Notice of Award

*EXPLORATORY/DEVELOPMENT GRANT*
Department of Health and Human Services
National Institutes of Health

**Federal Award Date:** 03/03/2017

NATIONAL INSTITUTE ON AGING

**Grant Number:** 1R21AG054973-01A1
**FAIN:** R21AG054973

**Principal Investigator(s):**
Marcia A Blackman (contact), PHD
William W Reiley, PHD

**Project Title:** An improved mouse model for aging immunology

Mr. Chapin, William
Chief Administrative Officer
154 Algonquin Avenue
Saranac Lake, NY 129832100

**Award e-mailed to:** grants@trudeauinstitute.org

**Period Of Performance:**
**Budget Period:** 03/15/2017 – 02/28/2018
**Project Period:** 03/15/2017 – 02/28/2019

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $297,000 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to TRUDEAU INSTITUTE, INC. in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

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

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute On Aging of the National Institutes of Health under Award Number R21AG054973. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

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

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

Sincerely yours,

Ryan Blakeney
Grants Management Officer
NATIONAL INSTITUTE ON AGING

Additional information follows

## SECTION I – AWARD DATA – 1R21AG054973-01A1

**Award Calculation (U.S. Dollars)**

| | |
|---|---|
| **Federal Direct Costs** | $150,000 |
| **Federal F&A Costs** | $147,000 |
| **Approved Budget** | $297,000 |
| **Total Amount of Federal Funds Obligated (Federal Share)** | $297,000 |
| **TOTAL FEDERAL AWARD AMOUNT** | $297,000 |
| **AMOUNT OF THIS ACTION (FEDERAL SHARE)** | $297,000 |

| SUMMARY TOTALS FOR ALL YEARS | | |
|---|---|---|
| **YR** | **THIS AWARD** | **CUMULATIVE TOTALS** |
| 1 | $297,000 | $297,000 |
| 2 | $247,500 | $247,500 |

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

**Fiscal Information:**

| | |
|---|---|
| **CFDA Name:** | Aging Research |
| **CFDA Number:** | 93.866 |
| **EIN:** | 1141401413A1 |
| **Document Number:** | RAG054973A |
| **PMS Account Type:** | P (Subaccount) |
| **Fiscal Year:** | 2017 |

| IC | CAN | 2017 | 2018 |
|---|---|---|---|
| AG | 8470692 | $297,000 | $247,500 |

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

**NIH Administrative Data:**
**PCC**: 1BIMMRF / **OC**: 414A / **Released**: eRA Commons User 02/27/2017
**Award Processed:** 03/03/2017 01:34:55 AM

## SECTION II – PAYMENT/HOTLINE INFORMATION – 1R21AG054973-01A1

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

## SECTION III – TERMS AND CONDITIONS – 1R21AG054973-01A1

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

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

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

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

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

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

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

This award has been assigned the Federal Award Identification Number (FAIN) R21AG054973. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

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

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

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

**Treatment of Program Income:**
Additional Costs

## SECTION IV – AG Special Terms and Conditions – 1R21AG054973-01A1

This is a Modular Grant Award without direct cost categorical breakdowns issued in accordance with the guidelines published in the NIH Grants Policy Statement. See: http://grants.nih.gov/grants/policy/nihgps/HTML5/section_13/13_modular_applications_and_awards.htm# . Recipients are required to allocate and account for costs related to this award by category within their institutional accounting system in accordance with applicable cost principles.

In keeping with NOT-OD-06-054 (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-06-054.html), as this grant has multiple Principal Investigators (PIs), although the signatures of the PIs are not required on prior approval requests submitted to the agency, the grantee institution must secure and retain the signatures of all of the PIs within their own internal processes.

**STAFF CONTACTS**

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

**Grants Management Specialist**: Mitchell Whitfield
**Email**: whitfieldm@od.nih.gov **Phone**: 301-435-0969 **Fax**: 301-480-2304

**Program Official:** Rebecca A Fuldner
**Email**: fuldnerr@nia.nih.gov **Phone**: (301) 496-6402 **Fax**: (301) 402-0010

**SPREADSHEET SUMMARY**
**GRANT NUMBER:** 1R21AG054973-01A1

**INSTITUTION:** TRUDEAU INSTITUTE, INC.

| Facilities and Administrative Costs | Year 1 | Year 2 |
|---|---|---|
| F&A Cost Rate 1 | 98% | 98% |
| F&A Cost Base 1 | $150,000 | $125,000 |
| F&A Costs 1 | $147,000 | $122,500 |

| | | |
|---|---|---|
| PI: **Blackman, Marcia A** | Title: An improved mouse model for aging immunology | |
| Received: 08/15/2016 | FOA: PA16-161 | Council: 01/2017 |
| Competition ID: FORMS-D | FOA Title: NIH EXPLORATORY/DEVELOPMENTAL RESEARCH GRANT PROGRAM (PARENT R21) | |
| **1 R21 AG054973-01A1** | Dual: AI | Accession Number: 3963024 |
| IPF: 8409401 | Organization: TRUDEAU INSTITUTE, INC. | |
| Former Number: | Department: | |
| IRG/SRG: ASG | AIDS: N | Expedited: N |
| Subtotal Direct Costs<br>(excludes consortium F&A)<br>Year 1: 150,000<br>Year 2: 125,000 | Animals: Y<br>Humans: N<br>Clinical Trial: N<br>Current HS Code: Evaluative Info<br>HESC: N | New Investigator:<br>Early Stage Investigator: |
| | | |
| *Senior/Key Personnel:* | *Organization:* | *Role Category:* |
| Marcia Blackman Ph.D | TRUDEAU INSTITUTE, INC. | PD/PI |
| William Reiley Ph.D | Trudeau Institute | MPI |
| Lawrence Johnson Ph.D | Trudeau Institute | Other (Specify)-Other Significant Contributor |
| HERBERT VIRGIN M.D. | WASHINGTON UNIVERISTY | Other (Specify)-Other Significant Contributor |
| Raymond Welsh Ph.D | University of Massachusetts Medical School | Other (Specify)-Other Significant Contributor |

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

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

| 3. DATE RECEIVED BY STATE | State Application Identifier |
|---|---|
| | |

**1. TYPE OF SUBMISSION***

❍ Pre-application ● Application ❍ Changed/Corrected Application

**4.a. Federal Identifier**
AG054973

**b. Agency Routing Number**

**2. DATE SUBMITTED**
2016-08-15

**Application Identifier**

**c. Previous Grants.gov Tracking Number**

**5. APPLICANT INFORMATION**

**Organizational DUNS*:** 0206589690000

Legal Name*: TRUDEAU INSTITUTE, INC.
Department:
Division:
Street1*: 154 ALGONQUIN AVENUE
Street2:
City*: SARANAC LAKE
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 129832100

Person to be contacted on matters involving this application
Prefix: Mr. First Name*: William Middle Name: Last Name*: Chapin Suffix:
Position/Title: Chief Administrative Officer
Street1*: 154 Algonquin Avenue
Street2:
City*: Saranac Lake
County: Franklin
State*: NY: New York
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 12983-2100
Phone Number*: 518-891-3080 ext. 510 Fax Number: 518-891-5126 Email: wchapin@trudeauinstitute.org

**6. EMPLOYER IDENTIFICATION NUMBER** *(EIN) or (TIN)** 14-1401413

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

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

**8. TYPE OF APPLICATION***

❍ New ● Resubmission
❍ Renewal ❍ Continuation ❍ Revision

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

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

**9. NAME OF FEDERAL AGENCY***
National Institutes of Health

**10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER**
TITLE:

**11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT***
An improved mouse model for aging immunology

**12. PROPOSED PROJECT**
Start Date* 04/01/2017
Ending Date* 03/31/2019

**13. CONGRESSIONAL DISTRICTS OF APPLICANT**
NY-021

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



**14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION**

Prefix: Dr. First Name*: Marcia Middle Name: A Last Name*: Blackman Suffix: Ph.D

Position/Title: Member
Organization Name*: TRUDEAU INSTITUTE, INC.
Department:
Division:
Street1*: 154 ALGONQUIN AVENUE
Street2:
City*: SARANAC LAKE
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 129830000
Phone Number*: (518) 891-3080 Fax Number: (518) 891-5126 Email*: MBLACKMAN@TRUDEAUINSTITUTE.ORG

**15. ESTIMATED PROJECT FUNDING**

| | |
|---|---|
| a. Total Federal Funds Requested* | $544,500.00 |
| b. Total Non-Federal Funds* | $0.00 |
| c. Total Federal & Non-Federal Funds* | $544,500.00 |
| d. Estimated Program Income* | $0.00 |

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

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

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

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

● I agree*

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

**18. SFLLL or OTHER EXPLANATORY DOCUMENTATION** File Name:

**19. AUTHORIZED REPRESENTATIVE**

Prefix: Ms. First Name*: Suzanne Middle Name: M. Last Name*: Chapman Suffix:

Position/Title*: Grants Administrator
Organization Name*: Trudeau Institute
Department:
Division:
Street1*: 154 Algonquin Avenue
Street2:
City*: Saranac Lake
County: Franklin
State*: NY: New York
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 12983-2100
Phone Number*: 518-891-3080 Fax Number: 518-891-5126 Email*: schapman@trudeauinstitute.org

**Signature of Authorized Representative***
Suzanne Chapman

**Date Signed***
08/15/2016

**20. PRE-APPLICATION** File Name:

**21. COVER LETTER ATTACHMENT** File Name:CoverLetter.pdf

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

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

# Project/Performance Site Location(s)

**Project/Performance Site Primary Location**

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

| | |
|---|---|
| Organization Name: | TRUDEAU INSTITUTE, INC. |
| Duns Number: | 0206589690000 |
| Street1*: | 154 ALGONQUIN AVENUE |
| Street2: | |
| City*: | SARANAC LAKE |
| County: | |
| State*: | NY: New York |
| Province: | |
| Country*: | USA: UNITED STATES |
| Zip / Postal Code*: | 129832100 |
| Project/Performance Site Congressional District*: | NY-021 |

File Name

**Additional Location(s)**

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

# RESEARCH & RELATED Other Project Information

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

1.a. If YES to Human Subjects

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

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

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

IRB Approval Date:

Human Subject Assurance Number

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

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ● No

IACUC Approval Date: 04-26-2016

Animal Welfare Assurance Number A3075-01

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

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

4.b. If yes, please explain:

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

4.d. If yes, please explain:

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

5.a. If yes, please explain:

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

6.a. If yes, identify countries:

6.b. Optional Explanation:

| | Filename |
|---|---|
| **7. Project Summary/Abstract*** | Abstract.pdf |
| **8. Project Narrative*** | Relevance.pdf |
| **9. Bibliography & References Cited** | LiteratureCited.pdf |
| **10.Facilities & Other Resources** | Facilities.pdf |
| **11.Equipment** | Equipment.pdf |

Immune function declines with age. In order to extend quality of life, it is critical that we understand mechanisms underlying the age-associated decline in immune function. Humans are riddled throughout life with a variety of acute and chronic infections which trigger the immune system. The accumulating effect of acute and chronic infections throughout the lifespan profoundly impacts the T cell repertoire and immune response of aged individuals. Although the aging mouse model provides a robust experimental model amenable to addressing mechanisms, it is increasingly realized that an important limitation of the mouse model is that mice are typically housed in specific pathogen free conditions. Immune senescence cannot be appropriately modeled in mice in which antigen experience has been deliberately constrained. In addition, optimal immune responses to new infections are thought to be dependent on a diverse repertoire of naïve T cells. With age, the numbers and diversity of naïve T cells decline and the ratio of memory to naïve T cells greatly increases. It has been determined that T cell recognition of antigen/MHC is highly degenerate and T cell responses exhibit extensive and unexpected cross reactivity. We hypothesize that, with the declining numbers of naïve T cells with age, the response to new infections become increasingly dependent on memory cells that accumulated with antigen experience and are fortuitously cross reactive. The first goal of this proposal is to develop a better mouse model for aging by defined exposure early in life to sequential infection with chronic and acute viruses. The second goal of this developmental R21 is to test the hypothesis that sequentially-infected aged mice, by virtue of enhanced antigen experience, will manifest increased diversity in the memory T cell repertoire capable of cross-reacting with new infections. Accomplishing the goals of this *developmental R21* will be an important advance for aging research. Studies in antigen-experienced aged mice will benefit understanding the impact of antigen experience on immunity and senescence in elderly humans and has important implications for vaccination strategies for the elderly, supporting the concept that vaccines in young and middle age are important for maintaining immunity in later life.

The elderly population is increasing and people are living longer. The ability to respond to infection and vaccination declines with age. It is important to discover underlying mechanisms so therapeutic interventions and better vaccines can be developed. The mouse model has been important in pre-clinical experimentation, but the model has drawbacks, one of which is that experimental mice are maintained in specific pathogen free conditions whereas humans are exposed to a variety of pathogens throughout life. The proposed studies are directed toward developing a mouse model of aging in which mice are sequentially infected with pathogens to generate a more relevant model for human aging.

## FACILITIES AND OTHER RESOURCES

Laboratory: The Principal Investigators have lab space fully equipped for the work planned in this application. Equipment includes: PCR thermocycler, microscopes, incubators, centrifuges, refrigerators, freezers, laminar-flow hoods, biohazard hoods, ELISA reader, various small benchtop equipment and animal handling equipment. Shared lab space includes a dark room, cold room, radioisotope room and a flow cytometry facility, which is managed by a technical specialist (for list of equipment, see Major Equipment section). Specialized biosafety level 2 and 3 laboratories are also available.

Animal: Experimental animal facilities at the Trudeau Institute (12,190 sq. ft.) are located within the main research building and include housing for uninfected mice housed in individually ventilated cage racks (ABSL-1) and infected mice housed in negatively pressurized ventilated cage racks (ABSL-2 and ABSL-3). This space includes a state of the art experimental animal wing (7,000 sq. feet) attached to the main building which was completed in 2010 that has facilities for housing mice under ABSL-2 and ABSL-3 (M.tb-infected mice) conditions. Procedure rooms are located conveniently within or adjacent to animal holding rooms. Also, one suite within this newer facility is a CDC registered Select Agent Suite. All caging and supplies utilized in the experimental animal facilities are autoclave-sterilized before and after animal use. All animal handling is done within certified biological safety cabinets and strict facilities traffic patterns are enforced to maintain biosecurity. Quarantine space is available for the importation of mice from non-approved vendors. Additionally, quality control is ongoing and includes comprehensive health screening with dirty bedding sentinel mice on every animal rack, tested at least quarterly. The animal facilities have been fully AAALAC accredited for over 35 years and were most recently inspected in March, 2016. The facilities are managed by a Certified Manager of Animal Resources (CMAR) full-time manager and 80% of animal care staff are certified at the AALAS Assistant Laboratory Animal Technician level or higher. Veterinary care is provided on a daily basis by animal health technicians with >30 years' experience and by an ACLAM board certified part time veterinarian, who is available 24/7 for consultation. A back-up veterinarian is available as well.

Computer: The Principal Investigators' offices and laboratories are equipped with computers necessary to complete these studies. Flat bed scanners, printers and a network file system are also available. Communication with the scientific community is available via direct access to the internet and e-mail.

Office: PI's are allocated private office space. Post-doctoral fellows and research assistants have shared office space.

Other: Library: The Hirsch Memorial Library contains 40,000 volumes and subscribes to 40 of the most applicable immunology and related journals with access to approximately 85% of those online. Inter-library loans are acquired by the Librarian-usually within 48 hours. An internal intranet provides on-line access to journal holdings. Internet access to the National Library of Medicine's Medline database is available Institute-wide from NCBI's web site.

MBCF: The molecular biology core facility supports research in the field of immunology at the Trudeau Institute by providing molecular biology oriented services, products and consultation in such areas as automated DNA Sequencing, Taqman real-time PCR, Spectratyping and production of recombinant proteins. In addition, the core generates MHC class I and class II tetrameric reagents for labs which are charged back at cost.

IMCF: The imaging and microscopy core facility supports the Institute's research effort by providing services and consultation in such areas as image preparation, advanced imaging manipulation, graphics and publication material preparation, microscopy (confocal, bright field and fluorescence), as well as histology. Services are charged back at cost.

