymphoid tissue (BALT).

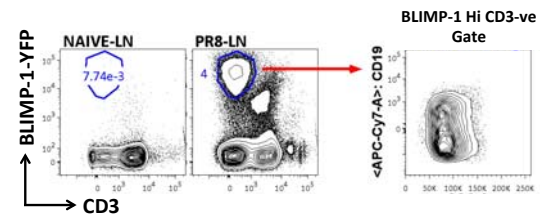
Interestingly, we may see differences in the number of mutations between the BRM subsets. For example, since CD73 is a marker of GC-emigrants<sup>8</sup> we may find more mutations in subsets A and B compared to subsets C and D, which are likely derived from the EFA. Since the extrafollicular reaction is rapidly resolved after infection, we may find that subsets C and D fail to accumulate more mutations over time, but that subsets A and B continue to accumulate mutations, suggesting that BRMs in these subsets are still being seeded by GC-derived precursors. To test this possibility, we will ablate GCs starting on day 15 by treating the mice with a blocking antibody to CD40L<sup>24</sup> and assay the number of mutations in BRMs in the lung on day 30. If the number of mutations increases in control mice, but fails to increase in CD40L-blocked mice, then we will conclude that at least some BRMs are continually seeded by GC-derived precursors.

In addition to seeding of BRMs from cells outside the lung, the number and quality of BRMs in the lung is likely to be affected by local homeostatic proliferation and cell death. To better understand the dynamics of BRM turnover in the lung, we will quantify apoptotic cells by staining with Annexin V and proliferating cells by BrdU (or EdU) incorporation. We will also determine the expression of survival markers like Bcl-2. If we find that some populations have low Annexin V, high Bcl-2 and incorporate BrdU, whereas others have high Annexin V, low Bcl-2 and fail to incorporate BrdU, then we would conclude that the former are likely to outcompete the latter and to dominate the BRM populations in the lung over time.

*Pitfalls and alternative approaches:* I have spent the last year developing the parabiosis model following influenza infection and characterizing influenza-specific BRMs in the lung using tetramer reagents and flow cytometry. Moreover, I have extensive experience with preparative and analytic flow cytometry, including analysis of Annexin V, BrdU and intracellular staining. Moreover, I will have help with the single cell sequencing from Dr. Rodney King here at UAB, who routinely performs similar experiments to amplify mouse



and human VH and VL sequences for recombinant antibody expression. Thus, we do not anticipate any insurmountable technical problems. One issue we will have to deal with is the number of cells we can sort from each BRM subset and how many sequences we will be able to analyze. Our preliminary data show that we can get about between  $1-10 \times 10^3$  antigen-specific BRMs from one lung, depending on the subset and the timepoint. We plan to sort around 100 cells (a 96 well plate) of each BRM subset per mouse and to use four or five animals per experiment. Amplification is about 80% efficient for IGVH, which seems to be due to the efficiency of the initial reverse transcriptase reaction rather than, for example, poor amplification of some VH families. Regardless of the reason, this limitation would be the same in all subsets and should not adversely impact our results. We expect to analyze 100 sequences per group, taking about 20-25 from each mouse. We will amplify more VH regions than we will actually have sequenced, so that if something goes wrong, we will always have back-up amplicons. Importantly, this method will allow us to obtain the entire VDJ sequences for every cell. Dr. King will also assist me in the use of the IMGT platform to assign sequences to particular VH family members so that I can determine the number of mutations. Ideally, I would like to determine the number of mutations across the entire VDJ region, but may have to exclude regions where I cannot unambiguously differentiate between junctional diversity and bona fide mutations.



**Figure 4. BLIMP-YFP mice upregulate YFP in the CD3-CD19 low and hi B cell population.** Two left panels show naive and PR8 infected mice. PR8 infected mouse at 12 dpi. Plots gated on LIVE, doublet excluded CD3- YFP+ population. Plot on the right shows CD19 expression on the CD3-YFP+ population, showing that the B cells have a CD19 hi and a CD19 downregulated population.

**AIM 1.2 Which subset of BRMs in the lung has the greatest propensity to form ASCs?** *Rationale:* Our preliminary data show that IgM+ BRMs in the lung are about 80% CD73- and 20% CD73+, whereas the swlg+ BRMs in the lung are about 65% CD73- and 35% CD73+ (Fig 3), suggesting a mixture of cells derived from both the GC and the EFA. The GC-derived CD73+ BRMs likely went through a very different differentiation and selection process than the CD73- BRMs from the EFA, which may imprint a differential ability to differentiate into ASCs following challenge. Furthermore, swlg+ memory B cells seem to more efficiently become ASCs than IgM+ memory B cells<sup>35</sup>, whereas the IgM+ memory B cells should more efficiently proliferate than the swlg+ memory B cells. *Thus, we hypothesize that the swlg+CD73+ cells (subset A) will form ASCs more robustly than the other BRM populations, but that the IgM+CD73- cells (subset D) will most efficiently proliferate.*

**Approach:** Cells from each of the 4 BRM subsets in the lung will be sorted from PR8-infected, Blimp-YFP reporter mice at day 60 after infection and cultured at 1, 5 and 10 cells/chamber in microscopic imaging chambers with anti-kappa, IL-21, BAFF and CD40L. We will use a live cell-imaging microscope to image these cells for 3 days and to quantify cell proliferation, cell death and YFP expression (differentiation into ASC).

**Results, interpretations & alternatives:** Blimp-1 is a critical transcription factor for ASC differentiation and is highly expressed in ASCs<sup>25</sup>. Therefore, we would expect that ASCs from Blimp-YFP reporter mice will have high expression of YFP (**Figure 4**). As hypothesized, we expect cells in subset A to form YFP+ ASCs, whereas we expect cells in subset D to proliferate, but to predominantly remain YFP-. As the CD73- populations are not expected to be as differentiated as A and B, we expect them to behave like A and B respectively but less effectively. Contrary to our hypothesis, if we observe that the CD73- C and D form YFP+ cells as optimally or better than the CD73+ A and B, it would be because these cells may be forming a more effector memory like population. This will be a novel find because we don't expect the extrafollicular response to generate a better effector population than the GC response. Importantly, since the microscope keeps sequential digital images (mini-movies) of each chamber over the full course of the experiment, we will be able to tell whether ASC differentiation is coupled (or not) to proliferation. Moreover, we will be able to tell whether individual BRMs consistently give rise to only YFP+ or YFP- cells or whether they produce mixed progeny. Previous results would predict that single BRMs will only generate a single type of progeny.

The in vitro studies will give us a single cell resolution to determine the propensity of the BRMs to form ASCs. However, we will confirm our conclusions in vivo by adoptively transferring CD45.2+ cells into naive CD45.1+ donors, which have been primed with intranasal CpG for 6 days<sup>26</sup>, and co-transfer bulk CD4+ cells from these donors, as well. Then we could infect the mice with PR8 intranasally at day 0. At 3 and 6 days post infection, the AT BRMs from the lungs studied for YFP upregulation and proliferation. If our hypothesis is correct we should observe the most of population A to be YFP+, while the others should have less numbers of YFP+. The IgM+ B and D should show YFP+ and a large number of CD45.2+YFP- cells, indicative of GC seeding. To test this we could stain the cells from the mLN and lung with PNA and Fas and test by flow cytometry to determine if these cells express these GC markers. In D if we observe that they form similar numbers of YFP-CD45.2+ cells as B, we will stain for their CD73, PNA and Fas expression to determine if they are seeding GCs by

upregulating CD73 expression. If we observe that the IgM<sup>+</sup> are higher in YFP compared to the swlg<sup>+</sup>, it would indicate that the IgM<sup>+</sup> cells are forming more ASCs than the swlg<sup>+</sup>. This will contradict published work<sup>8</sup>, so it may be because this experiment is in the context of a viral infection unlike previous studies which used immunization models.

**Pitfalls and alternative approaches:** We are well-experienced with the influenza model system and sorting BRMs. Moreover, the microscopic cell culture system is available in our laboratory and the BLIMP-1-YFP reporter mice are breeding the Randall/Lund animal colony, so we do not anticipate any technical difficulties with this experiment. Moreover, we can sort more than  $1 \times 10^4$  NP-specific IgM<sup>+</sup> and swlg<sup>+</sup> live memory cells on day 70 from 2-3 mice. Given that there are 125x125 chambers per slide and that we will culture the cells at small numbers per chamber (less than 10), we will have plenty of cells for this experiment. We have chosen to trigger the BCRs of sorted BRMs with anti-kappa so that BRMs will be stimulated regardless of antigen specificity or isotype expression. However, an alternative approach is to stimulate cells with inactivated virus or recombinant NP or HA. Although most of the BRMs in the lung are influenza specific, the inactivated virus is most likely to cross-link HA and NA-specific cells, whereas the recombinant proteins will only stimulate those NP or HA-specific cells, which may only be 20-50% of the cells. Thus, we may sort NP or HA-specific BRMs. Although sorting antigen-specific BRMs is dicey due to the potential for triggering activation-induced cell death, other investigators have successfully sorted viable antigen-specific B cells using reagents that bind the BCR<sup>27</sup>. Importantly, inactivated virus is likely to elicit a stronger BCR response than recombinant NP or HA, due to BCR crosslinking by repetitive epitopes on the virion. We also chose to use BAFF as a B cell survival factor and IL-21 and CD40L to mimic T cell help. These may or may not be the best conditions to stimulate BRMs. Thus, an alternative is to add OTII T cells and OVA<sub>323-339</sub> so that the BRMs receive cognate help from T cells.