## MAJOR EQUIPMENT

Shared instrumentation includes three FACS Calibur dual-laser analytic flow cytometers capable of 4-color flow cytomtery, a BD Influx/4 laser cell sorter capable of 8-color flow cytometry based sorting of live lymphocytes isolated from infected mice, two BD FACS Canto cytometers and a BD LSR analytic flow cytometer. For microscopy**,** we have a Zeiss Axiophot II upright microscope with brightfield and fluorescent capabilities and a Zeiss Axiovert 200M inverted microscope with DIC optics, fluorescent capabilities, and environmental chamber for live cell imaging. This scope is also equipped with a Zeiss Apotome that allows for 3D reconstruction of tissues or cells and an Eppendorf MicroDissector. We also have a Leica SP5 TCS Laser Scanning Confocal Microscope with AOBS and nine laser excitation lines. There are five spectral detectors for simultaneous detection of up to five fluorochromes from 400-800nm. This microscope also has an environmental chamber for live cell imaging and a scanning stage with mark/find and tile scanning capabilities. Software includes modules for 3D Visualization, Colocalization, Deconvolution, as well as FRET and FRAP Wizards. The software is also loaded on an offline workstation for convenience.

We also have available a Buxco Plethysmograph (pulmonary measurement system for mice), ultracentrifuges, refractometer, cryostat, Vibrotome, analytical balances, biohazard hoods, centrifuges (benchtop, free-standing, refrigerated, microfuges), electrophoresis equipment and power supplies, HPLC apparatus, incubators (humidified, CO2), -80 and -150 freezer storage, luminometer, ELISPOT reader, ELISA plate readers, microplate fluorometer, PCR apparatus (standard and real-time), pH meters, Gene gun, photomicroscopes, spectrophotometers, water purification units, Luminex multiplex cytokine analysis system, Middlebrook airborne infection apparatus, Bio-Rad phosphoimager, ABI Prism 310 capillary performance optimized genetic analyzer.

Facilities are available for $^{137}$Cesium irradiation of animals and cells. Radiation monitoring equipment is available for measuring commonly used isotopes, including $^{3}$H, $^{14}$C, $^{125}$I, $^{32}$P and $^{51}$Cr, including a Trilux beta/gamma counter. The Institute maintains a 1,500 sq. ft workshop, staffed by mechanics and electricians for the internal manufacturing, and maintenance of the Institute and its equipment. All investigators and laboratories are housed in a single building.

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

# RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Marcia Middle Name A Last Name*: Blackman Suffix: Ph.D

| | |
|---|---|
| Position/Title*: | Member |
| Organization Name*: | TRUDEAU INSTITUTE, INC. |
| Department: | |
| Division: | |
| Street1*: | 154 ALGONQUIN AVENUE |
| Street2: | |
| City*: | SARANAC LAKE |
| County: | |
| State*: | NY: New York |
| Province: | |
| Country*: | USA: UNITED STATES |
| Zip / Postal Code*: | 129830000 |

Phone Number*: (518) 891-3080 Fax Number: (518) 891-5126 E-Mail*: MBLACKMAN@TRUDEAUINSTITUTE.ORG

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

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

Degree Type: Ph.D. Degree Year: 1985

| | File Name |
|---|---|
| Attach Biographical Sketch*: | BlackmanBiosketch.pdf |
| Attach Current & Pending Support: | |

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: William Middle Name W Last Name*: Reiley Suffix: Ph.D

Position/Title*: Assistant Member
Organization Name*: Trudeau Institute
Department:
Division:
Street1*: 154 Algonquin Ave
Street2:
City*: Saranac Lake
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 129832100

Phone Number*: 518-891-3080 Fax Number: 518-891-5126 E-Mail*: wreiley@trudeauinstitute.org

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

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

Degree Type: PHD,BS Degree Year: 2005

File Name

Attach Biographical Sketch*: ReileyBio.pdf

Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Lawrence Middle Name L. Last Name*: Johnson Suffix: Ph.D

Position/Title*: Distinguished Professor Emeritus
Organization Name*: Trudeau Institute
Department:
Division:
Street1*: 154 Algonquin Ave.
Street2:
City*: Saranac Lake
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 129832100

Phone Number*: 518 891 3084 Fax Number: E-Mail*: ljohnson@trudeauinstitute.org

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

Project Role*: Other (Specify) Other Project Role Category: Other Significant Contributor

Degree Type: PHD,MA,BA Degree Year: 1980

File Name

Attach Biographical Sketch*: JohnsonBio.pdf

Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: HERBERT Middle Name W Last Name*: VIRGIN Suffix: M.D.

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Department: PATHOLOGY & IMMUNOLOGY
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Project Role*: Other (Specify) Other Project Role Category: Other Significant Contributor

Degree Type: MD,PHD Degree Year: 1985

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

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Raymond Middle Name M Last Name*: Welsh Suffix: Ph.D

Position/Title*: Professor
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City*: Worcester
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 016052324

Phone Number*: 508-856-5819 Fax Number: E-Mail*: Raymond.Welsh@umassmed.edu

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Project Role*: Other (Specify) Other Project Role Category: Other Significant Contributor

Degree Type: PHD Degree Year: 1972

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

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

## BIOGRAPHICAL SKETCH

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

NAME: Blackman, Marcia A.

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

POSITION TITLE: Member

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

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
| Upsala College, East Orange, NJ | B.S. | 05/1969 | Biology |
| University of Maryland, College Park, MD | M.S. | 05/1972 | Microbiology |
| University of California, Berkeley, CA | Ph.D. | 05/1985 | Immunology |
| National Jewish Center for Immunology and Respiratory Medicine and Howard Hughes Medical Institute, Denver, CO | Postdoctoral | 08/1990 | Immunology |

### A. Personal Statement

I am a viral immunologist with 25 years' experience running an NIH-funded basic research laboratory, studying T cell tolerance and thymic selection, T cell recognition of Ag/MHC and superantigens, T cell repertoire, and viral immunity. Recently my laboratory has focused on the impact of aging on T cell immunity and repertoire, using the mouse influenza virus model. The mouse model has been useful for research on the immune impacts of aging, but there are acknowledged shortcomings in terms of a model for human aging. A major problem, which we hope to address in the current R21 proposal, concerns the non-physiological specific pathogen free (SPF) husbandry of aging mice, which makes them not representative of aging humans who are exposed to many pathogens throughout their life. We propose to sequentially infect mice with chronic and acute pathogens, including γ-herpesvirus-68, MCMV, Sendai virus, and a persistent intestinal helminth, *Heligmosomoides polygyrus* (*H. polygyrus*). We will examine transcriptional profiles of sequentially-infected and SPF-housed aged mice. Dr. Skip Virgin will serve as a consult for the sequential infections and the transcriptional profiles, which will be carried out with the Gene Expression and Genomics unit of the Laboratory of Genetics of the National Institutes of Aging. Dr. Laura Haynes will provide PBL samples from young and aged humans for transcriptional profiling. We will also determine the T cell and inflammatory phenotypes of the sequentially-infected and SPF-housed aged mice. We will challenge the mice after aging with influenza virus and determine the impact of sequential infections on the repertoire of cross-reactive memory T cells that respond to influenza virus epitopes. I have direct experience with the mouse influenza virus, γ-herpesvirus and Sendai virus models. Dr. Ray Welsh will serve as a consultant to offer expertise in infection with MCMV. *H polygyrus* is a widely-used pathogen at Trudeau Institute. Dr. Larry Johnson will provide statistical expertise. This proposal is being submitted as an MPI with a young Trudeau Institute investigator, William Reiley. Bill brings an expansive knowledge and experience with multiple mouse infection models, including *H polygyrus*, which he has acquired in the role of Senior Contract Research Organization Program Manager at Trudeau Institute. As head of the Trudeau Institute Molecular Biology core, he generates tetramers and has experience with tetramer pull-down experiments. My background in viral immunity and aging research coupled with Dr. Reiley's experience with a variety of infectious disease models and our location at Trudeau Institute which specializes in infectious disease research in mouse models combine to make a powerful experimental team. All the infection models proposed are currently or have recently been in use at Trudeau Institute. In addition, I currently maintain an aging mouse colony. We feel that our experience and collective expertise position us to successfully carry out the experiments proposed in the current R21 and achieve our goal of developing a better mouse model for studying the impact of aging on immune function.

**B. Professional Positions:**

1992-1998 Assistant Professor, Department of Pathology, University of Tennessee, Memphis, TN
1990-1996 Assistant Member, Dept. of Immunology, St. Jude Children's Research Hospital, Memphis, TN
1996-1999 Associate Member, Dept. of Immunology, St. Jude Children's Research Hospital, Memphis, TN
1998-1999 Associate Professor, Department of Pathology, University of Tennessee, Memphis, TN
1999-2003 Associate Member, Trudeau Institute, Inc., Saranac Lake, NY
2000-present Adjunct Associate Professor, Albany Medical College, Albany, NY
2002-2005 Adjunct Associate Professor, University of Vermont College of Medicine, Burlington, VT
2003-present Member, Trudeau Institute, Inc., Saranac Lake, NY
2005-present Adjunct Professor, University of Vermont College of Medicine, Burlington, VT
2012-present Adjunct Professor, National Jewish Health, Denver, CO
2015-present Adjunct Professor, Clarkson University, Potsdam, NY

**Memberships and Activities:**

1996-2001 Associate Editor, Journal of Immunology
1996-2001 Member, NIH Immunological Sciences Study Section
2004-2006 Waksman Foundation for Microbiology Lecturer
2004-2008 Member, NIH Virology B Study Section
2006-2008 Member AAI Programming Committee
2006-2015 Faculty of 1000
2009-2011 AAI Nominating Committee (elected position)
2009-present Ad hoc, mail reviewer and internet assisted reviewer for ASG study section, ZAG1 ZIJM1, SBIR "Translational Research in Aging," ZRG1 IDM-U (03) M, "Viral Pathogenesis and Aging," ZRG1 IDM-M 02 IAM "Virology" study section (chairman), ZRG1 IDM-S Topics in Infectious Disease and Microbiology, IDM M-02 Virus study section, ZRG1 AARR-K(03), and K08 reviews.
2016-present Member, NIH AITC study section,

**C. Contribution to Science**

1. **Impact of aging on T cell repertoire, T cell function and vaccination**. The ability of the immune system to respond to infections and vaccination declines with age. We are studying the impact of aging on the repertoire of T cells and T cell effector function in a mouse influenza virus model. We showed that the decline in CD8 T cell repertoire diversity in aged mice resulted in impaired immunity to influenza virus and in some cases caused "holes" in the repertoire. These observations prompted the hypothesis that in the absence of a diverse naïve T cell repertoire, responses in the elderly to newly encountered antigens are dominated by cross-reactive memory CD8 T cells. We further predicted that the responses would be stochastic in individuals and of lower avidity compared to naïve responses. However, unexpectedly, we found that the response to *de novo* influenza infection of SPF aged mice was mediated almost exclusively by virtual memory cells, which are dramatically increased in aged mice. We are currently testing the response and function of cross-reactive "true memory" and virtual memory CD8 T cells in aged mice in adoptive transfer studies. In addition, it is well known that the elderly are impaired in their ability to be successfully vaccinated, and they are highly susceptible to influenza virus infections, Thus it is important to work toward developing better vaccines and adjuvants for the elderly. It has been shown that CD8 T cell responses are better immune correlates of successful vaccination against influenza in the elderly than antibody, so we are studying the efficacy of vaccination of young and aged mice with recombinant influenza nucleoprotein (rNP) in association with a new TLR4 agonist adjuvant developed by the Infectious Disease Research Institute (IDRI). We are examining the response of CD8 and CD4 T cells to rNP and the effectiveness of the response on survival and viral clearance after challenge with a heterosubtypic influenza viral challenge. These studies are in progress.

   Woodland, D.L. and **Blackman, M.A**. 2006. Immunity and age: Living in the past? Trends Immunol. 27: 303-307.

   Yager, EJ, Ahmed, M, Lanzer, K, Randall, TD, Woodland, DL and **Blackman, MA**. 2008. Age-associated decline in T cell repertoire diversity leads to holes in the repertoire and impaired immunity to influenza virus. *J. Exp. Med.* 205: 711-723.

Ahmed, M., Lanzer, K. G., Yager, E. J., Adams, P. S., Johnson, L. L., and **Blackman, M. A.** 2009. Clonal expansion and loss of receptor diversity in the naïve CD8 T cell repertoire of aged mice. *J. Immunol.* 182:784-792.

Lanzer, KG, Johnson, LL, Woodland, DL and **Blackman, MA**. 2014. Impact of ageing on the response and repertoire of influenza virus-specific CD4 T cells. *Immunity & Ageing* 11:9. PMCID: PMC4082670.

2. **T cell memory and recall responses to respiratory viruses.** We have studied the generation, maintenance, phenotype and anatomical distribution of CD8 memory T cells. As it has been shown that memory generated in young mice stays good into old age, but memory generated in aged mice is poor, we are examining the development of functional memory and protective recall responses in young and aged mice. The goal is to determine the mechanisms underlying the poor development of memory and the impaired recall response in aged mice with the goal of developing strategies to improve the development and maintenance of memory following infection and vaccination of the elderly.

Brincks, EL, Roberts, AD, Cookenham, T, Sell, S, Kohlmeier, JE, **Blackman, MA**, Woodland, DL. 2013. Antigen-specific memory $T_{reg}$ control memory responses to influenza virus infection. *J. Immunol*, 190: 3438-3446. PMCID: PMC3608733.

Kohlmeier, JE, Reiley, WW, Perona-Wright, G, Freeman, ML, Yager, EJ, Conner, LM, Brincks, EL, Cookenham, T, Roberts, AD, Burkum, CE, Winslow, GM, **Blackman, MA**, Mohrs, M and Woodland, DL. 2011. Inflammatory chemokine receptors regulate CD8+ T cell contraction and memory generation following infection. J. Exp. Med. 208:1621-1634. PMCID: PMC3149221

Ely, KH, Roberts, AD, Kohlmeier, JE, **Blackman, MA**, Woodland, DL. 2006. Aging and CD8+ T cell immunity to respiratory virus infections. Exp. Gerontol. 42, 427-431, 2007.

Hikono, H., Kohlmeier, J.E., Ely, K. H., Scott, I., Roberts, A. D., **Blackman, M. A**. and Woodland, D. L. 2006. T cell memory and recall responses to respiratory virus infections. Immunol Rev. 211:119-132.

3. **The impact of aging on immunity to persistent viruses.** The oncogenic γ-herpesviruses establish life-long latent infections in their hosts and constant immune surveillance is required to prevent reactivation of latency and the development of malignancies. As immunity declines with aging, we measured parameters of cellular and humoral immunity over time. Unexpectedly, the results showed that aging had differential impact on cellular and humoral immunity but there was no enhanced viral reactivation. We are continuing to study immune control of γ-herpesvirus latency with the goal of developing therapeutic treatments or vaccines to prevent the reactivation of latency.

Yager, E. J., Kim, I. J., Freeman, M. L., Lanzer, K. G., Burkum, C. E., Cookenham, T., Woodland, D. L. and **Blackman, M. A**. 2010. Differential impacts of aging on cellular and humoral immunity to a persistent murine γ-herpesvirus, Immunity and Ageing 7 (1):3. PMCID- PMC2843645.

4. **Vaccination to prevent γ-herpesvirus latency**. We have tried two approaches to prevent the establishment of latency by vaccination. First, we showed that peptide vaccination with CD8 epitopes reduced the level of lytic virus but failed to have an impact on long-term latency. Second, in collaboration with Ren Sun and Ting-Ting Wu at UCLA, we carried out protection studies with a recombinant replication deficient virus (AC-RTA), in which a viral transcription activator (RTA) is over-expressed and there is a deficiency in several genes required for latency and reactivation. We showed that infection with the latency-deficient virus prevented infection following subsequent challenge with wild type virus. Sterilizing immunity required both T cells and antibody. In the absence of antibody-mediated control, the AC-RTA virus amplified in the brain and resulted in fatality, suggesting important considerations in the development of vaccination strategies based on live-attenuated viruses.

Liu, L, Usherwood, EJ, **Blackman, MA**, and Woodland, DL. 1999. T cell vaccination alters the course of MHV-68 infection and the establishment of viral latency in mice, *J. Virol*. 73: 9849-9857.