## **AIM 2 To test the metabolic pathways and transcriptional regulators employed by BRMs.**

**AIM 2.1 Do BRMs forming ASCs have a greater glycolytic flux upon stimulation than those that seed GCs? Rationale:** Cells that need to rapidly proliferate or have higher metabolic demands have been compared to cancer cells. Like cancer cells, these cells have been shown to exhibit the Warburg effect and employ glycolysis to meet their energetic needs<sup>16</sup>. T cell studies also show that, in addition to energetic requirements, the metabolic switch provides metabolites that may enhance effector functions<sup>17</sup>. Moreover, upon activation, B cells increase glucose uptake, Glut1 transporter expression and glycolysis<sup>18</sup>. Further inhibition of glycolysis by the pyruvate dehydrogenase kinase inhibitor, dichloroacetate, reduces antibody production<sup>18</sup>. Since we propose that the 4 subsets of BRMs are different in their ASC function, *we hypothesize that they will differ in their ability to harness the glycolytic pathway.*

**Approach:** Cells from each BRM subset will be sorted from the lungs of Blimp-YFP mice and cultured with anti-kappa, BAFF, IL-21 and CD40L. We will measure ADP/ATP ratios at 18 hours using a bioluminescent detection kit (Abcam ab65313). We will measure uptake of 2-NBDG (fluorescent glucose analog)<sup>28</sup> and glucose transporter -1 (Glut-1) expression by flow cytometry. We will also measure mitochondrial volume with Mito Tracker Red and Glut-1 expression by imaging flow cytometry. All of these parameters will be correlated with ASC differentiation as measured by increased YFP expression.

**Results, interpretations & alternatives:** Increased glycolysis should result in lower ADP/ATP ratios, higher glucose uptake (2-NBDG signal), higher Glut-1 expression and increased cytoplasmic volume with scattered mitochondria. If our hypothesis is correct, then we will observe that the largest increase in glycolysis correlates with the most efficient ASC differentiation. Given that BRMs in subset A (CD73+swlg<sup>+</sup>) have transited through a GC and isotype-switched, we expect that these cells will be most inclined to differentiate into ASCs and will use glycolysis to accomplish this function. In contrast, we predict that BRMs in subset D (CD73-IgM<sup>+</sup>) will be most likely to proliferate, but will form fewer YFP-expressing ASCs and will use oxidative phosphorylation (OX-PHOS). Thus we would expect these cells to have higher ADP/ATP ratios, reduced glucose uptake and Glut-1 expression, smaller cytoplasmic volume with distinct areas of mitochondrial concentration and will poorly express the BLIMP-1 reporter, YFP. These data would fit with the idea that IgM<sup>+</sup> memory cells are predisposed to proliferate and seed new GC, whereas swlg<sup>+</sup> memory cells are predisposed to differentiate into ASCs. Alternatively, we may find that glycolysis poorly correlates with the propensity to differentiate into ASCs or that there is a temporal shift in metabolism that occurs progressively following activation as cells differentiate into ASCs. If so, we will have to evaluate these same parameters at multiple times (6, 12, 24 and 48 hours). Finally, although we are using multiple parameters to infer increases in glycolysis, we will confirm our conclusions using the XF Seahorse analyzer, which can directly measure increased extracellular H<sup>+</sup>s in glycolytic cells and O<sub>2</sub> consumption in OX-PHOS exploiting cells and is available in the lab of Dr. Victor Darley-Usmar at UAB.

**Pitfalls and alternative approaches:** As mentioned above, I am proficient at sorting BRMs from the lung and the required instrumentation is in our lab (luminometer) or in the imaging core (Amnis ImageStream). Since the

assays needed for this experiment are published or are available in kit form, we do not anticipate any technical difficulties. However, we will need sufficient BRMs of each subset to perform these assays. For example, we will need around  $10^3$ - $10^4$  cells to set up the ATP/ADP assay in a 96-well plate. We can easily get about  $10^4$  NP-specific IgM+ and swIg+ live BRMs on day 70 from 2-3 mice. Thus, we will sort cells from 5-10 mice and have sufficient cells for each of the assays. Importantly, the ImageStream requires only  $10^3$ - $10^4$  cells per sample and can easily resolve the signals from Mito Tracker Red and YFP<sup>29</sup>, so we expect to get good resolution of the mitochondria in the YFP+ ASCs.

**AIM 2.2: Do BRMs use the mTORC1/HIF1a-dependent, PI3K-independent pathway to suppress Bach2, and promote glycolysis to form ASCs?** *Rationale:* CD8 T cell effector functions require the glycolytic shift<sup>30</sup>, as do B cells in carrying out ASC functions<sup>18</sup>. Further, CD8 T cell studies have revealed that the glycolytic shift is mediated by mTORC1 signaling in a PI3K-independent, HIF1a-dependent manner<sup>22</sup>. Thus, this signaling pathway is likely to be involved in ASC differentiation. In addition, the transcription factor, Bach2, which heterodimerizes with small Maf proteins and binds Maf recognition elements on DNA, represses the Prdm1 locus thus reducing Blimp-1 expression<sup>20</sup>. Given that Blimp-1 is an important inducer of plasma cell differentiation<sup>13</sup>, Bach2 expression in memory B cells suppresses ASC differentiation. Importantly, this regulation involves mTORC1 signaling, as rapamycin mediated mTORC1 blockade reduces the Bach2 suppression<sup>1</sup>. Therefore, *we hypothesize that BRMs that are poised to become ASCs will have low Bach2 levels and use the mTORC1 pathway to upregulate glycolysis upon challenge.*

*Approach:* We will sort cells of each of the BRM populations in the lung from PR8-infected Blimp-YFP mice on day 60 and culture them with anti-kappa, BAFF, IL-21 and CD40 with and without rapamycin. Bach2 expression will be measure by qPCR directly ex vivo and after 24 hours of culture. We will also measure 2-NBDG (glucose) uptake and Glut-1 expression by flow cytometry. We will also culture BRMs in the microscopic imaging chambers and collect images for three days.

*Results, interpretations & alternatives:* We expect the cells that rapidly form ASCs to express high levels of Glut-1, increase glucose uptake, suppress Bach2 expression and robustly become YFP+ cells when imaged. As per our hypothesis, rapamycin treatment should reduce Glut-1, glucose uptake, and YFP+ cell formation while increasing Bach2 expression. Further, we expect the impact of rapamycin-mediated blockade to have the greatest impact on the population that is the most prone to become ASCs.

We hypothesize that population A should be the most competent in forming ASCs rapidly, therefore we expect these cells to maintain their readiness to become ASCs by suppressing Bach2. If this is correct we expect the ex vivo sample of population A to have the lowest relative expression of Bach2. Further, we expect this population to show the greatest difference in expression when stimulated and treated with rapamycin. For example, we expect the Rap+ / Rap- ratio of Bach2 expression to be the highest in this population as the Rap- Bach2 would have been the lowest, as they upregulate Blimp-1 and form ASCs without mTORC1 blockade and then the blockade of this pathway should allow for enhanced Bach2 expression in the Rap+. Alternatively, we may not observe differences in Bach2 expression, which would indicate that there is another regulator(s) that may be involved in maintaining the state of readiness. We would need to do a transcriptome analysis to explore the possible regulators in an unbiased manner.

We expect that rapamycin will reduce Glut-1 expression and glucose uptake as well as ASC differentiation (YFP expression). We expect population C to be similar to A, but it may not be as fit, therefore we expect to see a milder impact compared to A. As population B and D are expected to mostly seed GCs, we don't expect to observe significant changes in these measured parameters. If we observe that population A is highly impacted by rapamycin treatment we would do further experiments to prove that all these parameters are interconnected to make these be poised in a state of readiness. We would stain these cells post cytospin and show that they have Bach2 nuclear localization, reduced Glut-1 expression and 2-NBDG uptake when treated with rapamycin. Further, if we observe an impact of rapamycin in the Bach2 expression and glycolytic shift in stimulated cells, we will determine the impact of Bach2 blockade (using siRNA or Bach2 KO cells<sup>31</sup>) alone on the glycolytic shift to determine if mTORC1 works via Bach2 to regulate glycolysis.

*Pitfalls and alternative approaches:* Our lab has successfully used the mouse Influenza infection model for many years and I am proficient at cell sorting lymph node and lung cells. I have previous experience successfully using rapamycin in vivo and in in vitro settings (unpublished). As previously stated, we will further confirm our glycolytic shift data on the XF Seahorse analyzer with and without rapamycin treatment. These data will only determine the pathways that memory B cells use to either become central memory like GC seeding cells or effector memory like ASC forming cells. We will follow up with experiments to determine the specific steps of the metabolic pathways that are impacted by mTORC1 signaling, by carrying out profiling experiments with labeled glucose to identify targets for vaccines.

## Vertebrate animals

1. The experiments herein proposed require the use of laboratory mice to evaluate the protective potential, origin and metabolic and transcriptional regulation of lung resident memory B cells post influenza infection. We propose to use adult male and female mice (>8 weeks) for the experiments since gender is not reported to have a significant effect on B cell differentiation. The experiments proposed in this grant will include 2-3 groups of mice/experiment, 2-4 time points/experiment and 5-8 mice/time point/group. Some of these experiments will require the adoptive transfer of immune cells; therefore congenically mismatched (CD45.1 and CD45.2) mice are needed. We will use ~500 experimental mice to complete the studies proposed in this application. Furthermore, we expect that, depending on the outcome of the initial experiments, we will further refine our approach and additional experiments will be performed. Experimental mice will be generated in the Randall and Lund animal breeding core. Experimental mice will be infected via the intranasal route with different strains of influenza. The mice will be sacrificed at various time points and tissues and cells will be isolated for biochemical, molecular, and immunological assays. For infusion studies, mice will be lightly anesthetized with isoflurane and Pacific Blue conjugated anti B220 antibody administered intravenous and left in their cage for 5min. Our tests show that less than 5 min is sufficient for the antibody to circulate through the mouse. We will also use isoflurane to anesthetize mice that need to have cells adoptively transferred intraorbitally. Although we do not expect to observe significant morbidity or mortality after influenza infection at our sub lethal dose, animals that reach IACUC approved morbidity endpoints will be euthanized.

2. Justification and Group size determinations. We must use laboratory animals for these experiments because we are studying immune responses in mice that have been exposed to live influenza virus. It is impossible to measure complex immune responses *in vitro* because *in vitro* experiments cannot replicate the cell-cell interactions that occur directly within lymphoid organs and cannot replicate the complex interplay between the pathogen, and the B and T cells responding to these pathogens. In this proposal, we will use inbred B6 (CD45.1 and CD45.2) animals as well as reporter mice for the Blimp-1 gene expression to determine the function and formation of memory in the lymphoid organs and lung. For optimal memory B cell differentiation the cells have to proceed through a germinal center response. The germinal center response cannot be replicated in tissue culture or modeled mathematically as the interactions that take place within this site are highly dependent on the underlying stromal components of the organ which provide the architecture required to support the ongoing processes of B cell affinity maturation, class-switching, and differentiation; all of which occur in this site. Thus, the only option is to use experimental animal models for these studies. Further, the protective potential of cells in the lung upon rechallenge require the cells to be in the lung when the virus is introduced to determine cell intrinsic and extrinsic mechanisms. The laboratory mouse is ideal for these studies because there are a number of tools available that will allow us to manipulate the germinal center microenvironment and lung seeding of memory B cells.

We proposed studies that will utilize a minimum number of animals to obtain the maximum amount of information from each animal. For some experiments, we only need to consider two-group tests (either independent or paired). However, even in experiments where more than two groups are compared, the following justifications are still applicable since we will be using a post hoc test, which indicates which specific pairs of means are significantly different, as part of an analysis of variance test. We found in our preliminary experiments, that sample sizes of 5-10 mice are sufficient to detect differences of 1.5 fold change between independent groups with 80% power, and in experiments in which paired analyses are used, 3-6 mice are sufficient to detect differences of at least 1.3 fold change between groups with 80% power. The proposed experiments are structurally similar to the preliminary experiments, and as such, we will use between 5-8 mice/group/timepoint. We will also need to include experimental and one or more control groups of mice so that we can interpret whether our experimental variables have any effect on the immune response that we are measuring. Finally, we will need to repeat the experiments that we perform a minimum of 2-3 times to ensure that the results are reproducible. Thus, we propose to use ~500 mice over a 2-3 year period to complete the initial studies outlined in this application.

3. Veterinary Care. The UAB Animal Resources Program (ARP) is the primary service component of the UAB Animal Care and Use Program and provides the daily husbandry and veterinary care to animals being used in research at UAB. The UAB Animal Care and Use Program has been accredited by the Association of Assessment and Accreditation of Laboratory Animal Care International (AAALAC) since 1971, has maintained continuous Assurance with the National Institutes of Health, Office of Laboratory Animal Care (OLAW, #A3255-01) and is permitted by the United States Department of Agriculture (#64-R-004). The UAB ARP facilities and procedures meet all the requirements of the Public Health Service *Policy on the Humane Care and Use of Laboratory Animals*, the National Research Council Institute of Laboratory Animal Resources *Guide for the Care and Use of Laboratory Animals* and the United States Department of Agriculture *Animal Welfare Regulations*. The UAB ARP is fully staffed with animal care technicians, veterinarians, veterinary technicians,

safety technicians, financial management personnel, trainers, facility managers and administrative support staff. ARP staff provides all necessary services for animal procurement, care and transportation. UAB ARP husbandry, veterinary, and facility staff provides monitoring and care 24 hours a day, 7 days a week, 365 days a year. The veterinary staff, through protocol planning and review as well as post-approval monitoring, ensures that analgesics, anesthetics, tranquilizing drugs, restraint techniques and methods of euthanasia comply with IACUC policy to minimize discomfort, distress, pain and injury. The animals to be used in this proposal will be housed in Research Support Building (RSB) animal facility, which is approximately 75,000 nsf and includes 45,000 nsf of animal housing. RSB includes a fully equipped cage wash facility with rodent cage washer, rack washer, and 2 bulk sterilizers. The animal x-ray irradiator is located in RSB and is available to all researchers requiring animal or cell irradiation. All animal housing rooms are equipped for Animal Biosafety Level 2 (ABSL-2) activities. Rodents are housed in autoclaved microisolator units in individually ventilated caging systems (ventilated racks). Mice receive irradiated diet and ultrafiltered acidified water in disposable pouches. The pathogen status of all rodent colonies (~250) is monitored quarterly using soiled bedding sentinels. All colonies are clear of the most common murine pathogens. Colonies are segregated according to pathogen status and only colonies with same status are housed in the same room.

4. Pain and Distress. Every effort will be made to reduce the pain and distress of experimental mice. Anesthetics will be used to minimize the distress of mice that are undergoing invasive procedures (i.e. i.v. injections). Analgesics are not typically used because these drugs may change the immune parameters (i.e. inflammation in the infected tissue) that we are measuring. In all experiments, the goal is to monitor the development and maintenance of the virus-specific memory B cell compartments following influenza infection. The infection protocols are relatively non-invasive and only require the animal to be lightly anesthetized (30 sec) with isoflurane. The animals recover within 2 minutes and do not exhibit signs of distress. In mice that are infected with influenza virus, it is critical that they receive a non-lethal dose of virus so that we will have animals to study after the virus infection is cleared. The animals will be euthanized at multiple time points between days 30 and 70 following resolution of the infection. Infected animals will be monitored daily for the first 14 days or until the infection is cleared.

5. Euthanasia. All mice in the proposed experiments will be euthanized by CO<sub>2</sub> narcosis. This method is rapid and, if performed correctly, results in only minimal distress to the animals. This method is consistent with the recommendations of the Panel on Euthanasia of the AVMA.

## Select Agents Research

None.

*There is work with BSL-2.*

**Biohazards.** The influenza virus isolate used in this application, A/PR8, is a commonly used mouse adapted laboratory strain that has been attenuated for infectivity in humans due to repeated passaging in mice. This strain is not considered highly pathogenic to humans and does not fall under select agent research guidelines. Although the strain does not pose a significant risk to *healthy* individuals, the virus does require BSL-2 and ABSL-2 level containment. The shared laboratory of Drs. Randall and Lund is approved for studies using BSL-2 samples infected with influenza virus. All lab members are trained to safely handle potentially infectious samples and are retrained annually. When working with viral stocks and infected animal tissues, investigators are required to wear appropriate PPE and must handle these samples within a biosafety cabinet. All animals infected with viruses are housed in Dr. Randall's dedicated A-BSL2 animal housing space and are manipulated within biosafety cabinets located in the A-BSL2 procedure room space.

## Resource Sharing Plan

Data Sharing Plan: All research findings will be disseminated to the scientific community through publications in peer-reviewed journals and presentations at local and national scientific meetings, in adherence with the publication regulations on open access policies set forth by the NIH. The data generated in this project will also be shared with the UAB immunology scientific community during in-house seminars and meetings.

Sharing Tools and protocols: We will share any tools that we generate with the larger research community following publication in peer-reviewed research journals. We have generated B cell tetramers to the nucleoprotein, hemagglutinin and NS1 epitopes of influenza virus, which we have started sharing with various groups at the University of Minnesota and UC Davis.

Sharing Model Organisms: We and our institution will adhere to the NIH Grants Policy on Sharing of Model Organisms for Biomedical Research (NOT-OD-04-042), as detailed: any new model organisms generated in this Project will be made available to the larger research community following publication in peer-reviewed research journals.

Requests from for-profit organizations will be handled through UAB Foundation, which is the technology sharing organization on our campus. The UAB Foundation will negotiate any licensing in accordance with institutional policy and will report any invention disclosures submitted by our group to the NIH. Material transfers will be made with no more restrictive terms than in the Simple Letter Agreement (SLA) or the Uniform Biological Materials Transfer Agreement (UBMTA) and without reach through requirements. Should any intellectual property arise which requires a patent, we will ensure that the technology (materials and data) remains widely available to the research community in accordance with the NIH Principles and Guidelines document.

## Respective Contributions

During my first year in Dr. Randall's lab, I tested the B cell tetramers and identified NP and HA specific B cells in the mediastinal lymph node, spleen and lung. I carried out extensive studies characterizing and studying the kinetics of the various populations in these organs. In my second year, I have now identified the resident memory B cells in the lung using combined antibody infusion and parabiosis studies. As this is a novel discovery, we are currently trying to complete the manuscript on the formation and trafficking of these cells. As my results reveal CD73+ and CD73- populations in the lung, after repeated discussions with Dr. Randall, we have decided to test the origin and seeding of these cells in the lung. Due to my own personal interest I have put forth the studies of the transcriptional and metabolic regulation of these cells as my future direction for this project and I have received Dr. Randall's complete support. He has also found multiple people at UAB and Dr. Teresa Fan at the University of Kentucky (UK) to help me establish this arm of my project. Further, he funded a significant portion of the cost of attending the metabolism workshop at the metabolism core at UK.

The research proposal was entirely conceived and drafted by me, the applicant, revised by my mentor Dr. Randall, and written as an F32 grant application. Dr. Rodney King was consulted to determine the feasibility of the repertoire analysis experiments. The proposal herein was refined based on discussions with Dr. Randall.