Jia, Q, Freeman, ML, Yager, EJ, McHardy, I, Tong, L, Martinez-Guzman, D, Rickabaugh, T, Hwang, S, **Blackman, MA**, Sun, R, Wu, T-T. 2010. Induction of protective immunity against murine gammaherpesvirus-68 infection in the absence of viral latency. *J. Virol.* 84:2453-2465. PMC2820913

Freeman, ML, Burkum, CE, Yager, EJ, Woodland, DL,Sun, R, Wu, T-T, and **Blackman, MA**. 2011. Importance of antibody in virus infection and vaccine-mediated protection by a latency-deficient recombinant murine gammaherpesvirus-68, J. Immunol. 188: 1049-1056. PMCID: PMC3262927

5. **Immune control of γ-herpesvirus latency and reactivation.** We showed that γHV68 latency in the spleen was established in three types of MHC class II-positive antigen presenting cells- B cells, macrophages and dendritic cells. We have also developed an *in vivo* reactivation model which allows us to study immune control of viral reactivation. We found that T cell depletion allowed viral reactivation as measured by increased viral loads, whereas full-blown recrudescence with infectious lytic virus and enhanced transfer of virus to new cells only occurred in the absence of antibody. In collaboration with Alex Sette at La Jolla Institute for Allergy and immunology, we identified 33 (22 not previously identified) CD8 and 17 CD4 T cell epitopes, including a latency-specific CD4 T cell epitope. Using intracellular cytokine secretion assays and tetramers, we have monitored their differential expression during lytic infection, latency and reactivation. Our overall goal is to understand immune control of latency so that we can develop therapeutic strategies and vaccines to prevent reactivation of these oncogenic viruses.

   Flaño, E, Kim, I-J, Woodland, DL, and **Blackman, MA**. 2002. γ-herpesvirus latency is preferentially maintained in splenic germinal center and memory B cells. *J. Exp. Med*. 196:1363-1372.

   Freeman, ML, Burkum, CE, Wu, T-T, Sun, R, Woodland, DL, and **Blackman, MA.** 2011. Gammaherpesvirus reactivation differentially stimulates epitope-specific CD8 T cell responses, J. Immunol. 188:3812-3819. PMCID: PMC3324632

   Freeman, ML, Lanzer, KG, Cookenham, T, Peters, B, Sidney, J, Wu, T-T, Sun, R, Woodland, DL, Sette, A, and **Blackman, MA**. 2010. Two kinetic patterns of epitope-specific CD8 T cell responses following murine gammaherpesvirus-68 infection. *J. Virol*. 84:2881-2892. PMC2826075

   Freeman, ML, Roberts, AD, Burkum, CE, Woodland, DL, **Blackman, MA.** 2014. Promotion of a subdominant CD8 T cell response during murine γ-herpesvirus-68 infection in the absence of CD4 T cell help. *J Virol.* 88: 7862-7869. PMCID: PMC4097778.

   Freeman, ML, Burkum, CE, Cookenham, T, Roberts, AD, Lanzer, KG, Huston, GE, Jensen, MK, Sidney, J, Peters, B, Kohlmeier, JE, Woodland, DL, van Dyk, LF, Sette, A and **Blackman, MA**. 2014. CD4 T cells specific for a latency-associated γ-herpesvirus epitope are polyfunctional and cytotoxic, *J. Immunol* 193: 5827–5834. PMCID:PMC4301266.

Complete List of Published Work in MyBibliography

**http://www.ncbi.nlm.nih.gov/sites/myncbi/marcia.blackman.1/bibliography/40486726/public/?sort=date&direction=descending**

D. Research Support

**Active**

5P01AG021600-12 (Haynes) 12/2/02-5/31/17
NIA
Program Title: “Aging and immunity to infections" (Haynes)
Project 4 Title: "Impact of aging on the T cell repertoire and cellular immunity to influenza virus" (Blackman)
In the context of other projects in the program, the studies in project 4 will address mechanisms underlying the age-associated decline in cellular immunity which is essential for the goal of designing better therapies and vaccines for the elderly.
Role: PI of project 4

**Completed**

R01AG039485 (Blackman) 8/1/11-5/31/16
NIA
Aging, T cell repertoire, and cellular immunity to influenza virus
The major goal of this project is to address the role of aged CD4 T cells in the defective generation of CD8 memory in aged mice.
Role: PI

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

## BIOGRAPHICAL SKETCH

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

NAME: Reiley, William W.

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

POSITION TITLE: Assistant Member

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

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
| Rochester Institute of Technology, Rochester, NY | B.S. | 05/2000 | Biotechnology |
| Pennsylvania State University, Hershey Medical Center, Hershey, PA | Ph.D. | 07/2005 | Cell & Molecular Biology |
| Pennsylvania State University, Hershey Medical Center, Hershey, PA | Postdoctoral | 08/2006 | Microbiology & Immunology |
| Trudeau Institute, Saranac Lake, NY | Postdoctoral | 08/2006 | Immunology |

**A. Personal Statement**

I am an immunologist starting the process of running my own NIH-funded research laboratory. The major objective of my research is to define how T cell immunity, specifically CD4 T cell, is generated and maintained during acute and persistent infections such as influenza, herpes virus and *Mycobacterium tuberculosis* (Mtb) to name a few. In addition to this program, for the past three years I have been running the Contract Research Organization at Trudeau Institute. This organization provides our Industrial and Academic partners the ability to examine novel therapeutic compounds and vaccines against 20 different pathogens in a mouse model. With respect to the current proposal, my expertise with the growth and infection of mice with a variety of pathogens, along with the generation of tetramer reagents and my experience with tetramer pull-down experiments, will be directly applicable to the proposed studies.

A majority of studies on mice have predominantly characterized the effects that a pathogen imparts on the immune system under specific pathogen free (SPF) conditions. However, such models do not take into account the complication that previous infections might have on the immune system. While there exist a tractable aging model in mice, which has provided many relevant findings for aging research, these studies have been exclusively carried out under SPF conditions. Our goal will allow for us to determine how the consequences of multiple infections over the course of a mouse life impacts immune function of aged mice. These proposed studies will address what cannot be performed in humans and help to generate a better aging mouse model. The present proposal is being submitted as an MPI grant with Dr. Blackman. The combined expertise of my research and contract research experience with Dr. Blackman's multiple years of expertise studying viral immunity will allow for us to push the field forward and to address important gaps in aging research.

**B. Professional Positions:**

2010 – 2013 Research Scientist at Trudeau Institute, Saranac Lake, NY
2013 – Present Senior Contract Research Organization Program Manager at Trudeau Institute, Saranac Lake, NY
2013 – Present Assistant Member, Trudeau Institute, Inc., Saranac Lake, NY
2014 – Present Adjunct Assistant Professor, University of Vermont College of Medicine, Burlington, VT
2014 – Present Adjunct Professor, Clarkson University, Potsdam, NY

## C. Contribution to Science

1. Was the first to demonstrate the tumor suppressor CYLD, a deubiquitinating enzyme, is regulated by the IκB kinase. I went on to demonstrate that this regulation, through phosphorylation as specific serine residues, blocks its suppressive ubiquitination of TRAF2 and TRAF6 thereby allowing for activation of downstream kinases such as the JNK pathway.
   a. **Reiley WW**, Zhang M, Sun SC. 2004. Negative regulation of JNK signaling by the tumor suppressor CYLD. *J Biol Chem* 279(53): 55161-7
   b. **Reiley WW**, Zhang M, Wu X, Granger E, Sun SC. 2005. Regulation of the deubiquitinating enzyme CYLD by IkappaB kinase gamma-dependent phosphorylation. *Mol Cell Biol* 25(10): 3886-95

2. Was the first to describe the pleotropic roles the deubiquitinating enzyme CYLD *in vivo*. I accomplished the characterization of this protein and its *in vivo* phenotypes through the genetic deletion of the gene in embryonic stem cells and subsequent generation of the CYLD KO mouse. The phenotype of the genetic deletion of this protein ranged from immunological defects, altered T and B cell development, to osteoclast development, spermatogenesis and numerous signal transduction defects within many cell types.
   a. **Reiley WW**, Zhang M, Jin W, Losiewicz M, Donohue KB, Norbury CC, and Sun SC. 2006. Regulation of T cell development by the deubiquitinating enzyme CYLD. *Nat Immunol* 7(4): 411-7
   b. Jin W, **Reiley WW**, Lee AJ, Wright A, Wu X, Zhang M, and Sun SC. 2007. Deubiquitinating enzyme CYLD regulates the peripheral development and naive phenotype maintenance of B cells. *J Biol Chem* 282(21): 15884-93
   c. **Reiley WW**, Jin W, Lee AJ, Wright A, Wu X, Tewalt EF, Leonard TO, Norbury CC, Fitzpatrick L, Zhang M, and Sun SC. 2007. Deubiquitinating enzyme CYLD negatively regulates the ubiquitin-dependent kinase Tak1 and prevents abnormal T cell responses. *J Exp Med* 204(6): 1475-85
   d. Wright A, **Reiley WW**, Chang M, Jin W, Lee AJ, Zhang M, and Sun SC. 2007. Regulation of early wave of germ cell apoptosis and spermatogenesis by deubiquitinating enzyme CYLD. *Dev Cell* 13(5): 705-16
   e. Jin W, Chang M, Paul EM, Babu G, Lee AJ, **Reiley W**. Wright A, Zhang M, You J, and Sun SC. 2008. Deubiquitinating enzyme CYLD negatively regulates RANK signaling and osteoclastogenesis in mice. *J Clin Invest* 118(5): 1858-66

3. We demonstrated for the first time that T-bet through the induction of IL-27 regulates the ability of CD8 T cells to produce INFγ rather than IFNγR signaling after infection with various viral, parasitical, and bacterial infections.
   a. Mayer KD, Mohrs K, **Reiley W**, Wittmer S, Kohlmeier JE, Pearl JE, Cooper AM, Johnson LL, Woodland DL, and Mohrs M. 2008. Cutting edge: T-bet and IL-27R are critical for in vivo IFN-gamma production by CD8 T cells during infection. *J Immunol* 180(2): 693-7

4. Published the analysis for the roles of different chemokines in regulating antigen-specific CD4 and CD8 T cell responses during both acute and chronic respiratory infections.
   a. Kohlmeier JE, **Reiley WW**, Perona-Wright G, Freeman ML, Yager EJ, Connor LM, Brincks EL, Cookenham T, Roberts AD, Burkum CE, Sell S, Winslow GM, Blackman MA, Mohrs M, Woodland DL. 2011. Inflammatory chemokine receptors regulate $CD8^+$ T cell contraction and memory generation following infection. *J Exp Med* 208(8): 1621-34

5. I showed that during *Mycobacterium tuberculosis* (Mtb) infection multiple mechanisms are utilized to maintain the antigen-specific CD4 T cell responses. My data demonstrated that during Mtb infection naïve CD4 T cell only first detect antigen in the mediastinal lymph node 10 days after infection. After the initiation of the CD4 T cell response the effector response develops normally but distinct functions of antigen-specific CD4 T arise. I demonstrated that the antigen specific response are maintained by the highly proliferative population of PD-1 expressing cells with only minimal contributions from recent thymic emigrants. Which is in part due to the decreased antigen levels that naïve and effector T cells can access during the chronic infection.
   a. **Reiley WW**, Calayag MD, Wittmer ST, Huntington JL, Pearl JE, Fountain JJ, Martino CA, Roberts AD, Cooper AM, Winslow GM, Woodland. 2008. ESAT-6-specific CD4 T cell responses to aerosol Mycobacterium tuberculosis infection are initiated in the mediastinal lymph nodes. *Proc Natl Acad Sci U S A* 105(31): 10961-6
   b. Winslow GM, Cooper A, **Reiley W**, Chatterjee M, Woodland DL. 2008. Early T-cell responses in tuberculosis immunity. *Immunol Rev* 225: 284-99

c. Khader SA, Rangel-Moreno J, Fountain JJ, Martino CA, **Reiley WW**, Pearl JE, Winslow GM, Woodland DL, Randall TD, Cooper AM. 2009. In a murine tuberculosis model, the absence of homeostatic chemokines delays granuloma formation and protective immunity. *J Immunol* 183(12): 8004-14
d. **Reiley WW**, Shafiani S, Wittmer ST, Tucker-Heard G, Moon JJ, Jenkins MK, Urdahl KB, Winslow GM, Woodand DL. 2010. Distinct functions of antigen-specific CD4 T cells during murine *Mycobacterium tuberculosis* infection. *Proc Natl Acad Sci* U S A 107(45): 19408-13
e. **Reiley WW**, Wittmer ST, Ryan LM, Eaton SM, Haynes L, Winslow GM, and Woodland DL. 2012. Maintenance of peripheral T cell responses during *Mycobacterium tuberculosis* infection. *J Immunol* 189 (9): 4451-4458

**Complete List of Published Work in MyBibilography**

http://www.ncbi.nlm.nih.gov/sites/myncbi/william.reiley.1/bibliograpahy/48448813/public/?sort=date&direction=descending

**D. Research Support**

None

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

## BIOGRAPHICAL SKETCH

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

NAME: Lawrence L. Johnson

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

POSITION TITLE: Distinguished Professor Emeritus

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

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
| University of Maine, Orono, ME | B.A. | 06/1964 | Zoology |
| University of Maine, Orono, ME | M.A. | 06/1973 | Mathematics |
| University of Maine, Orono, ME | Ph.D. | 12/1980 | Zoology |
| University of Wisconsin, Madison, WI | Postdoc | 1980-1984 | |

**A. Personal Statement**. I believe I am well-suited for the role of statistical consultant for Dr. Marcia Blackman's R21 proposal. I have worked closely with Dr. Blackman at the Trudeau Institute for more than a decade and been a co-author with her and many others on publications during my 31 years at the Trudeau Institute. I currently serve on the Administrative Core for an NIH Program Project Grant to provide statistical advice and analysis to all members of that grant including Dr. Blackman. I am formally trained in mathematics and have provided analytical advice to members of the faculty and postdoctoral staff of the Trudeau institute for many years. I periodically give lectures to Trudeau Institute postdocs and staff on problems and pitfalls to avoid in the statistical analysis of experimental data and the importance of power considerations in experimental design.

**B. Professional Experience**

1969-1976 Laboratory Technician, Dept. An. Vet. Sci., University of Maine, Orono, ME
1976-1980 Graduate Student, Donald W. Bailey, Thesis Advisor, The Jackson Laboratory, Bar Harbor, ME.
1980-1983 Postdoctoral Trainee, Laboratory of William F. Dove, Dept. of Oncology and Genetics (McArdle Laboratory), University of Wisconsin-Madison
1983-1984 Lecturer in Medical Genetics, Dept. of Genetics, University of Wisconsin-Madison
1984-1989 Assistant Member, Trudeau Institute, Saranac Lake, NY
1986-2001 Chairperson, Trudeau Institute Animal Care and Use Committee
1990-2000 Associate Member, Trudeau Institute, Saranac Lake, NY
1998-present Adjunct Associate Professor, Albany Medical College, Albany, NY
2000-2015 Member, Trudeau Institute, Saranac Lake, NY
2005-present Adjunct Associate Professor, University of Vermont Medical School
2006-2015 Faculty Advisor to Trudeau Institute Mouse Operations
2008-2015 Chairperson Animal Operations Committee
2011-2015 Chief Operating Officer, Trudeau Institute, Saranac Lake, NY
2013-2015 Vice President, Trudeau Institute, Saranac Lake, NY
2015-present Member Trudeau Institute Board of Trustees
2015-present Distinguished Professor Emeritus, Trudeau Institute

**C. Contribution to Science**.

1. Transplantation Immunology and Genetics. During my graduate studies with Dr. Donald Bailey at the Jackson Laboratory, my postdoctoral studies with Dr. William Dove at the University of Wisconsin, and my first years as a faculty member at the Trudeau Institute, I studied aspects of transplantation immunobiology – especially the role of non-major histocompatibility antigens. My work focused on the distribution on those antigens and their interactions in transplantation. I was among the first to demonstrate that the context in which the immune system encounters antigens can profoundly influence the immunological response to them.

Johnson, L.L. (1979) Genetic influences other than H-2 on the rejection of male skin by female hosts. Immunogenetics 8:373-376.

Johnson, L.L., Bailey, D.W., and Mobraaten, L.E. (1980) Genetics of histocompatibility in mice. II. Survey for interactions between minor (non-H-2) antigens by skin grafting. Immunogenetics 11:363-372.

Johnson, L.L. (1981) At how many histocompatibility loci do congenic mouse strains differ? Probability estimates and some implications. J. Hered. 72:27-3 1.