I will complete the research proposed with help from UAB and UK core facilities, and some technical assistance from Dir. John Edwin Bradley, a technical scientist in Dr. Randall's lab. He prepares the tetramers and antigens for ELISAs and ELISPOTs. In addition, I will work with Uma Mudunuru, a veterinary technician in Dr. Randall's lab, who assists with the parabiosis surgeries. We will consult Dr. Rodney King in Dr. John Kearney's lab on our repertoire analyses as he has accrued extensive experience in these experiments in human and mouse work. We will consult with Dr. Teresa Fan at UK on our metabolism experiments and use the UK core for our future profiling experiments.



## Selection of Sponsor and Institution

When I started my PhD at Dartmouth College, I realized that my human immunology background had given me many fundamental experiences to carry forth into my PhD, like extensive ELISA and flow cytometry skills, data analysis and management skills. Yet, I felt that I wanted to fill the gap that I always experienced in human immunology to answer more mechanistic questions. Thus, when I joined Dr. Usherwood's lab, I was thrilled to finally tackle questions in a hypothesis driven manner. Most questions that I would put forward to Dr. Usherwood would be met with questions about the soundness of my experimental design, alternatives if I did not observe results that proved my hypothesis etc. Thus, I enjoyed and thrived in his lab and learned to think critically, which I felt was the main goal of obtaining a PhD.

My findings on how PD-1 expression or retinoic acid signaling impacted CD8 differentiation lead to many more questions about the rest of the immune response which was outside the scope of my thesis and our lab. Thus as I was wrapping up the last of my requirements for my PhD at Dartmouth College, I began to realize that I wanted to think broadly about immune responses as a whole. I wanted to either join a lab that studied a completely different cell type or a lab that studied multiple cell types to expand my knowledge base of the immune responses to viruses.

The extensive reading for my thesis and journal clubs, combined with scientific meetings I had attended, had exposed me to the work of multiple people who were asking question about immune responses to viruses. Most of the well-known names in the memory field worked on T cells, thus I had to look beyond memory into researchers studying immune responses to viruses. During my classes at Dartmouth, I had come across Dr. Randall's work, as he has been a vital contributor to the field of dendritic cell responses and to responses in the lung. Reading his more recent work made me realize that his lab studied T cell, B cell and dendritic cell responses to influenza virus infection, in addition to the impact of ectopic lymphoid organs on infections and allergies. His lab filled all my requirements for postdoctoral training, 1) he was well funded to carry out the project that he was offering, 2) his lab was so diverse in projects that I could passively learn more than a single project could ever teach me, 3) my project on B cell memory in the lung post influenza infection was ideal to allow me to carry on with my passion in immune memory differentiation while broadening my experience with the study of the B cell, 4) his other mentees were extremely agreeable and enthusiastic scientists and with the interactions and discussions with them I would expand my knowledge and cultivate my own interest in a secure and healthy environment . Finally the most important factor for choosing his lab was Dr. Randall himself. While being extremely knowledgeable and youthfully enthusiastic about all things immunology, he is an excellent postdoctoral mentor (having trained many post docs in his career). During my interview, one of the assistant professors in his lab who had also been his postdoc told me that he had "worked for Troy for 6 years and would move wherever Troy moved to continue working with him". This is a rare thing to hear from any postdoc about his mentor and having worked for him now, I completely concur with these sentiments. Dr. Randall combines the drive to do good science with a relaxed and informal demeanor, which allows his mentees to move forward to ask exciting and novel questions in their projects without objections or doubts. I feel that this quality is the reason for some of the high quality publications, which have come from his people, many of which are paradigm shifting.

By the end of 2012, *The Scientists* ranked UAB as #8 for the best places to work as a postdoc compared to national and international institutions. Amongst public universities, UAB was ranked #1. This was the outcome of a survey which took in the opinions of actual postdocs. This ranking was based on the quality of research, mentorship and career development opportunities that UAB provides. After becoming a postdoc, I have realized that this has a lot to do with the caliber of the faculty and the Office of Postdoctoral Education (OPE). The faculty members, especially in Clinical Immunology & Rheumatology and in Microbiology are the leaders in their fields like Dr. Casey Weaver. Due to the distinction of the faculty members, UAB also attracts many outstanding speakers for its regular immunology and other special seminars. Thus, in the short time that I have been here, I have had the opportunity to meet founders of memory and MHC restriction like Drs. Oldstone and Zinkernagal, respectively. Further, as there are many Max Cooper progeny like Dr. John Kearney, UAB is known for its outstanding contributions to the field of B cells. This gives me an environment filled with experts in my field to consult on my project. As mentioned above, the OPE plays an important role in making UAB a haven for postdocs as it provides many training sessions for grant writing, like the Writing a K99 workshop last year which was extremely helpful for me. They were able to bring in a former program officer to give us the dos and don'ts of writing a K99. Additionally they provide a lot of funding opportunities for training and travel like the Career Enhancement Award, which partly funded the RCSIRM workshop, which I attended earlier this year.

Therefore I feel that having Dr. Randall as a mentor combined with the extremely enthusiastic and knowledgeable scientific community within our lab and UAB at large is the best possible environment for me to train to become a contributing member of the immunology research community.

## **Training in the Responsible Conduct of Research**

Dr. Allie will take a formal course (GRD 717) in responsible conduct of research (RCR) at UAB and has participated in multiple mandatory training sessions focusing on RCR during her doctoral work at Dartmouth College.

### **Principles of Scientific Integrity (GRD 717)**

*Format:* The organization of the course is through a new pedagogic approach, called “Team Based Learning.” Team based learning organizes the course so that the materials that normally would be presented in a lecture format are made available approximately one week before the class meeting. Class time is spent in teams of 6 to 7 students each who will meet each week to discuss the course materials.

*Subject Matter:* Includes the nature, extent and causes of fraud in science; UAB policies on fraud; ideals of good science; the responsibilities of authorship and peer review; conflict of interest; mentor/mentee relationships; bias and sloppy practices; responsible use of the press; potential problems raised by the commercialization of research; scientists as public policy advisors; and ethical issues involved in animal experimentation and in clinical trials. Famous cases from the history of science as well as fictional case studies are used to involve students in discussion of the above issues. Readings come from multiple different scientific publications, as well as selected cases from “Classic Cases in Medical Ethics” by UAB faculty member Gregory Pence.

*Faculty Participation:* The primary instructor is Jeff Engler, PhD, and Associate Dean for Academic Affairs, and UAB’s Research Integrity Office. Other faculty members provide content specific contributions as in-class guest facilitators.

*Frequency and Duration of Instruction:* This course is over the course of a semester offering 48 contact hours of instruction. Attendance is required weekly over the course of the semester for 2.5 hours at each class meeting to discuss assigned course work.

## Goals for the Fellowship and Training

I intend to become an independent research scientist at an academic institution. My objective is to establish a lab that studies the establishment of immune memory and the transcriptional and metabolic mechanism that drive their differentiation. My current interest in studying these mechanisms is to benefit vaccine design and immune therapy. Yet, the marrying of B cell memory and metabolomics opens up a highly unexplored area of study, so I foresee that future discoveries down this path may lead to this research making an impact on a wider area of science and health. The *metabolome* (the sum of all small molecules associated with living systems) has been shown to influence more than just the energy requirements of cells but to broadly impact genes and cellular functions including immune modulation. Thus, the study of the impact of these small metabolites on immune memory differentiation and establishment post viral infection may give us information which is more generalizable to other immune cells and disease models.

The proposed project and training exercises will allow me to attain my long-term goals in multiple ways. 1) The proposed immunology experiments will allow me to further solidify my training as an immunologist with the help of Dr. Troy Randall, who is an internationally renowned immunologist who has made crucial contributions in the study of immune responses (T cell, B cell and DCs) to influenza virus. His lab has many excellent postdocs and junior faculty who do and will continue to help me grow as an independent immunologist. 2) The proposed training and collaborations in the field of metabolomics will help me get trained in carrying out sound metabolomics experiments and in eventually handling the large amounts of data that such experiments generate. Thus our collaborations with Dr. Teresa Fan's group will help me gain the bioinformatics skills that I will need to carry out advanced studies on the metabolome, in the future. 3) As we are proposing to set up multiple collaborations and to consult with many faculty members at UAB, this would allow me to establish my network in the scientific community within and without UAB, as a support for my independent career. 4) The metabolomics arm of the proposal was entirely due to my interest and reading, thus the stories that arise from these experiments will help me establish my own niche in the world of immunology and metabolism. 5) The proposal will further help the establishment of my own niche because it will draw from two different fields which is rather unique, at present.

The formal training in the RCSIRM workshop that I attended has already helped me in asking questions about the quality of immunology experimental design to answer metabolomics questions. I feel that the Special Topics in Metabolism class (at UAB), the advanced data analysis workshop (at UKy) combined with the weekly sessions with the metabolism group at UAB will allow me to collectively establish a formal foundation in metabolism research.

Another aspect of the activities that we have proposed in this grant that is vital to an independent career in academia is to develop a teaching portfolio and mentoring experience. My current experience in teaching the graduate level Cancer Immunology class provides an excellent platform for me to polish my teaching skills. Additionally, my experience in mentoring junior students with Dr. Usherwood and my current mentee, Michael Dixon (rotation student), in Dr. Randall's lab have helped me gain the skills that I will need to establish my own research group. Therefore, I feel that combined with my thorough training in immunology with Drs. Edward Usherwood and Troy Randall during my doctorate and postdoc, respectively, combined with the formal training in metabolism proposed in this grant, and my mentoring/teaching experience will help drive and establish my independent research career.

**Activities Planned Under This Award**

| <b>Activity</b> \ <b>% Time</b>   | <b>2015</b> | <b>2016</b> | <b>2017</b> |
|-----------------------------------|-------------|-------------|-------------|
| <b>Research</b>                   | 85          | 85          | 90          |
| <b>Teaching and Mentoring</b>     | 5           | 2.5         | 5           |
| <b>Meetings and Seminars</b>      | 5           | 2.5         | 2.5         |
| <b>Training and Grant Writing</b> | 5           | 10          | 2.5         |

**Year 1**

- **Research:** completion of Aim 1, complete the in vitro live imaging studies to determine ASC formation on a cellular level and the in vivo adoptive transfer studies to determine ASC formation
- **Teaching & Mentoring:**
  - Teach the T cell section of the Cancer Immunology (GBS774) graduate class
  - Oversee and direct discussions in the Cancer Immunology Journal Club (GBSC 700-01A)
  - Mentor first year graduate students in the lab
- **Training Activities:**
  - Attend RCSIRM advanced analysis workshop at the Mackay Cancer Center Metabolism Core at the University of Kentucky, Lexington, KY
  - Attend the “Principles of Scientific Integrity” GRD 717 class to meet the training in research ethics requirements.
  - Attend the weekly ad hoc Metabolomics group meeting, headed by Stephen Barnes.
- **Seminars:**
  - Present at the annual Research in Progress meeting in the Department of Clinical Immunology and Rheumatology at UAB.
  - Present at the annual Virology Group meeting - Microbiology Department at UAB.
  - Attend the *Program in Immunology* weekly seminars featuring internationally renowned immunologists.
- **Scientific Meetings:**
  - B cell meeting – Keystone Symposium “The Golden Anniversary of B cell Discovery”
  - Southeastern Immunology Symposium

**Year 2**

- **Research:** completion of Aim 1 & Aim 2, complete the repertoire analysis experiments and carry out in vitro assays on sorted populations to determine the glycolytic shift potential of the various populations.
- **Teaching & Mentoring:**
  - Teach the T cell section of the Cancer Immunology graduate class
  - Direct discussions in the Cancer Immunology Journal Club (GBSC 700-01A)
- **Training Activities:**
  - Attend the “Advanced Special Topics Course in Metabolism” (GBS748). A three-month long intensive training course at UAB run by Dr. Stephen Barnes, a leader in the field of metabolomics.
  - Attend the weekly ad hoc Metabolomics group meeting, headed by Stephen Barnes.
- **Grant writing Activities:**
  - Attend the “Grant writing for postdoctoral Fellows” course offered by the UAB-Office of Postdoctoral Education
  - Write the K99 training grant focusing on the metabolic pathways of B cells.
- **Seminars:** Same as Year 1
- **Scientific Meetings:**
  - American Association of Immunology Symposium
  - Southeastern Immunology Symposium

**Year 3**

- **Research:** completion of Aim 2, complete the metabolic pathway determination studies
- **Teaching & Mentoring:**
  - Teach the T cell section of the Cancer Immunology graduate class
  - Direct discussions in the Cancer Immunology Journal Club (GBSC 700-01A)
- **Training Activities:**
  - Attend the weekly ad hoc Metabolomics group meeting, headed by Stephen Barnes.
- **Seminars:** Same as Year 1
- **Scientific Meetings:** Same as Year 1

## Doctoral Dissertation and Other Research Experience

### **1. Identification and phenotyping of autoreactive human effector memory CD4 T cells in multiple sclerosis (MS)**

- Mentor: Peter A. Calabresi, Department of Neurology, at University of Maryland at Baltimore.
- Laboratory Technician 2001-2003

Dr. Calabresi's lab studied the trafficking patterns and functions of antigen specific human T cells based on their chemokine receptor expression profile. My initial work in the Calabresi lab identified CXCR6 as a good marker of effector memory T cell function and migration in autoimmune disease. We then characterized the lymph node homing chemokine receptor – CCR7 – expression on Th1 and Th2 polarized population and the kinetics showed that Th1 cells expressed CCR7 earlier than Th2 cells implicating CCR7 expression in the rapid priming of Th1 cells in autoimmune diseases like MS. In collaboration with Dr. George Chandy's group at UC Irvine we also identified a effector memory phenotype which was indicative of a highly autoreactive population in myelin specific CD4 T cells from MS patients. Our studies determined that Ag specific effector memory T cells from MS patients that had undergone repeated stimulation expressed high levels of the voltage gated potassium channel Kv1.3 and that it could be used as a target for immunotherapies for MS. This experience served as a good introduction to basic science research and to human immunology studies, and I was able to interact with other investigators in lab meetings and departmental journal clubs.

- 1- Calabresi PA, Yun SH, **Allie R**, Whartenby KA. Chemokine receptor expression on MBP-reactive T cells: CXCR6 is a marker of IFN $\gamma$ -producing effector cells. *J Neuroimmunol.* 2002 Jun 1; 127(1):96–105.
- 2- Calabresi PA, **Allie R**, Mullen KM, Yun SH, Georgantas RW, Whartenby KA. Kinetics of CCR7 expression differ between primary activation and effector memory states of T(H)1 and T(H)2 cells. *J Neuroimmunol.* 2003 Jun; 139(1-2):58–65.
- 3- Wulff H, Calabresi PA, **Allie R**, Yun S, Pennington M, Beeton C, et al. The voltage-gated Kv1.3 K(+) channel in effector memory T cells as new target for MS. *J Clin Invest.* 2003 Jun; 111(11):1703–13.

### **2. Glatiramer acetate mediated modulation of autoreactive human CD4 T cells in MS patients**

- Mentor: Peter A. Calabresi, Department of Neurology, at Johns Hopkins University.
- Laboratory Manager 2003-2007

I was promoted to the position of lab manager in Dr. Calabresi's lab when he was recruited to Johns Hopkins University. My first project in which I carried out the studies and analyses independently was determining the mechanism of action of Glatiramer acetate (a well established MS therapeutic drug) on autoreactive CD4 T cells. The study showed that the Ag specific T cells were Th2 biased as previously shown and that the cellular expression of the Th1 chemokine receptors, CXCR3, CXCR6 and CCR5, was significantly reduced. Additionally we showed that the CD4 response as a whole had been diminished. This was an excellent exposure to independent work and to the management of complicated data sets. This project allowed me to take a number of graduate statistics courses at Johns Hopkins and apply my knowledge to the management and analysis of the patient data sets that I used for this study.

- 1- **Allie R**, Hu L, Mullen KM, Dhib-Jalbut S, Calabresi PA. Bystander modulation of chemokine receptor expression on peripheral blood T lymphocytes mediated by glatiramer therapy. *Arch Neurol.* 2005 Jun; 62(6):889–94.

### **3. Immune mediated neuronal cytotoxicity in MS**

- Mentor: Peter A. Calabresi, Department of Neurology, at Johns Hopkins University.
- Laboratory Manager 2003-2007

My expertise in isolating and culturing T cells from MS patients, identifying Ag specific T cells and studying their proliferation and function lead to multiple collaborations within the department of Neurology. In collaboration with Dr. Avindra Nath, we showed that Granzyme B mediated the T cell mediated neurotoxicity in a perforin independent manner. This collaboration gave me experience in culturing neurons, astrocytes and carrying out biochemical analyses and mitochondrial integrity studies.

In collaboration with Dr. Jerome Graber in our lab we studied mechanisms of immune mediated neurotoxicity showing that IL-17 and IL-6 produced by T cells caused copious amounts of IL-6 production by astrocytes, which resulted in neuronal toxicity. This study resulted in a co-authored publication. This experience lead to a platform presentation at the Rare Neurological Diseases symposium in Maryland and it allowed for my training to orally communicate my findings and to discuss these basic science findings with clinicians.

- 1- Wang T, **Allie R**, Conant K, Haughey N, Turchan-Chelowo J, Hahn K, et al. Granzyme B mediates neurotoxicity through a G-protein-coupled receptor. *FASEB J Off Publ Fed Am Soc Exp Biol.* 2006 Jun; 20(8):1209–11.
- 2- Graber JJ\*, **Allie SR\***, Mullen KM, Jones MV, Wang T, Krishnan C, et al. Interleukin-17 in transverse myelitis and multiple sclerosis. *J Neuroimmunol.* 2008 May 30; 196(1-2):124–32. **\*CO-AUTHORSHIP**

### **4. Impact of autoimmune primed effector memory CD8 T cells on melanoma**

- Mentor: Dr. Mary Jo Turk, Geisel School of Medicine, at Dartmouth College
- Graduate research rotation, Winter 2008

Dr. Mary Jo Turk works on immune responses to poorly immunogenic tumors and immunotherapy targets to these tumors. My rotation in her lab led to the observation of B16 melanoma surgically removed mice that were CD4 T cell depleted developing Vitiligo. Further studies led to the observation that the Vitiligo induced damage primed a CD8 effector memory T cell population, which was protective against tumor challenge. This experience allowed me to explore tumor immunology. Unlike my work in autoimmunity, which focused on *suppressing* the immune response I learned to focus on immune *activation* against tumors in the Turk lab.

1- Byrne KT, Côté AL, Zhang P, Steinberg SM, Guo Y, Allie R, et al. Autoimmune melanocyte destruction is required for robust CD8+ memory T cell responses to mouse melanoma. *J Clin Invest*. 2011 May; 121(5):1797–809.