Johnson, L.L. (1988) Properties of iv-infused donor cells that prolong the survival of H-Y-disparate skin grafts. Transplantation 46:167-170.

VanderVegt, F.P. and Johnson, L.L. (1993) Induction of long term H-Y-specific tolerance in female mice depleted of CD4+ or CD8+ T cells. J. Exp. Med. 177:1587-1592.

2. Immunity to *Toxoplasma gondii*. Beginning in the early 1990's and during most of my career at the Trudeau Insitute, I focused on immunity to the protozoan parasite, *Toxoplasma gondii,* a pathogen of major clinical importance to congenitally infected humans and AIDS patients. My laboratory revealed key roles of cell-mediated immunity, humoral immunity, and cytokine in immunity to the parasite.

Johnson. L.L. (1992). A protective role for endogenous tumor necrosis factor in *Toxoplasma gondii* infection. Infect. Imm. 60, 1979-1983.

Johnson, L.L (1992) SCID mouse models of acute and relapsing Toxoplasma gondii infections. Infect. Imm. 60:3719-3224.

Sayles, P.C., Rakhmilevich, A.L., and Johnson, L.L. (1995) T-cells and acute primary *Toxoplasma gondii* infection in mice. J. Infect. Dis. 171:249-252.

Johnson, L.L. and P.C. Sayles. (1997) (Commentary) Interleukin-12, dendritic cells, and the initiation of host-protective mechanisms against *Toxoplasma gondii.* J. Exp. Med. 18.6:1-4.

Johnson,L.L., Lanthier, P., Hoffman, J., and Chen, W. (2004). Vaccination protects B cell-deficient mice against an oral challenge with mildly virulent Toxoplasma gondii. Vaccine 22:4054-4061.

3. Fibrin, coagulation and infection. A collaboration with Dr. Stephen Smiley revealed the importance of fibrin and coagulation in resistance to infection.

Johnson, L.L Berggren, K.N., Szaba, F.M., Chen, W. Smiley, S.T. (2003) Fibrin-mediated protection against infection-stimulated immunopathology. J. Exp. Med. 197:801-806.

Mullarky, I.K., Szaba, F.M., Berggren, K.N., Parent, M.A., Kummer, L.W., Chen, W., Johnson, L.L., Smiley, S.T. 2005. Infection-stimulated fibrin deposition controls hemorrhage and limits hepatic bacterial growth during listeriosis. Infect. Immun. 73:3888-3895.

Mullarky, I.K., Szaba, F.M., Berggren, K.N., Kummer, L.W. Wilhelm, L.B., Parent, M.A., Johnson, L.L., and Smiley, S.T. 2006. Tumor necrosis factor alpha and gamma interferon, but not hemorrhage or pathogen burden, dictate levels of protective fibrin deposition during infection. Infect. Immun. 74:1181-1188.

4. Immunity to *Yersinia pestis (*plague). A collaboration with Dr. Smiley and Dr. Jr-Shiuan Lin revealed key immunological features of immunity to *Y. pestis*.

Kummer LW, Szaba FM, Parent MA, Adamovicz JJ, Hill J, Johnson LL, Smiley ST. 2008. Antibodies and cytokines independently protect against pneumonic plague. Vaccine 26:6901-7.

Luo D, Szaba FM, Kummer LW, Johnson LL, Tucker EI, Gruber A, Gailani D, Smiley ST. 2012. Factor XI-deficient mice display reduced inflammation, coagulopathy, and bacterial growth during listeriosis. Infect Immun. Jan;80(1):91-9.

**Complete List of Published Work in MyBibliography**
http://www.ncbi.nlm.nih.gov/sites/myncbi/lawrence.johnson.2/bibliography/49621950/public/?sort=date&direction=ascending.

**D. Research Support**
**Active**

5P01AG021600-11 (Haynes) 12/2/02-5/31/17
NIA
Program Title: “Aging and immunity to infections" (Haynes)
Core A Title: "Administration" (Haynes)
The Administrative Core provides oversight of the program project, assists the program director and project leaders and provides data analysis support.
Role: Statistical Consultant providing advice in experimental design and statistical data analysis.

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

## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Herbert Whiting Virgin IV

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

POSITION TITLE: Edward Mallinckrodt Professor and Chair Department of Pathology and Immunology, Washington University School of Medicine

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

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
| Harvard University, Boston, MA | AB | 05/1977 | Biology, *magna cum laude* |
| Harvard University Special Student, Boston, MA | | 1977-79 | Biology |
| Harvard Medical School, Boston, MA | M.D.<br>Ph.D. | 1985<br>1985 | Medicine<br>Immunology:ER Unanue |
| Harvard Medical School (HMS) | Postdoctoral | 1985-90 | Virology: BN Fields |
| Brigham and Women's, HMS | Residency | 1985-90 | Internal Medicine |
| Barnes Hospital, Washington University School of Medicine | Fellowship | 1990-91 | Infectious Diseases |

**A. Personal Statement**

We seek to understand the interactions between virus and host in molecular and immunologic terms, how these interactions are disrupted during disease and how the mechanisms involved may be targeted therapeutically. We focus on host pathways that are important for human disease susceptibility such as autophagy, interferon responses and the function of genes that regulate human disease susceptibility. Our primary interest is in chronic virus infection and the virome, but we also study acute virus infection, bacteria, helminths, apicomplexan pathogens and commensals as needed to define mechanisms of immunity. We use genetic, computational, sequencing, and structural approaches to test hypotheses. The nature of this type of research is highly collaborative as we frequently require the expertise of our colleagues to get to the bottom of a scientific question. Below are four recent reviews related to this body of work (**papers a-d**). We are particularly proud to have trained investigators including: Mark Heise (UNC, tenure); Karen Weck (UNC); Margaret MacDonald (Rockefeller), Sharookh Kapadia (Genentech); Barbara Haller (UCSF); Linda van Dyk (Univ. Colorado, tenure), Scott Tibbetts (Univ. Florida, tenure); Stephanie Karst (Univ. Florida, tenure); Felipe Suarez (Université Paris Descartes), Linda Van Dyk (Univ. Colorado, tenure); Christiane Wobus (Univ. Michigan, tenure); Becky Sparks-Thissen (Univ. S. Indiana); Larissa Thackray (Washington Univ.); Nat Moorman (UNC); Rachel Presti (Washington University); Doug White (Gundersen Health System/Univ. of Wisconsin); Vera Tarakanova (Medical College of Wisconsin); Shivaprakash Gangappa (CDC); Deborah Lenschow (Washington Univ., tenure); Ken Cadwell (NYU School of Medicine), Dan Popkin (Case Western), Seungmin Hwang (Univ. Chicago), Tiffany Reese (UTSW School of Medicine), Tim Nice (OHSU), and Megan Baldridge (Washington University School of Medicine).

a. Levine, B, N. Mizushima, and **H. W. Virgin**. Autophagy in Immunity and Inflammation. **2011. Nature.** 469(7330):323-35. PMID: 21248839. PMCID: PMC31311688.
b. **Virgin, H. W.** The virome in mammalian physiology and disease. **2014 Cell.** 157(1):142-150. PMID 24679532. PMCID PMC3977141.
c. Pfeiffer, J. K. and **H. W. Virgin**. Transkingdom control of viral infection and immunity in the mammalian intestine. **2016. Science.** Jan 15;351(6270):239. PMID: 26816384. PMCID: 4751997. http://science.sciencemag.org/content/351/6270/aad5872.full

d. Stappenbeck, T. S. and **H. W. Virgin**. Accounting for reciprocal host-microbiome interactions in experimental science**. 2016. Nature.** June 9 534;191-199. PMID: 26816384. PMCID: PMC4751997.

**B. Positions and Honors**

**Positions:** (Washington University School of Medicine, St. Louis, MO USA): 1990-91 Instructor in Medicine and Pathology and Immunology; 1991-96 Assistant Professor of Medicine, Pathology and Immunology, and Molecular Microbiology; 1996-98 Assistant Professor of Pathology and Immunology and Molecular Microbiology: 1998-01 Associate Professor of Pathology and Immunology and Molecular Microbiology; **Present Position:** 2002- Professor of Pathology and Immunology and Molecular Microbiology; 2006- Edward Mallinckrodt Professor and Chair of Pathology and Immunology; 2008- Professor of Medicine.

**Honors & Awards:** 1985 Sheard Sanford Award, American Society of Clinical Pathologists; 1991 Burroughs Wellcome Young Investigator in Virology; 1995 Mallinckrodt Scholar; 1998 American Society for Clinical Investigation; 2001 Mentorship Award, Academic Women's Network, Washington University; 2002 Outstanding Faculty Mentor Award, Washington University; 1998 American Society for Clinical Investigation; 2004 Fellow American Association for the Advancement of Science; 2007 American Association of Physicians; 2010 American Academy of Microbiology; 2016 National Academy of Sciences.

**Editorial:** 2006- Editorial Board, *Cell Host & Microbe;* 2010- Board of Reviewing Editors, *Science*; 2016- Editorial Board, *Cell*.

**Selected Professorships and Keynote Addresses:** 2000 Bernard N. Fields Lecturer, American Society of Virology; 2004 Maurice Hilleman-Merck Research Laboratories Lecturer, American Society for Virology; 2004 Bernard N. Fields Lecturer, FASEB Conference; 2009 NIH Salzman Award Symposium; 2009 Allan Granoff Lectureship in Virology, St. Judes; 2010 Ernst A. H. Friedheim Memorial Lecture, Rockefeller University; 2010 Henle Professor Lecture, University of Pennsylvania School of Medicine; 2011 Sandra Clark Endowed Lecture in Immunology, University of Washington; 2011 Keystone Immunologic Memory, Persisting Microbes and Chronic Disease; 2012 Lamb Professorship, Vanderbilt University School of Medicine; 2013 Cell Symposium Microbiome and Host Health; 2013 Keystone Positive Strand RNA Viruses; 2013 Lya Cordova Latta Lectureship, UCLA; 2013 Pierre Grabar Lecture, French Society for Immunology; 2015 Keystone Viral Immunity; 2015 Keystone Gut Microbiota Modulation of Host Physiology; 2015 Sid Leskowitz Professor, Tufts School of Medicine; 2015 Rolla E. Dyer Lecture, NIH; 2015 Gairdner Symposium on the Microbiome. 2016, Erskine Lecture St. Jude, Memphis TN; 2016 Hilleman Lecture, University of Chicago.

**Current Scientific Advisory and Other Boards:** 2009- Chair SAB Ragon Institute of MGH, Harvard, and MIT; 2012- SAB Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery (Scripps); 2014- Principle Investigator NIH Center for Excellence in Translational Research; 2014- Steering Committee NIH Genomic Centers for Infectious Diseases; 2014- SAB Institut Pasteur.

**C. Contributions to science (200 peer reviewed primary studies published or in press)**

**1. Discovery, culture, pathogenesis, and genetics of murine norovirus (mNoV):**

We discovered the murine NoV (**paper a**). NoVs are responsible for >90% of epidemic non-bacterial gastroenteritis worldwide. This led to the first culture (**paper b**) and reverse genetics systems for a NoV, and to increasing use of this model system. We have identified steps in NoV replication, mapped mNoV genes involved in virulence and persistence, defined components of adaptive immunity responsible for vaccination against enteric mNoV infection and reported the first structural studies of an infectious NoV. mNoV has been used by our trainee Ken Cadwell to demonstrate that enteric virus infection can substitute for commensal bacteria in gut immune development. We have identified a novel sterilizing function of gut innate immunity that depends on interferon-λ but not adaptive immunity (**paper c**), and discovered a proteinaceous receptor for the virus that, interestingly, requires a soluble co-factor to function in infection (**paper d**).

a. Karst, S.M., Wobus, C. E., Lay, M., Davidson, J., and **H. W. Virgin**. STAT1-dependent innate immunity to a Norwalk-like virus. **2003. Science.** 299(5612):1575-8. PMID 12624267.
b. Wobus, C. E., S. M. Karst, L. Thackray, K.-O. Chang, S. V. Sosnovtsev, G. Belliot, A. Krug, J. M. Mackenzie, K. Y. Green, and **H. W. Virgin.** Replication of a *Norovirus* in cell culture reveals a tropism for dendritic cells and macrophages. **2004. PLOS Biology.** E432. PMID: 15562321. PMCID: PMC532393.
c. Nice, T. J., M. T. Baldridge, B. T. McCune, J. M. Norman, H. M. Lazear, M. Artyomov, M. S. Diamond and **H. W. Virgin**. Interferon λ cures persistent murine norovirus infection in the absence of adaptive immunity. **2015. Science.** 347(6219):269-73. Epub 2014 Nov 27. PMID: 25431489. PMCID: PMC4398891.

d. Unpublished

**2. Defining the physiologic importance of virus-host genetic interactions:**

Our interest in genetic interactions between viruses and the host arose from efforts to prove the *in vivo* mechanisms of action of herpesvirus immune evasion molecules (e.g. complement regulator, PKR inhibitor, v-cyclin, v-bcl-2, MHC regulators, chemokine binding protein). We developed the concept of 'host complementation' to prove mechanisms of such proteins *in vivo* (**paper a**). Studies in pursuit of defining host-virus genetic interactions led to the discovery of symbiotic effects of chronic viral infection-mediated stimulation of innate immunity to (**paper b**), and to the 'virus-plus-host gene' concept that host phenotypes can be induced by the combination of chronic viral infection and host mutation when neither virus infection nor host mutation alone suffices (**paper c**). We extended these findings to show that genetic immunodeficiency can be complemented by chronic herpesvirus infection (**paper d**), supporting the concept that the virome contributes to normal immune function.

a. Leib, D. A., Machalek, M. A., Williams, B. R., Silverman, R. H. and **H. W. Virgin.** Specific phenotypic restoration of an attenuated virus by knockout of a host resistance gene. **2000. PNAS (USA).** 97(11):6097-6101. PMID 10801979. PMCID PMC18564.
b. Barton. E. S., D. W. White, J. S. Cathelyn, K. A. Brett-McClellan, M. Engle, M. S. Diamond, V. Miller, and **H. W. Virgin**. Herpesvirus latency confers symbiotic protection from bacterial infection. **2007. Nature.** 447(7142):326-9. PMID 17507983.
c. Cadwell, K., K. K. Patel, N. S. Maloney, T.-C. Liu, A. C. Y. Ng, C. E. Storer, R. D. Head, R. Xavier, T. S. Stappenbeck, and **H. W. Virgin**. Virus plus susceptibility gene interaction determines intestinal phenotypes of the Crohn's disease susceptibility gene Atg16L1. **2010. Cell.** 141(7): 1135-45. PMID 20602997. PMCID PMC2908380.
d. MacDuff, D. A., T. A. Reese, J. Kimmey, L. Weiss, C. Song, X. Zhang, A. Kambal, E. Duan, J. Carrero, B. Boisson, E. Laplantine, A. Israel, C. Picard , M. Colonna, B. T. Edelson, L. D. Sibley, C. Stallings, J.-L. Casanova, K. Iwai, and **H. W. Virgin**. Phenotypic complementation of genetic immunodeficiency by chronic herpesvirus infection. **2015. eLife.** 4:e04494. PMID: 25599590. PMCID: PMC4298697.