## 5. Mechanisms of CD8 memory priming and differentiation in the context of respiratory viral infections

- Mentor: Dr. Edward J. Usherwood, Geisel School of Medicine, at Dartmouth College
- Graduate research thesis, 2008-2012

Dr. Usherwood's lab studies the establishment and function of CD8 T cell memory in the context of acute and latent viral infections. My work in Dr. Usherwood's lab started with a collaborative study in which we rescued CD4 helpless memory defects during challenge infection by blocking PD-1 signaling, as we had observed high PD-1 expression on these cells. These findings led to an independent project, which looked at the impact of PD-1 expression on CD8 T cell memory differentiation and function. My experience in memory differentiation through the various precursors of memory took me to my next project, which was the impact of the morphogen – retinoic acid (RA) – on CD8 memory differentiation. In this study we reported that RA was vital for effector functions and effector memory formation. My established interest in memory differentiation further led to a collaborative project with Dr. Chin Yi Tsai, to look at the role of a microRNA (miR155) on CD8 memory differentiation and this led to a second author publication. My experience in Dr. Usherwood's lab has been instrumental in cultivating my interest in immune memory and in respiratory viral infection models. This experience allowed me to transition from human immunology to mechanistic studies using mouse models and influenced my choice of postdoctoral research.

1- Fuse S, Tsai C-Y, Molloy MJ, Allie SR, Zhang W, Yagita H, et al. Recall responses by helpless memory CD8+ T cells are restricted by the up-regulation of PD-1. *J Immunol Baltim Md* 1950. 2009 Apr 1; 182(7):4244–54.

2- Allie SR, Zhang W, Fuse S, Usherwood EJ. Programmed death 1 regulates development of central memory CD8 T cells after acute viral infection. *J Immunol Baltim Md* 1950. 2011 Jun 1; 186(11):6280–6.

3- Allie SR, Zhang W, Tsai C-Y, Noelle RJ, Usherwood EJ. Critical role for all-trans retinoic acid for optimal effector and effector memory CD8 T cell differentiation. *J Immunol Baltim Md* 1950. 2013 Mar 1; 190(5):2178–87.

4- Tsai C-Y, Allie SR, Zhang W, Usherwood EJ. MicroRNA miR-155 affects antiviral effector and effector Memory CD8 T cell differentiation. *J Virol*. 2013 Feb; 87(4):2348–51.

## 6. Identification and characterization of lung resident memory B cells post influenza infection

- Mentor: Dr. Troy D. Randall, Clinical Immunology and Rheumatology, at UAB
- Postdoctoral research project 12-2012 - present

As Dr. Randall's lab studied various immune responses (T cell, B cell and dendritic cell) to a respiratory virus (Influenza) I felt it would be an ideal setting for me to expand my horizons. Further, as my studies will focus on B cell memory establishment and maintenance in the lung, it was a marrying of my previous experience with a cell type that I had very little exposure to – the B cell. Our studies are extremely promising as we are able to use recombinant whole protein tetramers to identify Ag specific B cells at various stages of the response (germinal center and memory). Then we carried out antibody infused (anti B220 i.v.) parabiosis experiments and identified a novel Ag specific resident memory B cell population in the lung. To our knowledge we are the first to identify this population in the context of an actual infection. We are currently in the process of characterizing these cells and identifying the function of the subtypes, as we have observed that they exhibit quite a lot of heterogeneity. As our studies have revealed that the resident population is established early and does not recirculate, we are currently using infusion studies alone to determine the origin of these cells.

As our studies show distinct phenotypes of memory in the lymph node and lung, a question that I have begun to ask is, are they intrinsically different, as well? Since the cells in the lung are positioned to receive a pathogenic insult first and because they are required to respond speedily to resolve the infection, we feel that their energetic demands to meet all their functional demands would be distinct. Thus we are starting to establish protocols and techniques in the lab to study the metabolism of the B cells in the lung in comparison to the B cells in the lymph node. Therefore, in addition to the immune memory studies, my experience in Dr. Randall's lab is widening to include mechanisms of transcriptional and metabolic regulation of memory B cells.

1- Identification of an Ag specific resident memory B cell population in the lung, which establishes early post influenza A infection. *Manuscript in preparation*

## Sponsor and Co-Sponsor Information

### a. Research Support Available

| <b>Research support available (Randall, Sponsor)</b> |                 |  |                            |              |               |
|--|-----------------|--|----------------------------|--------------|---------------|
| <b>Source</b>  | <b>Number</b>   | <b>Title</b>   | <b>PI</b>                  | <b>Dates</b> | <b>Yearly</b> |
| NIH/NIAID  | R01<br>AI100127 | Pulmonary Immunity To Pathogens In Neonates  | Randall                    | 04/12-03/17  | \$211,000     |
| NIH/NIAID  | RO1<br>AI097357 | Central and effector memory B cells in the lung  | Randall                    | 4/12-3/17    | \$250,000     |
| NIH/NHLBI  | R01<br>HL069409 | Unique aspects of respiratory immunity   | Randall                    | 9/01-3/17    | \$250,000     |
| NIH/NIAID  | U19<br>AI109962 | Virus-induced cell fate decisions in anti-viral immunity. Core A   | Randall                    | 05/14-04/19  | \$77,214      |
| NIH/NIAID  | U19<br>AI109962 | Virus-induced cell fate decisions in anti-viral immunity. Project 1  | Randall                    | 05/14-04/19  | \$233,989     |
| NIH/NIAMS  | P30<br>AR048311 | Rheumatic Disease Core Center  | Mountz<br>(Randall co-inv) | 09/01-08/17  | \$400,000     |
| NIH/NIAID  | P01<br>AI078907 | B cells in Health and Disease. Project 3   | Sanz<br>(Randall co-inv)   | 08/10-07/15  | \$379,212     |
| <b>Research support available (Lund, co-Sponsor)</b> |                 |  |                            |              |               |
| <b>Source</b>  | <b>Number</b>   | <b>Title</b>   | <b>PI</b>                  | <b>Dates</b> | <b>Yearly</b> |
| NIH/NIAID  | R01<br>AI104725 | Controlling Th2 immunity by tuning CXCL13-dependent DC migration in lymph nodes                              | Lund                       | 03/13-02/18  | \$250,000     |
| NIH/NIAID  | R01<br>AI110508 | Control of anti-viral B cell responses by IFN $\gamma$ , T-bet and Eomes                                     | Lund                       | 03/13-02/18  | \$319,046     |
| Alabama Drug Discovery Alliance                      |                 | Treating B cell-derived neoplasms by targeting the ectoenzyme CD38: a regulator of the NAD metabolic pathway | Lund                       | 01/13-12/15  | \$50,000      |
| NIH/NIAID  | U19<br>AI109962 | Virus-induced cell fate decisions in anti-viral immunity. Core B   | Randall<br>(Lund co-inv)   | 05/14-04/19  | \$141,051     |
| NIH/NIAID  | U19<br>AI109962 | Virus-induced cell fate decisions in anti-viral immunity. Project 3  | Randall<br>(Lund co-inv)   | 05/14-04/19  | \$236,002     |
| NIH/NIAID  | P01<br>AI078907 | B cells in Health and Disease. Project 3   | Sanz<br>(Lund co-inv)      | 08/10-07/15  | \$379,212     |
| NIH/NIAID  | R01<br>AI097357 | Central and Effector B Cells in the Lung   | Randall<br>(Lund co-inv)   | 05/12-04/17  | \$250,000     |

### b. Sponsor's and Co-Sponsor's Previous Fellows/Trainees

**Troy Randall (Sponsor):** Dr. Randall began his career as a faculty member at the Trudeau Institute, which is not a degree-granting institution. As a result, he mentored only post-doctoral fellows during that time (1997-2008). He began accepting graduate students into his laboratory at the University of Rochester (2008-2012) and now at the University of Alabama at Birmingham (2012-present). Over the last 15 years, he has mentored ten post-doctoral fellows and three pre-doctoral trainees. His previous trainees currently have positions in academia or industry and continue to perform biomedical research. His previous trainees include the following:

1998-2005                      Byung O. Lee, Ph.D. (postdoctoral trainee). Assistant Professor at Vaccine Research Institute of San Diego

|           |   |
|-----------|---|
| 2002-2006 | Juan E. Moyron-Quiroz, Ph.D. (postdoctoral trainee). Scientist, Biogen Inc. San Diego, CA                                       |
| 2002-2012 | Javier R. Moreno, Ph.D. (postdoctoral trainee). Assistant Professor, Dept of Medicine, University of Rochester                  |
| 2004-2008 | Damian Carragher, Ph.D. (postdoctoral trainee). Scientist, Sanger Institute, Cambridge UK                                       |
| 2008-2012 | Andre Ballesteros-Tato, Ph.D. (postdoctoral trainee). Assistant Professor, Dept. of Medicine, University of Alabama, Birmingham |

**Frances Lund (co-Sponsor):** Dr. Lund also worked at Trudeau Institute between 1997-2008 and only mentored post-doctoral fellows and visiting graduate students during that time. After leaving Trudeau Institute, she began to take full-time graduate students into her lab and, over the last 15 years, has mentored nine post-doctoral fellows, two pre-doctoral trainees and five visiting graduate students who worked in her lab for 4-12 months at a stretch during their graduate training. Dr. Lund's previous trainees hold positions in academia or industry and continue to perform biomedical research. Her previous trainees include the following:

|           |  |
|-----------|--|
| 1998-2004 | Santiago Partida-Sanchez, Ph.D. (postdoctoral trainee). Associate Professor, Ohio State Univ.  |
| 2002-2003 | Miguel Moreno-Garcia, Ph.D. (visiting graduate student). Staff Scientist at Boehringer Ingelheim in Danbury CT   |
| 2004-2007 | Guixiu Shi, M.D., Ph.D. (postdoctoral trainee). Professor and Director of the Dept. of Rheumatology and Clinical Immunology at the First Hospital of Xiamen University in Xiamen China |
| 2006-2012 | Wojciech Wojciechowski, Ph.D. (postdoctoral trainee). Instructor, Dept. of Pediatrics at the University of Rochester.  |
| 2008-2013 | Beatriz Leon, Ph.D. (postdoctoral trainee). Assistant Professor, Dept. of Microbiology at UAB.   |

### c. Training Plan

Dr. Allie and I have developed an Individual Development Plan (IDP) that is specifically tailored to her career goals as an academic scientist working in the area of metabolic control of immune memory. This IDP incorporates classes offered by UAB, training opportunities provided by the UAB Office of Postdoctoral Education, participation in national and international conferences, journal clubs and lab meetings that will be beneficial for both her project and her future research goals. Importantly, the training plan incorporates specific training opportunities related to both metabolomics and immunology, which are the two focus areas of Dr. Allie's application.

The RCSIRM workshop: Dr. Allie has already attended (2014), and in 2015 will again attend the advanced analysis workshop at the Markay Cancer Center Metabolism Core at the University of Kentucky's Resource Center for Stable Isotope-Resolved Metabolomics. This 12-day, hands-on workshop will provide participants with practical knowledge in the application of stable isotope-resolved metabolomics and is preceded by a one-day symposium, in which a variety of metabolomics experts will present data and describe methodology relevant to metabolomics research. The symposium and workshop are organized by Dr. Teresa Fan, an internationally recognized expert in isotope-resolved metabolomics, and who is also a contributor on this application. Dr. Fan will also assist Dr. Allie in the design and interpretation of her metabolomics data. This training opportunity will uniquely allow Dr. Allie to develop skills in the detection of metabolites, the design of metabolism-related experiments and the interpretation of metabolomics data.

Metabolomics group meeting: Dr. Allie will also attend the weekly metabolomics group meeting headed by Stephen Barnes, who is the Director of the Targeted Metabolomics and Proteomics Laboratory at UAB and is a leader in the field of metabolomics. This group meeting will discuss topics of interest and each of the participants will present on a rotating basis their own experiments and the resulting data, which will be discussed and critically evaluated by the group. This forum will allow Dr. Allie to observe first-hand how other researchers design, perform and trouble-shoot metabolomics-related experiments and will allow her to get critical feedback from her peers in the metabolomics field.



Advanced Special Topics Course in Metabolism: Dr. Allie will also take the “Advanced Special Topics Course in Metabolism” (GBS748). This is a three-month long intensive training course at UAB that is also run by Dr. Stephen Barnes. This class will train students and postdocs in the scope, methodology, analysis and interpretation of metabolomics research.

Mentor/Trainee Meeting: Dr. Allie currently has and will continue to have a weekly, one-on-one meeting with Dr. Randall. This meeting will allow Dr. Allie to present her recent data, plan new experiments, refine study designs and to discuss how to interpret their results in light of our current understanding of the field. These meetings are also used as an opportunity to troubleshoot problems, to prioritize experiments, to plan for grant and manuscript submissions and to discuss scientific integrity – particularly as it relates to data collected for grant and manuscript submissions. Finally, this time may be used to discuss the latest or most interesting papers and to set goals for the coming weeks and months.

Lund/Randall Joint Laboratory Meeting: Dr. Allie will attend the Lund/Randall joint lab meeting, which occurs weekly on Friday afternoons for two-ish hours. Everyone in the lab (students, postdocs, technicians, faculty) is required to attend and, on a rotating basis, to give a presentation on their research project approximately every three months. The presentation is formal in that each trainee is expected to provide background, significance and rationale for the studies that they present. They also need to describe the experimental design, the data collection and analysis and their interpretation of the results. All members of the lab are encouraged and expected to ask questions and to push the presenter to think critically about their data. Thus, this is a great training experience for the students – regardless of whether they are the presenter or a member of the audience. The meetings also provide a great forum for the trainees to practice talks for upcoming meetings and to receive feedback from a critical but friendly audience.

Molecular and Cellular Immunology Journal Club: Dr. Allie will participate in the immunology journal club, which meets weekly and requires participants to present recent, high impact immunology papers. This journal club will keep Dr. Allie abreast of recent developments in the field of immunology.

Program in Immunology and Dept. of Microbiology Seminar Series: Dr. Allie will attend the Program in Immunology seminar series as well as the Microbiology weekly seminar series, both of which feature invited external speakers from the fields of immunology, infectious disease, virology and microbial pathogenesis. These seminar programs will allow Dr. Allie to learn about the most recent advances in her field. More importantly, she often has the opportunity to meet with the seminar speakers and tell them about her work. In the last 18 months she, along with some of her trainee colleagues, met with Dr. Eric Meffre (Yale), Dr. Arlene Sharpe (Harvard), Dr. Sue Pierce (NIH), Dr. Ann Rothstein (U. Mass) and Dr. Michael Oldstone (LIAI).

National and International Meetings: Dr. Lund and I require that all trainees attend at least one conference each year and to apply for travel awards for the conferences that they attend. For example, Dr. Allie received a travel award to attend the annual RIKEN RISP program that was held this summer in Japan, where she presented her work on resident memory B cells in the lung. She also received a travel award to attend the most recent RCSIRM metabolomics workshop. I expect that she will be attending AAI in New Orleans, one of the next B cell Keystone symposia and the Southeastern Immunology Symposium, where she will present posters and hopefully be chosen for a talk in a workshop. Dr. Allie will also participate in local poster sessions as part of department retreats or immunology-themed conferences at UAB. Each of these conferences or symposia will allow her to practice her presentation skills and to interact with her peers outside of UAB.

Lab Management Course for Postdoctoral Scholars: Dr. Allie will take the Lab Management course provided by the Office of Postdoctoral Education. This course will introduce every aspect of laboratory management, including managing start-up budgets and projects, effective hiring and mentoring practices, managing communication and conflict, data management and safe laboratory practices. This may be one of the most valuable courses for any postdoctoral scholar, given that most of them have never had a management class of any kind. Throughout the course, participants are expected to write and present a laboratory management plan to the class.

Scientific Writing: Dr. Allie is required to author manuscripts that will be submitted to high-quality, peer-reviewed journals. Dr. Allie’s draft manuscripts will be critiqued by Dr. Randall and the other authors, who will meet as a group to provide feedback and discuss data presentation and the logical flow of the manuscript. This iterative process is time consuming for everyone, but will be critical for Dr. Allie’s development as a scientific writer. I also ask Dr. Allie and other trainees in my lab to participate in reviewing manuscripts that have been submitted for publication. I am also a faculty member for Faculty of 1000 and Dr. Allie and the other postdocs in the lab are encouraged to pick papers for submission and write a critical evaluation of those papers that will be submitted to F1000. These activities will provide Dr. Allie with additional practice and experience with scientific writing.

**Biomedical Ethics:** Dr. Allie will take the required ethics course, Principles of Scientific Integrity (GBS 717), which was developed by Harold Kincaide, PhD, Director of UAB's Center for Ethics and Values in the Sciences, and is taught by Jeffrey Engler, Ph.D., UAB's Research Integrity Officer. This semester-long series meets weekly and is focused on ethical issues associated with all aspects of the scientific process.

**Research Environment and Facilities: Equipment and Cores:** Dr. Randall and Dr. Lund run a joint laboratory, which comprises about 3500 sq/ft of wet lab space in the Shelby Research Building on the UAB campus and is fully equipped for the work planned in this application. Cell imaging equipment available in the laboratory includes a BD Canto flow cytometer and a Nikon inverted 4-channel fluorescent microscope with humidified chamber for imaging cultured cells. The laboratory also has a Leica cryostat, microtome, cytospin and other basic histology and staining equipment. We also have plate readers that perform colorimetric, fluorescent and luminescent assays. We have a separate laboratory, which includes BSCII, centrifuge, ultracentrifuge, TC incubators, egg incubator, sonicators, water baths etc, for the preparation of large batches of influenza (or other pathogens). We have incubators and shakers for producing recombinant proteins in bacteria and eukaryotic cells and have a Biorad FPLC in a cold room for protein purification. We have three tissue culture rooms with BSCII, incubators, water bath, microscopes and other equipment needed for cell culture. Finally, we have numerous small equipment items, including thermocyclers, water baths, freezers, fridges, tissue disruptors etc.

In addition to the Shelby lab space, Dr. Randall and Dr. Lund have dedicated animal suites in the RSB mouse facility, which is connected to the Shelby building. Our animal suite includes two large mouse breeding rooms (together we breed about 95 strains of mice), several ABSL-2 rooms for housing experimentally infected mice and two ABSL-2 level procedure rooms, which is where laboratory personnel perform experimental infections, surgeries, euthanasia and tissue collection. The procedure rooms includes BSCII for working with infected mice and lab equipment needed to infect, monitor, and euthanize mice as well as equipment to harvest and process infected tissues. All work with mice and infectious influenza virus in either the lab or animal facility has been approved by the UAB IBC and IACUC committees and Dr. Allie has been trained to work with the infectious agents.

All other equipment that will be needed by Dr. Allie is available in either the Microbiology Department shared equipment core or within one of the many UAB Core Facilities located on the medical school campus. Of particular importance for Dr. Allie's project, the UAB *Comprehensive Flow Cytometry Core*, located partly in the Shelby Building and partly in the Bevell Building across the street, provides multiple analytic flow cytometry instruments (14-color and 20-color LSRIIs) and cell sorting (two 14-color FACSArialls). Two dedicated operators are available for sorting cells. The *Center for Clinical and Translational Science (CCTS)* provides access to ELISPOT readers and quantitative PCR instruments are available in the *Heflin Genomics Core*, the *Comprehensive Arthritis Musculoskeletal and Autoimmune Center (CAMAC)* and the *Dept. of Microbiology shared equipment core*. The Metabolomics core houses XXXX and the Shared Imaging facility houses the Amnis Imagestream flow cytometer.