**3. Autophagy and non-canonical functions of autophagy genes:**

Through our studies of autophagy (*Atg*) genes, we have defined important roles of both autophagy and non-canonical functions of *Atg* genes in host physiology and immunity. We found that *Atg* genes, in addition to their canonical role in envelopment and delivery of cytoplasmic cargo to the lysosome for degradation, play key roles in vesicle secretion in intestinal Paneth cells (**paper a**), osteoclasts and goblet cells. We have also shown that these genes play a role in the function and ontogeny and function of B cells and T cells including in the formation of anti-viral memory CD8 T cell responses. We were also the first to report non-canonical roles of *Atg* genes in control of infection (**paper b, c**). For example, studies of Mycobacterium tuberculosis have confirmed that not all essential *Atg* genes are required for such immune functions (**paper d**).

a. Cadwell, K., J. Y. Liu, S. L. Brown, H. Miyoshi, J. Loh, J. K. Lennerz, C. Kishi, W. Kc, J. A. Carrero, S. Hunt, C. D. Stone, E. M. Brunt, R. J. Xavier, B. P. Sleckman, E. Li, N. Mizushima, T. S. Stappenbeck, and **H. W. Virgin**, A unique role for autophagy and the autophagy gene Atg16L1 in murine and human intestinal Paneth cells. **2008. Nature.** 456:259-263. PMID 18849966. PMCID PMC2695978.
b. Zhao, Z., B. Fux, M. Goodwin, I. R. Dunay, D. Strong, B. C. Miller, K. Cadwell, M. Delgado-Vargas, M. Ponpuak, K. G. Green, R. E. Schmidt, N. Mizushima, V. Deretic, L. D. Sibley, and **H. W. Virgin.** Autophagosome-independent essential function for the autophagy protein Atg5 in cellular immunity to intracellular pathogens. **2008. Cell Host and Microbe.** 4:458-69. PMID 18996346. PMCID:PMC2682425.
c. Hwang, S., N. S. Maloney, M. W. Bruinsma, G. Goel, E. Duan, L. Zhang, B. Shrestha, M. S. Diamond, A. Dani, S. V. Sosnovtsev, K. Y. Green, C. Lopez-Otin, R. J. Xavier, L. B. Thackray, and **H. W. Virgin**. Non-degradative role of Atg5-Atg12/Atg16L1 autophagy protein complex in antiviral activity of interferon-γ. **2012. Cell Host and Microbe**. 11(4): 397-409. PMID 22520467. PMCID PMC3348177.
d. Kimmey, J. M., J. P. Huynh, L. A. Weiss, S. Park, A. Kambal, J. Debnath, **H. W. Virgin**, C. L. Stallings. Unique role for Atg5 in neutrophil-mediated immunopathology during *Mycobacterium tuberculosis* infection. **2015. Nature.** 528(7583):565-9. PMID: 26649827. PMCID: PMC4842313.

**4. Defining mechanisms of cytokine regulation of viral infection:**
We have had a long term interest in defining how interferons (IFNs) control virus infection, and have identified the role of IFNs, IFN receptors, and related transcription factors in norovirus, herpesvirus, poxvirus and other viral infections as well as in the anti-viral functions of T and NK cells. We have also defined the role of *Atg* genes in IFN mediated immunity (**see #1, #3 above**). We were the first to define the antiviral role in mice of *Isg15 in vivo* (**paper a**), identified a role for cGAS in control of RNA virus infection (**paper b**), and have identified a role for YM-1 in control of enteric immunity to murine NoV infection during helminth infection (**paper c**). Analysis of cytokine-mediated control of herpesvirus reactivation revealed that both murine and human herpesvirus promoters can sense the cytokine IL-4, resulting in reactivation from latency during helminth infection (**paper d**).

a. Lenschow, D. J., C. Lai, N. Frias-Staheli, N. V. Giannakopoulos, A. Lutz, T. Wolff, A. Osiak, B. Levine, R. E. Schmidt, A. Garcia-Sastre, D. A. Leib, A. Pekosz, K.-P. Knobeloch, I. Horak, and **H. W. Virgin**. ISG15 functions as a critical antiviral molecule against influenza, herpes, and Sindbis viruses. **2007. PNAS.** 104(4): 1371-1376. PMID: 17227866. PMCID: PMC1783119
b. Schoggins, J.W. D. A. MacDuff, N. Imanaka, M. D. Gainey, B. Shrestha, J. L. Eitson, K. B. Mar, R. B. Richardson, A. V. Ratushny, V. Litvak, R. Dabelic, B. Manicassamy, A. Aderem, R. M. Elliott, A. Garcia-Sastre, V. Racaniello, E. J. Snijder, W. M. Yokoyama, M. S. Diamond, **H. W. Virgin**, C. M. Rice. Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. **2014. Nature.** 505:691-5. PMID 24284630. PMCID PMC4077721.
c. Osborne L.C., L. A. Monticelli, T. J. Nice, T. E. Sutherland, M. C. Siracusa, M. R. Hepworth, V. T. Tomov, D. Kobuley, S. V. Tran, K. Bittinger, A. G. Bailey, A. L. Laughlin, J. L. Boucher, E. J. Wherry, F. D. Bushman, J. E. Allen, **H. W. Virgin**, D. Artis. Virus-helminth coinfection reveals a microbiota-independent mechanism of immunomodulation. **2014. Science**. 345(6196):578-82. PMID 25082704. PMCID: PMC4548887.
d. Reese, T. A., B. S. Wakeman, H. S. Choi, M. M. Hufford, S. C.C. Huang, X. Zhang, M. D. Buck, A. Jezewski, A. Kambal, C. Y. Liu, G. Goel, P. J. Murray, R. J. Xavier, M. H. Kaplan, R. Renne, S. H. Speck, M. N. Artyomov, E. J. Pearce, and **H. W. Virgin**. Helminth Co-infection Reactivates Gammaherpesvirus Infection Through Viral Promoter-targeted Cytokine Competition. **2014. Science.** 345(6196):573-7. PMID 24968940. PMCID: PMC4531374.

**5. Defining the virome and trans-kingdom interactions in control of the virome:**
Recognizing that most currently studied viruses were identified through growth in culture, we have sought to define the mammalian virome. To this end we developed sequencing and bioinformatic tools that have allowed us to move from identification of individual viruses such as murine NoV (**see #1 above**) to identification of populations of bacteriophages and eukaryotic viruses in disease situations. We identified many new eukaryotic viruses that may contribute to AIDs-associated enteropathy in SIV-infected macaques (**paper a**), and have provided the first demonstration that the fecal DNA virome is linked to a human disease, inflammatory bowel disease (**paper b**). We have discovered what we term 'trans-kingdom' interactions that control virus infection including the finding that enteric bacteria are required for the establishment of persistent enteric murine NoV infection (**paper c**). Study of bacterial populations revealed that the intestinal bacterial microbiome can also confer heritable immune phenotypes (**paper d**).

a. Handley, S., L. B. Thackray, G. Zhao, R. Presti, A. Miller, L. Droit, P. Abbink, L. F. Maxfield, A. Kambal, E. Duan, J. Kramer, S. C. Macri, S. R. Permar, J. E. Schmitz, K. Mansfield, J. Brenchley, R. S. Veazey, T. S. Stappenbeck, D. Wang, D. H. Barouch, and **H. W. Virgin**. Pathogenic simian immunodeficiency virus infection is associated with significant expansion of the enteric virome. **2012. Cell.** 151:253-266. PMID 23063120. PMCID 3490196.
b. Norman, J. M., S. A. Handley, M. T. Baldridge, L. Droit, C. Y. Liu, B. C. Keller, A. Kambal, C. L. Monaco, G. Zhao, P. Fleshner, T. S. Stappenbeck[1], D. P. B. McGovern[5], A. Keshavarzian, E. A. Mutlu, J. Sauk, D. Gevers, R. J. Xavier, D. Wang, M. Parkes and **H. W. Virgin.** Disease-specific Alterations in the Enteric Virome in Inflammatory Bowel Disease. **2015. Cell.** 160:447-68. PMID: 25619688. PMCID: PMC4312520.
c. Baldridge, M. T., T. J. Nice, B. T. McCune, C. C. Yokoyama, A. Kambal, M. Wheadon, M. S. Diamond, Y. Ivanova, M. Artyomov and **H. W. Virgin**. Commensal microbes and interferon-λ determine persistence of enteric murine norovirus infection. **2015. Science.** 347(6219):266-9. Epub 2014 Nov 27. PMID: 25431490. PMCID: PMC4409937.

d. Moon, C., M. T. Baldridge, M. A. Wallace, C.-A. D. Burnham, **H. W. Virgin***, T. S. Stappenbeck*. Vertically transmissible fecal IgA levels distinguish extra-chromosomal phenotypic variation. **2015. Nature. 521:90-3.** PMID: 25686606; PMCID: PMC4425643. *co-corresponding authors.

**D. Research Support**

**ACTIVE**

5 U19 AI109725-03 (Virgin) 3/1/14-2/28/19
NIH/NIAID
U19: Autophagy Modulators as Novel Broad-Spectrum Anti-Infective Agents
RP3: Genes/Pathways for ATG Gene-Dependent Inhibition of Virus and Parasite Infection
Goal: Stimulate autophagy and ATG genes to create broad-spectrum anti-infective agents.

5 R24 OD019793-02 (Virgin/Lackner) 4/1/15-2/28/19
NIH/OD
Primate Infectious Disease Resource (PIDR)
Goal: Develop next generation sequencing for primate health and infection diagnosis.

5 R01 OD011170-04 (Virgin/Barouch) 9/1/11-6/30/16 (NCE)
NIH/OD
Metagenomics of Enteric Disease in SIV-infected and Uninfected Macaques
Goal: Define the enteric virome and bacterial microbiome in SIV/AIDS.

5 R01 AI111918-03 (Kwon/Virgin) 3/11/14-2/28/18
NIH/NIAID - AIDS grant
Inflammation and the Vaginal Metagenome in HIV Acquisition
Goal: Define the vaginal microbiome and virome in HIV-at risk women.

5 R01 DK101354-02 (Kwon/Virgin) 7/10/14-6/30/19
NIH/NIDDK - AIDS grant
The Enteric Microbiome in Treated and Progressive HIV Infection
Goal: Define the enteric virome in HIV patients.

Private Source 9/1/12-12/31/17
Goals: Identify molecular mechanisms for risk gene contributions to inflammatory bowel disease.

Private Source 4/15/14-4/14/17
Goal: Define the molecular basis of HOIL-1 action

Private Source 12/1/14-11/30/16
Prospective Longitudinal Analysis of the Developing Gut Virome in Infants *en route* to Type 1 Diabetes
Goal: Determine the contribution of the enteric virome to Type 1 diabetes.

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

## BIOGRAPHICAL SKETCH

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

NAME: Raymond M. Welsh, Ph.D.

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

POSITION TITLE: Professor of Pathology & Microbiology & Physiological Systems

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

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
| University of Massachusetts, Amherst | B.S. | 05/1967 | Microbiology |
| University of Massachusetts, Amherst | Ph.D. | 08/1972 | Micro/Virology |
| University of Kansas, Lawrence | Postdoc | 1972-73 | Micro/Biochemistry |
| Scripps Clinic & Research Foundation, La Jolla, CA | Postdoc | 1973-75 | Virology/Immunology |

### A. Personal Statement

My laboratory has studied the immune response to a variety of viral infections using mouse models of infection since the 1970's, when we documented an early type 1 interferon (IFN) driven NK cell response, followed by a cytotoxic T cell response, reflective of innate and adaptive immunity. My lab documented the activation and role of NK cells in viral infections and showed that NK cells regulate murine cytomegalovirus infection by way of an activating NK receptor, Ly49H. My lab has also studied T cell apoptosis or attrition in the context of IFN-inducing viral infections, and I just completed a MERIT award (R37 AI017672) on this topic. Dr. Liisa Selin and I developed the field of T cell-dependent heterologous immunity, whereby memory T cells specific to one virus may participate in the control and pathogenesis of a second virus, often through the process of cross-reactivity. Dr. Eva Szomolanyi-Tsuda and I have collaborated on antibody responses to viral infections, analyzing polyclonal B cell activation and the need of TLR signaling for B cell memory responses. Having been an Editor for the Journal of Virology and Deputy Editor for the Journal of Immunology, I have a broad range of expertise that should be helpful for the completion of the projects proposed. Below are some recent papers demonstrating my expertise in these areas (of 275):

Waggoner, SN, M Cornberg, LK Selin, and RM Welsh. Natural killer cells act as rheostats modulating anti-viral T cells. 2011. Nature 481:394-398. PMC:3539796.

Kapoor, V, HM Shin, OH Cho, LJ Berg, J Kang, and RM Welsh. 2014. Regulation of tissue-dependent differences in CD8 T cell apoptosis during acute virus infection. J Virol 88: 9490-9503. PMC:4136305.

Seedhom, MO, KS Mathurin, S-K Kim, and RM Welsh. 2012. Increased protection from vaccinia virus infection in mice genetically prone to lymphoproliferative disorders. J Virol 86: 6010-6022. PMC:3372224.

Urban, SL, and RM Welsh. 2014. Out-of-sequence signal 3 as a mechanism for virus-induced immune suppression of CD8 T cell responses. Plos Path 10: e1004357. PMC:4177909.

**B. Positions and honors**

**Positions:**

1972-1973 Visiting Asst. Professor of Microbiology, Univ. of Kansas, Lawrence, KS; Dept. of Microbiology
1975-1980 Asst. Member, Scripps Clinic & Research Foundation, La Jolla, CA; Dept. of Immunopathology
1979 Visiting Scientist, Karolinska Institute; Scripps Clinic, 1987.
1980 Adjunct Assoc. Prof. of Pathology, Univ. California at San Diego Medical School, Dept. of Pathology
1980-1985 Assoc. Professor of Pathology, Molecular Genetics and Microbiology, Univ. Mass. Medical School, Worcester, MA
1985-2010 Professor of Pathology, Molecular Genetics and Microbiology, Univ. Mass. Medical School, Worcester, MA
2010–2015 Professor of Pathology, Microbiology & Physiological Systems, Univ. Mass. Medical School, Worcester, MA
2015-present Emeritus Professor of Pathology, UMMS

**Honors, Editorial Boards, and Advisory Groups:**

Recipient of RCDA AI-00253 (1978-1983); Recipient of NIH Merit Award R37 AI 17672 (2004 - 2014); Elected Fellow of AAAS (2011); Elected Fellow of the American Academy of Microbiology 2014; Editorial Boards: J. Immunol. (1982-1986; 1997-present). Section Editor (2001), Deputy Editor (2008-2012); Proc. Soc. Exp. Biol. Med. (1978-1987); J. Virol. (1986-present; Editor 1998-2007); Natural Immunity Cell Growth Regulation (1984-2000); J. Natl. Cancer Inst. (1987-1991); J. Exp. Med. (1995-present); Virology (1996-present); Study Sections: American Cancer Society (National) Immunology and Immunotherapy Section (1988-1991); American Cancer Society (Massachusetts)(1981-1991), Chairman (1985-1991); State of California AIDS Task Force (1985-1996); NIH Virology (1991-1995).

**C. Contributions to Science**

My laboratory has studied the immune response to a variety of viral infections using mouse models of infection since the 1970's and has been involved in the development of a number of concepts in viral immunology.

1. **Natural (innate) vs. adaptive immunity.** We showed in Mike Oldstone's lab that there were early and late cellular responses to infection, the first being a cytokine (interferon) driven NK cell activation followed by an antigen driven T cell activation; these are now referred to as the innate and adaptive immune responses (a,b) . We showed with Rolf Zinkernagel that T cells cleared virus in a syngeneic class 1 MHC antigen-restricted manner (c) , in contrast to NK cells, which did not require class 1 MHC recognition at all (a), and, with Rolf Kiessling, we showed that NK cells had an allogeneic antigen preference (b,d). Our drawings of what we now call the innate and adaptive responses to infection entered text books in the early 80s.

a. Welsh, R.M., Jr. 1978. Cytotoxic cells induced during lymphocytic choriomeningitis virus infection of mice: 1. Characterization of natural killer cell induction. J. Exp. Med. 148:163-181.

b. Welsh, R.M., Jr. and R.M. Zinkernagel. 1977. Heterospecific cytotoxic cell activity induced during the first three days of acute lymphocytic choriomeningitis virus infection in mice. Nature 268:646-648.

c. Zinkernagel, R.M. and R.M. Welsh. 1976. H-2 compatibility requirement for virus-specific T-cell mediated effector functions in vivo. I. Specificity of T cells conferring antiviral protection against lymphocytic choriomeningitis virus is associated with H-2K and H-2D. J. Immunol. 117:1495-1520.

d. Kiessling, R. and R.M. Welsh. 1980. Killing of normal cells by activated mouse natural killer cells: evidence for two patterns of genetic regulation of lysis. Int. J. Cancer 25:611-615.