**Environment:** In addition to the physical infrastructure that will be available for Dr. Allie's project, we will provide a nurturing intellectual environment that will help her to develop as a scientist. As mentioned above, Dr. Randall and Dr. Lund have run an integrated laboratory for more than 15 years. We have joint lab meetings, we jointly mentor students and postdocs, we serve as co-investigators on several NIH-sponsored projects and we share technical resources. The combined group includes Drs. Lund and Randall as well as 3 Assistant Professors, 5 post-doctoral fellows, 2 graduate students and 6 research technicians. This larger group will provide Dr. Allie with technical/training support and intellectual input. For example, Dr. Allie is exposed, through her interactions with the other members of the combined lab, to many different immunologically relevant infectious disease (viral, bacterial, fungal, and parasitic) and autoimmune/allergic disease models. She can collaborate with other fellows and students in the lab and learn about more than just B cells and influenza. Indeed, current members of the lab are examining the roles that neutrophils, macrophages, dendritic cells, B cells and T cells play following exposure to various types of pathogens. Importantly, this broad approach to immunology and host defense will allow Dr. Allie to visualize the full spectrum of immunity - from protection to immunopathology.

Dr. Allie will also be exposed to vibrant research community at UAB. UAB is one of the Southeast's premier biomedical research institutions with a world-renowned academic health center and more than 100 interdisciplinary research centers. UAB provides an environment in which collaborations between basic researchers, clinicians and clinician-scientists are encouraged and given the resources needed to succeed. The infectious disease and immunology research community at UAB is simply outstanding. There is a strong Program in Immunology (PII) that serves as the focal gathering point for the >100 UAB immunologists. PII sponsors a weekly seminar series and a yearly symposium and has funding to facilitate recruitment of

immunology faculty. Members of the UAB PII hold numerous P30 and P60 grants that provide financial and intellectual resources for immunology cores and equipment. UAB also has very strong infectious disease research community that is anchored by an extremely robust and effective CFAR, which has a long history of excellence in clinical care and research. Importantly, the UAB School of Medicine recognizes the importance of infectious disease and immunology in both patient care and research and invests heavily in both areas. These investments include hiring a new Chair for Microbiology (Lund) and providing resources for recruiting numerous investigators working in the areas of infectious disease and immunology. In addition, the School provides annual funding to the immunology and infectious disease steering committees, which, in turn, sponsor research symposia and infectious disease/immunology pilot projects. The committees also provide funding for UAB scientists to perform research sabbaticals at other labs outside of UAB. For example, graduate, MD, and MSTP students can apply for funding to perform TB and HIV research at K-RITH, a Howard Hughes Institute located in within the heart of the epidemic in South Africa. Collectively, these programs in infectious disease and host defense will be invaluable to Dr. Allie as she pursues her research project and should provide her with all of the tools needed for her to pursue her career as an immunologist.

#### **d. Number of Fellows/Trainees to be Supervised by Sponsor and Co-Sponsor during the Fellowship**

Dr. Randall currently mentors four postdoctoral fellows, two graduate students and one graduate MSTP student.

Dr. Lund currently mentors one graduate MSTP student engaged in thesis research. She also mentors two post-doctoral research fellows, three Assistant Professors (both clinical and basic researchers) and an MD/PhD pulmonary allergy resident in the internal medicine research residency program at UAB.

#### **e. Applicant's Qualifications and Potential for a Research Career**

Dr. Allie obtained her BS degree in Biology at the University of Kentucky and her MS degree in Applied Molecular Biology from University of Maryland in Baltimore. Dr. Allie then worked as a technician and laboratory manager for approximately four years. In 2007, Dr. Allie returned to school and in 2012 she obtained her PhD from the Department of Microbiology and Immunology at Dartmouth University. During the time that she worked as a technician in Dr. Peter Calabresi's lab she was an author on **nine** publications including two first author publications in *J. Neuroimmunology* and *Archives Neurology*. This is a phenomenal level of productivity for a technician and indicates that she learned very early in her career to work independently. Dr. Allie was also highly productive as a graduate student in Dr. Ed Usherwood's lab with **five** publications including two first author manuscripts. Within one year of being in the lab, she was an author on a *J. Immunology* article that examined the effect of PD-1 expression on virus-specific CD8 recall responses. In 2011 she was an author on a *J. Clinical Investigation* report from Mary Jo Turk's lab examining how the development of protective and long-lasting CD8 memory responses to melanoma tumor cells are dependent on the reactivity of these cells to the normal melanocytes. Dr. Allie also continued her studies examining the role of PD-1 in CD8 memory and in 2011 published a first author manuscript in *J. Immunology* that showed how PD-1 modulates the development of central and effector memory CD8 T cells following acute viral infection. Finally, Dr. Allie developed a project in Dr. Usherwood's lab examining how retinoic acid (RA) influences the development of terminally differentiated effector CD8 T cells and long-lasting effector memory CD8 T cells. This work, published in *J. Immunology* in 2013 showed that in the absence of RA signaling, the fate decision of CD8 T cells was altered, favoring the development of memory precursors that could give rise to central memory T cells.

Since arriving in the Randall/Lund lab, Dr. Allie has shown herself to be a critical thinker and careful scientist. She reads the literature and applies what she has learned to not only her own project, but to all of the projects in the lab. She established a lab journal club within weeks of joining the lab and is working closely with both students and other post-docs in the lab. She asks thoughtful and thought provoking questions in lab meeting and is a very active participant with a clear interest in all of the ongoing projects. Perhaps more importantly, she isn't afraid to ask questions – even during departmental seminars when most trainees are conspicuously silent. Thus, Dr. Allie has exhibited many of the qualities that we look for in trainees who will go on to have successful careers in science. Dr. Allie also quickly established an independent project in Dr. Randall's lab. She continues to maintain her interest in immune memory to viruses but has switched her focus from CD8 T cells to B cells. This is a big shift – both conceptually and technically. Despite the fact B and T cells are both lymphocytes, the methods used to functionally characterize these two populations can be quite distinct, the literature examining these two populations is for the most part non-overlapping and even the scientific meetings attended by scientists who study T cells and B cells are different. Furthermore, the "field" of B cell memory is relatively undeveloped compared to T cell memory. This is due in large part to the fact that it has been exceedingly difficult to characterize virus-specific memory B cells from mice. However, with technical

advances made by the Randall and Lund labs, we are now able to identify these cells using flow cytometry, which now allows for phenotypic, molecular and functional studies of these rare cells. Dr. Allie has capitalized on these new tools and is now combining these tools with the sophisticated mouse models that are available in the lab (including parabiotic animals) to study the development, trafficking and localization of the different subpopulations of virus-specific memory B cells that she has identified.

Dr. Allie is clearly capable of becoming a top-notch scientist as she has the necessary drive, the curiosity of a natural scientist, the creativity to develop new lines of research and confidence in her abilities. Importantly, I think that she has chosen the right scientific environment to further expand these qualities and to set her on the path to a successful career. UAB is an outstanding institution for immunologists and the environment is perfect for trainees at every level. We have a great immunology theme graduate program, an immunology PhD and post-doctoral training grant associated with our Program in Immunology and a weekly immunology seminar program. The facilities, cores and other resources needed for the types of *in vivo* memory studies that Dr. Allie proposes in her grant are available at UAB and are top notch. The immunology faculty includes prominent scientists in T and B cell biology, viral immunology and host-defense. Within the joint lab run by Dr. Randall and Dr. Lund are Assistant Professors, post-doctoral fellows, graduate students and technical support staff. Collectively, these individuals will provide Dr. Allie with technical and intellectual support needed for her to succeed in her project.

Dr. Allie has daily access to both Dr. Randall and Dr. Lund and she can (and does) use this access to seek advice as she pursues her project. However, the expectation in the Randall and Lund labs is that post-doctoral fellows are largely independent. We expect Dr. Allie to identify the questions that she thinks are worth pursuing, to establish the techniques and tools needed to address those questions, to design and perform the experiments, to analyze the data and to ultimately develop a complete story that is publication ready. Individuals come out of our lab as well-trained scientists who can carry a project from its inception to its conclusion. Given the nature of the experimental models that we use, this process can take 3-5 years but when the fellow leaves the lab, they will have one or more publications in high impact, top-ranked journals like *Nature Immunology*, *Immunity* and *J. Experimental Medicine*. Over the years, this lab-based support has facilitated the success of many post-doctoral fellows who have gone on to establish laboratories of their own. Thus, I feel confident that Dr. Allie, with her prior history of success in two different labs, will be able to take full advantage of the opportunities available to her in our lab and will be equally as successful as many of our past students and post-doctoral fellows.

In closing, I can say without reservation that Dr. Allie has already demonstrated her potential to be successful and that, with time, she will become a leader in the field of B cell metabolism and memory development. She has all of the ingredients necessary to become productive independent scientist – she is smart, works hard, has picked a tough but approachable project and has selected a lab where she will receive excellent training and mentoring. In short, all that she is missing from her CV is evidence that she can obtain her own funding for her work. Thus, we have encouraged her to aggressively apply for post-doctoral fellowships. I believe that a F32 fellowship will not only support her current research but will be a key component to the next phase of her career when she begins applying for independent research positions and grants. I give my very best recommendation and I urge you to seriously consider awarding a fellowship to Dr. Allie. I believe that it will help to advance a promising scientist on to an outstanding career in immunologic research.