2. **Regulation and role of NK cells in infection.** We went on to show with Christine Biron that NK cells were not end-stage cells, as they proliferated in response to infection-driven cytokines (a). We showed with Jack Bukowski that they entered sites of virus infection and mediated antiviral activity against some viruses, such as murine cytomegalovirus, but not others such as lymphocytic choriomeningitis virus (b). We subsequently defined Ly49H as a receptor on NK cells that regulates MCMV synthesis (c). More recent studies with Steve Waggoner

showed that NK cells control adaptive immunity (d). We believe that this demonstration of the antiviral roles of NK cells during viral infections could be considered a seminal observation in the field of viral immunology.

a. Biron, C.A., L.R. Turgiss and R.M. Welsh. 1983. Increase in NK cell number and turnover rate during acute virus infection. J. Immunol. 131:1539-1545.

b. Bukowski, J.F., J.R. Warner, G. Dennert and R.M. Welsh. 1985. Adoptive transfer studies demonstrating the antiviral effect of natural killer cells in vivo. J. Exp. Med. 161:40-52.

c. Daniels, K.A., G. Devora, W.C. Lai, C.L. O'Donnell, M. Bennett, and R.M. Welsh. 2001. Murine cytomegalovirus is regulated by a discrete subset of natural killer cells reactive with monoclonal antibody to Ly49H. J. Exp. Med. 194:29-44 (cover).

d. Rydyznski, C, KA Daniels, EP Karmele, TR Brooks, R Sutiwisesak, RM Welsh, and SN Waggoner. 2015. Antiviral memory T cell, germinal center, and neutralizing antibody responses are impaired by natural killer cells. Nat Commun 6:6375. PMID25721802.

3. **Heterologous immunity.** While studying the specificities of NK cells we noted that CD8 T cell responses to viruses often cross-reacted with allogeneic MHC antigens and with self-MHC-presented antigens encoded by other viruses (a). This, in collaboration with Liisa Selin, allowed us to develop the field of heterologous immunity, where memory T cells specific to one virus can alter the immune response to and pathogenesis of a second virus. These cross-reactive memory cells can also precipitate high variations in pathogenesis between individuals as a consequence of the private specificities of their immune repertoires (b,c,d). We believe that heterologous immunity explains some of the variations in viral pathogenesis seen between individuals (d) and may explain certain age-dependent differences in pathogenesis dependent on the accumulation of certain specific memory cell pools.

a. Nahill, S.R., and R.M. Welsh. 1993. High frequency of cross-reactive cytotoxic T lymphocytes elicited during the virus-induced polyclonal CTL response. J. Exp. Med. 177:317-327.

b. Selin, L.K., S.M. Varga, I.C. Wong, and R.M. Welsh. 1998. Protective heterologous antiviral immunity and enhanced immunopathogenesis mediated by crossreactive memory T cell populations. J. Exp. Med. 188:1105-1715

c. Chen, H.D., A.E. Fraire, I. Joris, M.A. Brehm, R.M. Welsh, and L.K. Selin. 2001. Memory CD8+ T cells in heterologous antiviral immunity and immunopathology in the lung. Nat. Immunol. 2:1067-1076 (cover, and commentary in Nature).

d. Kim, S.-K., X.Z. Wang, M. Cornberg, H.D. Chen, L.K. Selin, and R.M. Welsh. 2005. Private specificities of CD8 T cell responses control patterns of heterologous immunity. J. Exp. Med. 201:523-533. (with Commentary in JEM)

4. **Apoptosis and viral infections.** We did some of the original studies on T cell apoptosis during viral infection (a,b), showing that the decline of the CD8 T cell response at the resolution of infection was due to apoptosis (B), correlating virus-induced immune deficiency to the apoptosis of T cells (a), and documenting an early interferon driven apoptosis of memory T cells that can result in a long term loss of pre-existent memory at the resolution of an infection (c,d).

a. Razvi, E.S., and R.M. Welsh. 1993. Programmed cell death of T lymphocytes during acute viral infection: a mechanism for virus-induced immune deficiency. J. Virol. 67:5754-5765.

b. Razvi, E.S., Z. Jiang, B.A. Woda and R.M. Welsh. 1995. Lymphocyte apoptosis during the silencing of the immune response to acute viral infections in normal, *lpr*, and Bcl-2-transgenic mice. Am. J. Pathol. 147:79-91.

c. Selin, L.K., M.Y. Lin, K.A. Kraemer, D.M. Pardoll, J.P. Schneck, S.M. Varga, P. Santolucito, A.K. Pinto, and R.M. Welsh. 1999. Attrition of T cell memory: selective loss of LCMV epitope-specific memory CD8 T cells following infections with heterologous viruses. Immunity 11:733-742.

d. McNally, J.M., C.C. Zarozinski, M.Y. Lin, and R.M. Welsh. 2001. Attrition of bystander CD8T cells during virus-induced T cell and interferon responses. J. Virol. 75:5965-5976.

5. **B cell immunity during viral infections.** In collaboration with Dr. Szomolanyi-Tsuda, I have examined various aspects of virus-induced antibody formation, including (a) the demonstration of protective isotype-switched antibodies in the absence of T cells, (b,c) the requirement for MyD88 signaling in T-independent antibody responses and in the generation of T-dependent long term humoral immunity, and (d) the mechanism of polyclonal B cell activation during viral infections.

a. Szomolanyi-Tsuda, E. and R.M. Welsh. 1996. T cell-independent antibody-mediated clearance of polyoma virus in T cell-deficient mice. J. Exp. Med. 183:403-411.

b. Guay, H.M., T.A. Andreyeva, R.L. Garcea, R.M. Welsh, and E. Szomolanyi-Tsuda. 2007. MyD88 is required for the formation of long-term humoral immunity to virus infection. J. Immunol. 178:5124-5131. (Highlighted in JI)

c. Raval, FM, R Mishra, RL Garcea, RM Welsh, and E Szomolanyi-Tsuda. 2013. Long-lasting T cell-independent IgG responses require MyD88-mediated pathways and are maintained by high levels of virus persistence.. MBio e00812-13. PMID 24194540.

d. Jellison, E.R., H.M. Guay, E. Szomolanyi-Tsuda, and R.M. Welsh. 2007. Dynamics and magnitude of virus-induced polyclonal B cell activation mediated by BCR- independent presentation of viral antigen. Eur J Immunol 37:119-128.

**Complete list of published work in my bibliography:**
http://www.ncbi.nlm.nih.gov/sites/myncbi/raymond.welsh.1/bibliograpahy/40525136/public/?sort=date&direction=ascending

**D. Research Support.**
**ONGOING**

U19 AI109858-02 (Welsh, PD/PI) 03/01/2014 – 02/28/2019
NIH/NIAID
Project Grant: CD4 T cells in antiviral immunity and immune pathology.
Project 1 – NK cell regulation of CD4 T cell responses (Project Leader)
Core A – Administrative and quantitative core (Core Leader)
This project examines the control of T cell responses by NK cells as part of a program project on the role of CD4 T cells in viral pathogenesis. There also is an administrative core. Role: PD/PI/Core Lead.

2 T32 AI-007349-24A1 (Welsh-PI) 08/1/2014 – 07/31/19
NIH/NIAID
Training in immunology and virology
This is a training grant that awards pre-doctoral fellowships to trainees in the University of Massachusetts Immunology and Microbiology Program. Role: PI

P01 AI049320-13 Luzuriaga (PD/PI) 07/15/12 - 06/30/17
NIH/NIAID
Project Grant: Evolution & Maintenance of Memory CD8+ T Cells (Luzuriaga – Project Director)
Project 2: Crossreactivity of T cells in Human Virus Infection (Selin – Project Leader)
This is a program project to examine human T cell responses to viruses. The purpose of this project is to examine human virus-specific T cells that are cross-reactive between heterologous viruses and to determine if they can

be correlated with the pathogenesis of human disease. A focus is on T cell cross-reactivity between influenza virus and Epstein Barr virus.
Role: Co-Project Leader on project with Liisa Selin as primary Project Leader.

**COMPLETED (within the past three years)**

P01 AI046629-13 (Greiner -PD/PI) 06/01/10 - 05/31/15
NIH/NIAID
Project Grant : Viral infection influence on transplantation tolerance (Greiner – Project Director).
Project 2: Effect of Virus Infections on the Maintenance of Transplantation Tolerance (Welsh – Project Leader)
This is a program project grant in which Raymond Welsh is a Project Leader in a section designed to look at the ability of viral infections to induce rejection of skin allografts in mice tolerized by an anti-CD40 ligand protocol and to determine whether the rejection is mediated by T cells cross-reactive between viral and allo-antigens.
Role: Project Leader

R01 AI081675-27 Welsh (PI) 07/17/09 - 06/30/14
NIH/NIAID
Virus-induced immunopathology
This proposal examines the evolution of the T cell repertoire during viral infections of mice and examines how crossreactive T cells may regulate the control of infections by unrelated viruses.
Role: PI

R37 AI017672-34 Welsh (PI) 04/01/09 - 03/31/14
NIH/NIAID
Immunity and virus disease
This MERIT award examines T cell apoptosis and memory T cell loss during viral infection. The specific aims are to (1) determine the mechanism and significance of the early cytokine-induced lymphopenia and apoptosis of memory CD8 T cells during viral infections, (2) determine the mechanisms regulating the tissue-dependent differences in apoptosis of virus-specific CD8 T cells, and (3) determine the mechanism and significance of memory T cell attrition following acute and persistent viral infections.
Role: PI.

# PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

## 1. Human Subjects Section

Clinical Trial? ○ Yes ● No

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

## 2. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

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

● Yes ○ No

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

## 3. *Program Income Section

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

○ Yes ● No

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

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

# PHS 398 Cover Page Supplement

## 4. Human Embryonic Stem Cells Section

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

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

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

Cell Line(s) (Example: 0004):

## 5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ○ Yes ○ No

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

*Previously Reported: ○ Yes ○ No

## 6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

# PHS 398 Modular Budget

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

## Budget Period: 1

Start Date: 04/01/2017 End Date: 03/31/2018

### A. Direct Costs

| | Funds Requested ($) |
|---|---|
| Direct Cost less Consortium Indirect (F&A)* | 150,000.00 |
| Consortium Indirect (F&A) | |
| Total Direct Costs* | 150,000.00 |

### B. Indirect (F&A) Costs

| | Indirect (F&A) Type | Indirect (F&A) Rate (%) | Indirect (F&A) Base ($) | Funds Requested ($) |
|---|---|---|---|---|
| 1. | MTDC | 98.00 | 150,000.00 | 147,000.00 |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Regina DiGennaro

Indirect (F&A) Rate Agreement Date: 10/27/2015

Total Indirect (F&A) Costs: 147,000.00

### C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): 297,000.00





Funding Opportunity Number: PA-16-161. Received Date: 2016-08-15T11:26:57.000-04:00

# PHS 398 Modular Budget

| Budget Period: 2 | | | |
|---|---|---|---|
| Start Date: 04/01/2018 End Date: 03/31/2019 | | | |
| **A. Direct Costs** | | | Funds Requested ($) |
| | | Direct Cost less Consortium Indirect (F&A)* | 125,000.00 |
| | | Consortium Indirect (F&A) | |
| | | Total Direct Costs* | 125,000.00 |

**B. Indirect (F&A) Costs**

| | Indirect (F&A) Type | Indirect (F&A) Rate (%) | Indirect (F&A) Base ($) | Funds Requested ($) |
|---|---|---|---|---|
| 1. | MTDC | 98.00 | 125,000.00 | 122,500.00 |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Regina DiGennaro

Indirect (F&A) Rate Agreement Date: 10/27/2015

Total Indirect (F&A) Costs: 122,500.00

**C. Total Direct and Indirect (F&A) Costs (A + B)** Funds Requested ($) 247,500.00





Funding Opportunity Number: PA-16-161. Received Date: 2016-08-15T11:26:57.000-04:00

# PHS 398 Modular Budget

## Cumulative Budget Information

### 1. Total Costs, Entire Project Period

| | |
|---|---|
| Section A, Total Direct Cost less Consortium Indirect (F&A) for Entire Project Period ($) | 275,000.00 |
| Section A, Total Consortium Indirect (F&A) for Entire Project Period ($) | 0.00 |
| Section A, Total Direct Costs for Entire Project Period ($) | 275,000.00 |
| Section B, Total Indirect (F&A) Costs for Entire Project Period ($) | 269,500.00 |
| Section C, Total Direct and Indirect (F&A) Costs (A+B) for Entire Project Period ($) | 544,500.00 |

### 2. Budget Justifications

| | |
|---|---|
| Personnel Justification | PersonnelJustification.pdf |
| Consortium Justification | |
| Additional Narrative Justification | |



Tracking Number: GRANT12232147
Funding Opportunity Number: PA-16-161. Received Date: 2016-08-15T11:26:57.000-04:00

Budget Justification:

Personnel

Marcia A. Blackman, Ph.D. [EFFORT] effort) will share responsibility with Dr. Reiley for directing the experiments and communication of data in publications and at meetings. She will supervise the senior research associate and co-supervise the TBN post-doc. Dr. Blackman is a highly experienced viral immunologist and has expertise in analysis of the response of aged mice to influenza virus infection. Dr. Blackman is a Full Member of Trudeau Institute.

William Reiley, Ph.D. [EFFORT] effort) will share responsibility with Dr. Blackman for directing the experiments and communication of data in publications and at meetings. He will co-supervise the TBN post-doctoral fellow and the senior research associate. Dr. Reiley is an experienced infectious disease immunologist and has expertise in immunity to a variety of pathogens in the mouse model. In addition, as Director of the Molecular Biology Core at Trudeau Institute, Dr. Reiley has substantial expertise in growing and tittering viral stocks, passaging the *Heligmosomoides polygyrus* and in generating and optimizing MHC tetramers for detection of epitope-specific T cells and tetramer pull-down assays. Dr. Reiley is an Assistant Member of Trudeau Institute.

TBN postdoctoral fellow (100%) will be co-supervised by Drs. Blackman and Reiley. The TBN post-doc will carry out experiments outlined in the proposal, with the help of the research associate.

Tres Cookenham, B. S., [EFFORT], a senior research associate, will be supervised by Dr. Blackman and will provide technical support for all aspects of the project. He currently works in Dr. Blackman's lab on influenza vaccination studies in aged mice and is experienced in technical aspects of the project.

Other significant contributors:

Raymond M. Welsh, Ph.D. [EFFORT] alendar months) is an Emeritus Professor of Pathology at the University of Massachusetts with expertise in sequential viral infections, heterologous immunity and T cell cross-reactivity. He will serve as a consultant for our experiments and share his expertise with MCMV viral infections.

Lawrence L. Johnson, Ph.D. [EFFORT] alendar months) is a Distinguished Professor Emeritus at Trudeau Institute. He will serve as a statistical consultant for the proposed experiments.

Herbert (Skip) Virgin, M.D., Ph.D., [EFFORT] alendar months) is a Professor and Chair of Pathology and Immunology at Washington University. He will serve as a consultant for the sequential infections and the interpretation of the Bioinformatics data.

# PHS 398 Research Plan

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

| | |
|---|---|
| **Introduction** | |
| 1. Introduction to Application (Resubmission and Revision) | Introduction.pdf |
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| 8. Inclusion of Children | |
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| 11. Multiple PD/PI Leadership Plan | LeadershipPlan.pdf |
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| 13. Letters of Support | SupportLetters.pdf |
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| **Appendix** | |
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## INTRODUCTION

reviewers' comments

reviewers' comments

reviewers' comments

## SPECIFIC AIMS

Immune function declines with age. Therefore, it is important to understand mechanisms underlying immune dysfunction with age to enhance the quality of life in later years for the elderly. Humans are riddled throughout life with a variety of acute and chronic infections which trigger the immune system. The accumulating effect of acute and chronic infections throughout the lifespan profoundly impacts the T cell repertoire and immune response of aged individuals. Although the aging mouse provides an experimental model amenable to addressing immunological mechanisms, an important limitation is that mice are typically housed in specific pathogen free (SPF) conditions, which fail to mimic the human situation of a lifetime of infections. We propose to develop a better aging mouse model by generating aged mice with defined exposure early in life to sequential infections. Recently it was shown that sequentially-infected and "dirty" (co-housed with pet store mice) adult mice expressed gene expression profiles more similar to adult humans than SPF-housed mice, which had a gene expression profile more similar to immature, neonatal humans (Reese, et al., 2016. Cell Host and Microbe 19:713; Beura, et al., 2016. Nature 532: 512). The antigen-experienced adult mice also had different T cell phenotypes and responses to infection and vaccination, confirming the impact of accumulating antigen experience throughout the lifespan. Indeed, our preliminary data show no change in the T cell phenotype between SPF-housed adult and aged mice, confirming the importance of antigenic experience during aging. The first goal of this developmental R21 is to generate antigen-experienced (sequentially-infected) aged mice and to characterize differences with SPF-housed aged mice. We will compare transcriptional profiles to those of young and aged humans to determine if antigen experience makes the aged mice more "human-like". We will also compare the phenotype of memory T cells and cytokine markers of inflammation between the two groups.

Optimal immune responses to new infections are thought to be dependent on a diverse repertoire of naïve T cells. With age, the numbers and diversity of naïve T cells decline and the ratio of memory to naïve T cells greatly increases. It has been determined that T cell recognition of antigen/MHC is highly degenerate and T cell responses exhibit extensive and unexpected cross reactivity. We hypothesize that, with the declining numbers of naïve T cells with age, the response to new infections becomes increasingly dependent on memory cells that accumulate with antigen experience and are fortuitously cross reactive. The second goal of this developmental R21 is to test the hypothesis that sequentially-infected aged mice, by virtue of enhanced antigen experience, will manifest increased diversity in the memory T cell repertoire capable of cross-reacting with new infections. Thus, we will examine the numbers and epitope diversity of influenza-specific memory T cells from influenza-naïve aged mice that are antigen-experienced compared with SPF-housed aged mice.

**Aim 1. To generate and characterize "antigen experienced" aged mice that have been sequentially infected with pathogens that establish acute and chronic infections.** Young male and female mice will be sequentially infected with correlates of common human pathogens- two viruses that establish chronic infections (γHV68 and MCMV, mouse correlates of EBV and CMV), Sendai virus, a para-influenza virus that establishes an acute respiratory infection, and *Heligmosomoides polygyrus* (*H. polygyrus*), an intestinal parasite that chronically infects a large proportion of the world's population. We will compare the T cell response to each sequential pathogen with that of SPF mice given a single infection. Following the sequential infections, mice will be aged to 18-22 months and analyzed. First, transcriptional profiles will be compared between aged antigen-experienced and SPF mice, and also with PBL from aged and young humans. Second, the phenotype of memory T cells will be compared. Third, markers of inflammation will be compared. These experiments will test the hypothesis that antigen-experienced aged mice will more faithfully represent aged humans.

**Aim 2. To test the hypothesis that sequentially-infected compared to SPF-housed aged mice will manifest enhanced diversity in the cross-reactive memory T cell repertoire capable of responding to new infections.** We hypothesize that cross-reactive memory T cells make an important contribution to the response to new infections in the face of waning naïve T cells associated with aging. We will assess the diversity of the memory T cell repertoire in two ways. First, we will use tetramer pull-down assays to determine the magnitude of T cells specific for two immunodominant influenza virus epitopes in SPF-housed compared with sequentially-infected aged *influenza-naïve* mice. Second, we will assess the repertoire diversity of the cross-reactive memory in the T cell response to *de novo* infection with influenza virus by adoptive transfer experiments. These experiments will test the hypothesis that antigen-experience will enhance the diversity of the cross-reactive memory T cell response to new infections encountered in old age.

Accomplishing the goals of this *developmental R21* will be an important advance for aging research by *providing a more physiologically relevant, antigen-experienced mouse model for studying aging immunology.* Studies in antigen-experienced aged mice will benefit understanding the impact of antigen experience on immunity in elderly humans and has important implications for vaccination strategies for the elderly, supporting the concept that vaccines in young and middle age are important for maintaining immunity in later life.

## Research Strategy

### (a) Significance

Immune function declines with aging. As people are living longer, it is important that we understand the impact of aging on the immune system so that we can develop therapeutic strategies for improving survival and quality of life for the elderly. The aging mouse model has many experimental advantages in terms of dissecting mechanisms underlying impaired immunity in the elderly. However, there are acknowledged limitations of the model. One important limitation is that mice are typically maintained under specific pathogen free (SPF) conditions, whereas humans are exposed to a variety of pathogens throughout life. It has been proposed that the immune response to acute and chronic pathogens to which we are exposed throughout life generates a powerful imprint on immunity and that the use of SPF mice makes mice a less relevant model for human immunity (1-3). This issue may be especially important for models of aging, as immunological experience has a profound impact on immune function over time. Two recent studies illustrated dramatic changes in the immune profile of "dirty" mice (mice that had been sequentially infected with pathogens, wild mice, pet store mice and laboratory mice co-housed with pet store mice) (4, 5). In the current proposal we will sequentially infect mice with pathogens that establish chronic and acute infections, according to the recently published protocol (4), prior to aging the mice. We will compare sequentially-infected aged mice with SPF-hused aged mice in terms of transcriptional profiles, T cell phenotype and inflammatory cytokines. *We hypothesize that the generation of sequentially-infected aged mice will provide an improved mouse model for aging research.*

A diverse repertoire of naïve T cells is thought to be necessary for an optimal response to new infections (6, 7) However, there is a decline of numbers and diversity of naïve T cells with age, as a consequence of three factors- thymic arophy and generation of fewer naïve T cells, increase in memory cells as a consequence of accumulating antigen experience, and the development of T cell clonal expansions (TCE) in the memory pool (8-16). This decline in naïve T cells with aging correlates with impaired immunity (17-20). Our studies confirmed that with declining numbers of naïve CD8 T cells, aged mice respond poorly to *de novo* infection with influenza virus (21). We have hypothesized that with the age-associated decline in numbers and repertoire diversity of naïve CD8 T cells, the response to *de novo* infections in aged mice is mediated largely by fortuitously cross reactive memory CD8 T cells generated by exposure to a variety of pathogens throughout the lifespan (20). This hypothesis is supported by the demonstration that T cell recognition of antigen/MHC is highly degenerate and T cell responses exhibit extensive and unexpected cross reactivity (19, 22). In further support of this, unexpected cross-reactivity has been demonstrated between CD8 T cells specific for different viruses (23-31). In the current proposal, we will determine the magnitude and diversity of the repertoire of memory cells that can cross-react with influenza virus epitopes in sequentially-infected compared with SPF-housed aged mice.

*The first goal of the proposal is the generation of a better mouse model for aging research by sequentially infecting mice with pathogens prior to aging. We will compare these antigen-experienced aged mice to SPF-housed aged mice in terms of transcriptional profile, T cell immune phenotype and markers of inflammation. The second goal is to use these mice to determine the impact of sequential infections on the response of CD8 memory T cells to new infections in aged mice that more accurately model aged humans. These studies are significant because proof of our hypothesis, that antigenic experience and immunologic memory enhance the response of aged individuals to new infections, will provide experimental support for the concept of promoting increased vaccination in young and middle aged individuals to enhance immunity in old age.*

### (b) Innovation

The main innovative feature of this proposal is the development of an improved mouse model for aging research. This would be a significant contribution to the field by providing a mouse model which is more representative of human aging. It is worth trying to improve the mouse model, rather than abandoning it, because it provides a valuable experimental system for defining mechanisms underlying dysfunction of immunity in the elderly.

### (C) Approach

#### *Preliminary data*

***Phenotypic analysis of T cells from young and aged SPF-housed mice.*** It has recently been shown that there are major phenotypic differences in T cells from adult SPF-housed compared to antigen-experienced "dirty" mice (5). For example, the phenotype of memory cells in dirty mice showed down-regulation of CD62L, CD127, and CD27, whereas there was an upregulation of KLRG1, granzymeB and 1B11 compared with their SPF-housed cohorts. We extended this analysis to aged mice by comparing the phenotype of young (3-6 months) and aged (18-22 months) SPF-housed mice. The data in **Figure 1** show our results for young mice

are consistent with the Beura results for SPF-housed mice. Importantly, there is very little difference in phenotype in the aged compared to young SPF-housed mice! In the Beura study, this phenotype corresponded to a transcriptional profile consistent with neonatal humans, reinforcing the idea that SPF-housed aged mice are not good models for aged humans. These data strengthen our hypothesis that sequentially-infected aged mice will be more relevant models for aged humans. *In the current proposal we will generate aged mice that have been sequentially infected with four pathogens that establish chronic or acute infections, similar to the protocol of Reese, et al. (4). Experiments in Aim 1 will (1) generate a more physiologically-relevant mouse model for aging and (2) allow us to determine the impact of antigen experience during aging on memory populations and their contribution to new infections in aged mice.*

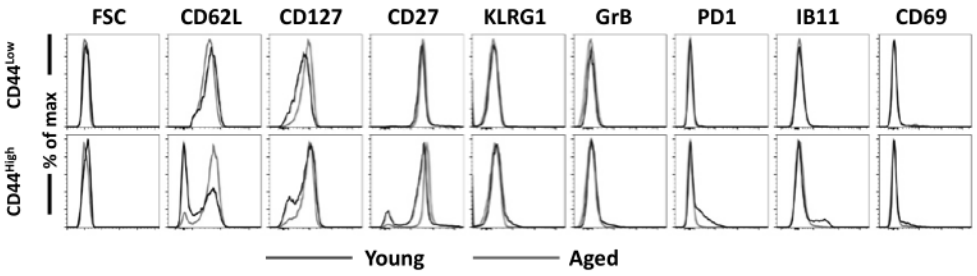


**Figure 1. Phenotypes of young and aged SPF mice.** CD44$^{low}$ (naïve) and CD44$^{high}$ (memory) CD8 T cells from young and aged mice maintained in SPF conditions show little difference in phenotypes that were previously shown to differ between "dirty" pet store and SPF laboratory mice by Beura, et al., 2016. Specifically, "dirty mice" showed strong down-regulation of CD62L, CD127, and CD27 and up-regulation of KLRG1, GrB and1B11. The absence of these phenotypic changes in aged SPF mice indicates that in the absence of antigenic experience their phenotype changes very little from young mice. We anticipate that phenotypic changes similar to those found in "dirty" mice will occur in sequentially-infected aged mice. The data are representative of three mice per group.

***Response of memory cells to de novo influenza infection in aged SPF-housed mice.*** We have a long standing interest in determining the impact of age on CD8 T cell immunity to new infections and CD8 memory, using the aged mouse influenza virus infection model. We and others have shown that the numbers and repertoire of naïve CD8 T cells declines with age (10, 21, 32-34). We have hypothesized that with the declining numbers of naïve T cells with age, the response to new infections become increasingly dependent on memory cells that accumulated over time with antigen experience and are fortuitously cross reactive. In support of this, analysis of the response of memory CD8 T cells from aged, influenza naïve SPF-housed mice has shown that memory CD8 T cells can fortuitously cross react with influenza virus epitopes (**Figure 2**). Analysis of the the response using tetramers showed that the epitope-specific response of memory cells from individual aged was less diverse than the response of young mice mediated by naïve CD8 T cells, and was distinct in individual mice. *Experiments in Aim 2 will test the hypothesis that sequentially-infected aged mice, by virtue of enhanced antigen experience and a more diverse memory T cell repertoire, will serve as better models for determining the role of cross-reactive memory in the response to new infections in old age.*

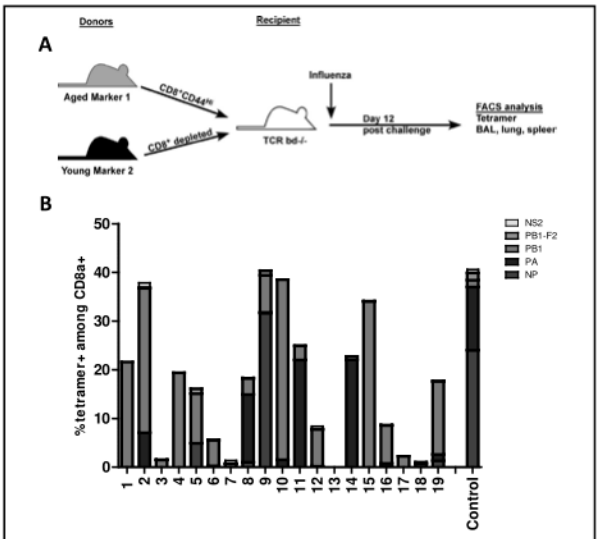


**Figure 2. Memory cells from aged SPF mice can respond to *de novo* influenza virus infection. A.** FACS-sorted memory (CD44$^{high}$) CD8 T cells from individual aged mice were adoptively transferred into T cell-deficient βδ-/- mice along with CD8-depleted splenocytes from young, congenically disparate mice. Recipient mice were challenged i.n. with x31 influenza virus (H3N2, 3000 $EID_{50}$). **B.** Bronchoalveolar lavage (BAL) was harvested at day 12 post infection and the epitope specificity of the flu-specific cells from individual aged mice and a control young mouse was determined using tetramers. T cells specific for individual tetramers are indicated by color.

**Aim 1. To generate and characterize "antigen experienced" aged mice that have been sequentially infected with pathogens that establish acute and chronic infections.**

**Rationale:** Concerns have been raised about the mouse aging model, namely that SPF mice are not relevant models for humans, who experience a wide variety of infections throughout their lifespan (1-3). We will generate an aging mouse model that is more relevant to human aging by sequentially infected young mice with four chronic and acute pathogens and then allowing the mice to age. These experiments will test the hypothesis that antigen-experienced aged mice will more faithfully represent aged humans.

*Experiment #1. To compare sequential with individual infection of young male and female mice.*

*Experimental Design:* Recently it was shown that a series of sequential infections rendered laboratory mice better models for adult humans (4). In the current proposal we will mimic these sequential infections, with one difference- we will substitute Sendai virus, a mose para-influenza virus, for influenza so that we can takeadvantage of the well-characterized immune response to influenza virus in the experimental read-out. Because of the success of the published studies, we have no concerns that the sequential infection regimine will generate mice with transcriptional profiles and immunological parameters more similar to humans. However, in the current experiment, we will analyze the response after each infection in the series compared with a single infection of SPF mice in order to better characterize the nature of the infection series. Thus, we will sequentially infect cohorts of young (3 month old) SPF C57BL/6 (B6) male and female mice from Jackson laboratories with two chronic viruses (γHV68 and MCMV, mouse correlates of EBV and CMV), an acute respiratory virus (Sendai virus) and an intestinal Helminth (*H. polygyrus*) as outlined in **Figure 3**. In order to confirm infectivity of each pathogen, we will compare the response of mice at each stage of infection with SPF mice given a single infection, using our panel of tetramers for γHV68, MCMV and Sendai viruses, and by determining the worm burden after *H. polygyrus* infection. The pathogens, doses and route of inoculation, and tetramers to be used are listed in **Table 1**. We have existing stocks of γHV68, Sendai virus and various strains of influenza virus that are currently in use. MCMV (Smith strain, ATCC:VR194) will be grown in salivary glands of BALB/c mice and titered on murine embryonic fibroblasts (35). *H. polygyrus* is continuously passaged at Trudeau Institute in BALB/c mice, so we have a source of L3 stage larvae (36). We have stocks of the relevant tetramers for γHV68, Sendai and influenza and we will generate the MCMV tetramers in our tetramer facility in the Trudeau Institute Molecular Biology Core.

| Pathogen | Dose and Administration | MHC tetramers | Reference |
|---|---|---|---|
| *H. polygyrus* | 50-200 L3 larvae; gavage | Not available | Reese, et al., 2016. Cell Host and Microbe 19:713. King, et al., 2010. J Immunol. 185: 6138. |
| γHV68 | 400 PFU; i.n. | $ORF6_{487-495}/D^b$ $ORF61_{524-531}/K^b$ $M2_{124-138}/IA^b$ | Stevenson, et al., 1998. PNAS, USA 95:15565.; Freeman, et al., 2014. J. Immunol. 193:5827. |
| MCMV | $3 \times 10^5$ PFU; i.p. | $M38_{316-323}/K^b$ $M45_{985-993}/D^b$ | Sims, et al., 2015. Eur J Immunol 45:113. |
| Sendai virus | 250 $EID_{50}$; i.n. | $NP_{324-332}/K^b$ | Kast, et al., 1991. PNAS USA 88:2283. |
| Influenza | 300 $EID_{50}$ A/PR/8/34 (PR8, H1N1); i.n. | $NP_{366-374}/D^b$ $PA_{224-233}/D^b$ $PB1_{703-711}/K^b$ $PB1\text{-}F2_{62-70}/D^b$ $NS2_{114-121}/K^b$ $NP_{311-324}/IA^b$ | Townsend, et al, 1986. Cell 44:959.; Belz, et al., 2000. J Virol. 74: 3486; Belz, et al., 2001. J Immunol 166: 4627; Chen, et al., 2001. Nature Medicine 7: 1306.; Vitiello, et al., 1996. J Immunol 157:5555; Lanzer, et al., 2014. Immunity and Ageing 11:9. |

**Table 1. Dose and route of administration of pathogens and relevant major MHC class I and II tetramers.**

*Anticipated results, discussion, pitfalls and future directions:* We are not concerned with our ability to carry out the sequential infections. We have a long publication history with infection of SPF mice with influenza virus, γHV68 and Sendai virus (21, 37-41) and we have large frozen stocks of these viruses. *H. polygyrus* has been previously used and is continuously passaged at Trudeau Institute so that larvae are readily available. Whereas MCMV has been used at Trudeau Institute in the past, we have not personally had experience with this virus. Dr. Ray Welsh has offered to help us and provide initial viral stocks (see letter of consultation). Trudeau Institute has a long-standing reputation of excellence in mouse models of infectious disease and the animal facilities at Trudeau Institute are ideally suited for housing of infected mice (see letter of support from the Animal Facilities Manager). After each infection, the CD4 and CD8 tetramer response of sequentially infected mice will be compared with age-matched SPF mice infected

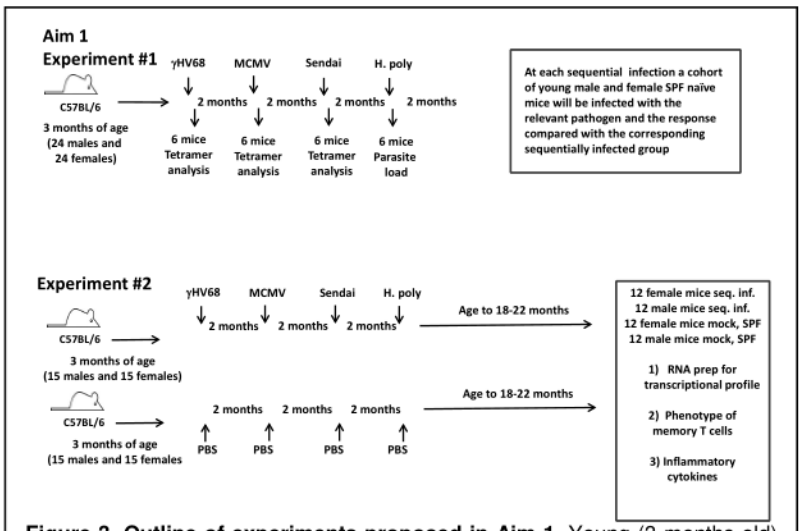


**Figure 3. Outline of experiments proposed in Aim 1.** Young (3 months old) male and female C57BL/6 mice will be sequentially infected with pathogens, as shown. In experiment #1, after each infection, the tetramer or pathogen load will be compared with naïve young SPF mice receiving only one infection. In experiment #2, the sequentially infected mice and SPF mock-infected mice will be housed until 18-22 months of age, at which time they will be analyzed for transcriptional profiles, phenotype of memory T cells and inflammatory cytokines, as outlined in the Materials and Methods.

with only the current virus to determine whether the previous infection history in the sequentially-infected mice is dramatically skewing the response. Subtly altered responses will not impact the development of memory T cells, as long as the infection "takes", which will be determined by measuring the CD8 tetramer response, or worm burden in the case of *H polygyrus*. We have no concern with success in making the mice more "human-like" because we are using a protocol of infection similar to that established by Reese, et al. (4). Because we are allowing 2 months between each infection, and infecting with sub-lethal doses of each virus, we hope to reduce the chances that a preceding infection will grossly impact the infectivity of the subsequent infection. *H. polygyrus*, γHV68 and MCMV establish life-long latency, and there have been both positive and negative impacts on subsequent infections reported. For example, both γHV68 and MCMV latency have been shown to enhance survival to subsequent influenza virus infection, with an enhanced CD8 T cell response (42, 43). Although it has been shown that MCMV dampens responses to LCMV, influenza, HSV and West Nile viruses in aged mice (44, 45), our infections will all be done in young mice. Dr. Ray Welsh, who has extensive experience wth sequential infections (29, 31, 46, 47) will serve as a consultant (see letter of support). We are not concerned because we are following the successful protocol recently published (4) and will consult with Dr. Skip Virgin if we have problems (see letter of support).

*Experiment #2. To characterize "antigen experienced" aged mice that have been sequentially infected with pathogens that establish acute and chronic infections.*

*Experimental design:* Cohorts of 15 each male and female young (3 months of age) C57BL/6 mice will be sequentially infected or sham infected as in experiment #1. They will then be housed under SPF conditions until 18-22 months of age. They will then be analyzed in three ways. First, transcriptional profiles will be determined and compared with those from young and aged humans. We will prepare RNA from PBL from sequentially infected and SPF-housed aged male and female mice and from young and elderly humans using a RiboPure RNA purification kit. RNA array analysis will be carried out on individual mice and humans in a collaboration with the Gene Expression and Genomics Unit of the Laboratory of Genetics of the National Institute on Aging (see letter of support from Dr. Kevin Baker). Bioinformatics analysis, guided by the analysis by Reese, et al. (4) will be carried out. Second, we will assess the phenotype of memory T cells from lung, spleen and peripheral lymph nodes of individual mice, as in preliminary data Figure 2. Third, we will measure inflammatory cytokines using Luminex assays to determine the impact of sequential infections on the proinflammatory status of aged mice.

*Anticipated results, discussion, pitfalls and future directions:* Transcriptional profile: The key question is whether we will identify age-associated changes in the transcriptional profile of aged compared to young humans, and whether similar changes are reflected in the aged sequentially-infected but not SPF-housed aged mice. Our collaborators require 1 μg of RNA from each sample for array analysis and feel that analysis of individual mice is better than pooling samples. This amount of RNA is readily attainable from individual mouse peripheral blood after terminal bleeding. Our collaborators will carry out the array analysis and the subsequent bioinformatics analysis, guided by the studies of Reese, et al. (4). Similar analysis of peripheral blood samples from aged and young humans, provided by Dr. Laura Haynes of the Center on Aging of the University of Connecticut, will also be carried out. T cell phenotype: We will assess phenotype by analysis of markers used in preliminary data, **Figure 1**. Tissues (spleen, lymph node, lung and peripheral blood) from the sequentially infected and SPF aged mice will also be analyzed in terms of naïve, central memory (CM), effector memory (EM) and resident memory (RM) T cells among total CD8 T cells and tetramer positive CD8 T cells. We will also monitor the frequency of total CD8 T cells and memory CD8 T cells specific for all the pathogens, using the panel of tetramers in **Table 1**. CMV infects the majority of the world's population and, with age, the human repertoire becomes dominated by CMV-specific cells (48-50). Therefore, it will be important to measure T cells specific the inflationary MCMV epitope, $M139_{419-426}/K^b$, in the sequentially infected mice to determine their degree of dominance (14, 51). Also, it has also been shown that *H. polygyrus* infection causes reactivation of latent γHV68 (52). Although this apparently was not a problem in the successful published generation of sequentially-infected mice (4), we will carefully examine expression of several γHV68-specific epitopes after infection with *H. polygyrus*. A potential concern is that sequential infections may shorten the lifespan of the mice, in the absence of an additional infection. We don't think this will be a problem with our low doses of pathogens, but if this is the case, we have set up extra cohorts of mice (Figure 5) and it is possible that mice will have to be considered aged and analyzed earlier than at 18-22 months. Inflammatory profile: We anticipate that sequentially-infected aged mice will demonstrate an enhanced inflammatory state, determined by customized Luminex analysis of inflammatory cytokines. We will assess pro-inflammatory cytokines, including IL-1, IL-2, IL-6, IL-12 TNFα, IFNγ, etc., and anti-inflammatory cytokines including TGFβ, IL-4, IL-10, etc. (53). It is important to assess the consequences of sequential infection on inflammatory cytokines, as inflammation is a key characteristic of human aging (54-56).

**Aim 2. To test the hypothesis that sequentially-infected compared to SPF-housed aged mice will manifest enhanced diversity in the cross-reactive memory T cell repertoire capable of responding to new infections.**

**Rationale**: We have hypothesized that as numbers and the repertoire diversity of naïve CD8 T cells decline with age, fortuitously cross reactive memory cells generated in response to previous infections dominate the response to new infections (20). This has significance in terms of encouraging early priming in humans by vaccination to boost the memory T cell repertoire before age-associated immunesenescence sets in, as early exposure has been shown to result in the long-term maintenance of memory (57, 58). Confirming the importance of memory established througout life on the ability of the elderly to respond to new infections is therefore important. Our preliminary data show that memory cells from aged SPF mice can respond to *de novo* infection with influenza (**Figure 2**). It can be seen that the repertoire of epitope specific cross-reactive responses is more restricted than the repertoire in young mice (presumably largely mediated by naïve T cells), and varies with individual mice. We hypothesize that over a lifetime of exposure to pathogens, the memory repertoire will increase in diversity and therefore the response of fortuitously cross reactive memory cells would also be more diverse. The specific goal of this Aim is to assess the contribution of infections throughout life to the cross-reactive memory response to *de novo* influenza infection in SPF and sequentially infected aged mice, using two distinct read-outs. First, we will determine the absolute number and frequency of cells specific for two immunodominant influenza epitopes, nucleoprotein (NP) and acid polymerase (PA), in influenza-naïve SPF-housed and sequentially infected aged mice, using tetramer pull-down assays. Second, we will determine the response of (cross-reactive) memory cells from influenza-naïve SPF-housed and sequentially-infected aged animals to influenza virus infection after adoptive transfer into "empty" hosts.

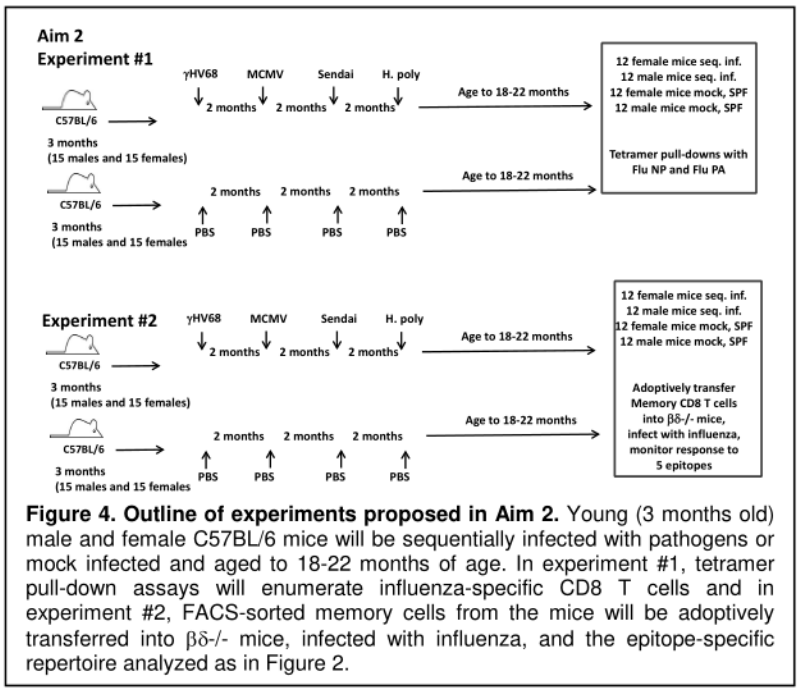


**Figure 4. Outline of experiments proposed in Aim 2.** Young (3 months old) male and female C57BL/6 mice will be sequentially infected with pathogens or mock infected and aged to 18-22 months of age. In experiment #1, tetramer pull-down assays will enumerate influenza-specific CD8 T cells and in experiment #2, FACS-sorted memory cells from the mice will be adoptively transferred into βδ-/- mice, infected with influenza, and the epitope-specific repertoire analyzed as in Figure 2.

*Experiment #1.* To assess the contribution of cross-reactive memory T cells generated in response to irrelevant infections to T cells cross-reactive to Flu NP and PA in influenza-naive aged mice by tetramer pulldown experiments.

*Experimental design*: We hypothesize that cross-reactive memory cells dominate the response to new infections upon decline of naïve T cell numbers and diversity associated with aging. Here we will directly quantitate influenza-specific T cells in sequentially infected and aged mice that have never been exposed to influenza virus. Tetramer pull-down analysis will be used to determine the frequency and absolute numbers of NP- and PA-specific cells in sequentially infected or SPF aged mice, according to standard protocols (59, 60). Single cell suspensions from major lymph nodes, lung, liver and spleen will be prepared using collagenase D, stained with the appropriate PE- and APC-conjugated tetramers (in the presence of azide to prevent tetramer internalization), washed, incubated with anti-PE- and APC-coupled MACS microBeads and isolated on a magnetized MACS column, as described (59). Cells will be released from the column and stained with antibodies including CD4, CD8, and CD44. Absolute numbers and frequencies of influenza epitope-specific cells in individual influenza-naïve sequentially infected and SPF-housed aged mice will be determined, as previously (59).

*Anticipated results, discussion, pitfalls and future directions:* We anticipate that there will be more memory cells that cross react with Flu NP and PA epitopes in the aged mice that have been sequentially infected. This would support the hypothesis that antigen experience enhances the pool of cross-reactive memory cells. We will extend the analysis by staining tetramer-positive cells with a panel of T cell receptor Vβ antibodies, to determine the repertoire diversity of the epitope-specific cells. One factor complicating the interpretation of the results is the development of T cell clonal expansions (TCEs), which are non-malignant, monoclonal populations of CD8 T cells that arise in both mouse and human. TCEs develop in approximately 50% of SPF aged mice, and it is likely that their development will be enhanced by the sequential infections, which include

chronic antigen stimulation thought to induce TCEs (61). We have also shown that TCE can arise from acute viral infections (62-64), further increasing the chances that they will be more frequent in sequentially infected aged mice. To assess TCEs we will pre-screen all aged mice for T cell receptor Vβ8 expression, using a Vβ8-specific mAb F23.1. Our criteria for a TCE in and Vβ population is if the percent of CD8 cells bearing Vβ8 TCR is 4 SD above or below the mean in young mice (37, 62). The development of TCEs will mean that we may need to analyze more mice, as we will divide our results into TCE and non-TCE mice. We have built in repeat experiments (**Figure 5**) to allow for a larger "n". As TCE cause severe constraints in the diversity of the peripheral repertoire, it is important to assess the contribution of TCE to the (cross-reactive) memory repertoire in both groups of mice separately. Importantly, TCE develop in both mice and humans, so this is not an artifact of the mouse aging model. We have built in repeat experiments (**Figure 5**) to allow for a larger "n" because of complications with TCE.

*Experiment #2. To assess the complexity of cross-reactive memory T cells elicited following influenza infection of sequentially-infected compared to SPF-housed, influenza-naïve, aged mice.*

*Experimental design*: To complement the above experiments, we will determine the (cross-reactive) T cell repertoire to a panel of Flu tetramers following adoptive transfer of memory CD8 T cells from sequentially-infected and SPF-housed aged mice into T cell-deficient "empty" hosts (βδ-/- mice) and infection with influenza virus, as in **Figure 2**. The response of CD8 T cells to 5 influenza epitopes (**Table 1**) will be determined at day 12 post infection.

*Anticipated results, discussion, pitfalls and future directions:* Experiment #2 is dependent on the ablity of aged, sequentially infected mice to respond to influenza. MCMV infected aged mice have been reported to have impaired CD8 immunity to a variety of pathogens, including influenza (14, 44, 45). Importantly, the response to these infections was reduced but not ablated, so we will be able to study the response of SPF and aged sequentially infected mice, and determine the contribution of pre-existing memory T cells. We will intranasally infect a cohort of sequentially-infected aged mice to confirm their ability to respond to Flu.

**Statistical analysis.** We will be in direct and frequent consultation with Dr. Larry Johnson for statistical analysis (see letter of support). In experiments where aged SPF mice are compared with sequentially infected aged mice, we will initially analyze groups of 12 mice. Analysis of the data we obtain will allow us to determine the actual statistical power achieved by experiments of that size. Thus, we will have data-based estimates for the inherent variability present in each type of experiment. With that information, we could adjust mouse numbers for future experiments, if necessary, to achieve adequate power of approximately 0.8. Data from different groups will ordinarily be compared statistically using Student's t-test or ANOVA with post-tests. Where appropriate, data will be log-transformed to satisfy requirements for use of the t-test. If the assumptions required for the use of parametric tests are not met, we will conduct appropriate nonparametric analyses. It is planned to replicate experiments at least twice to confirm reproducibility of results.

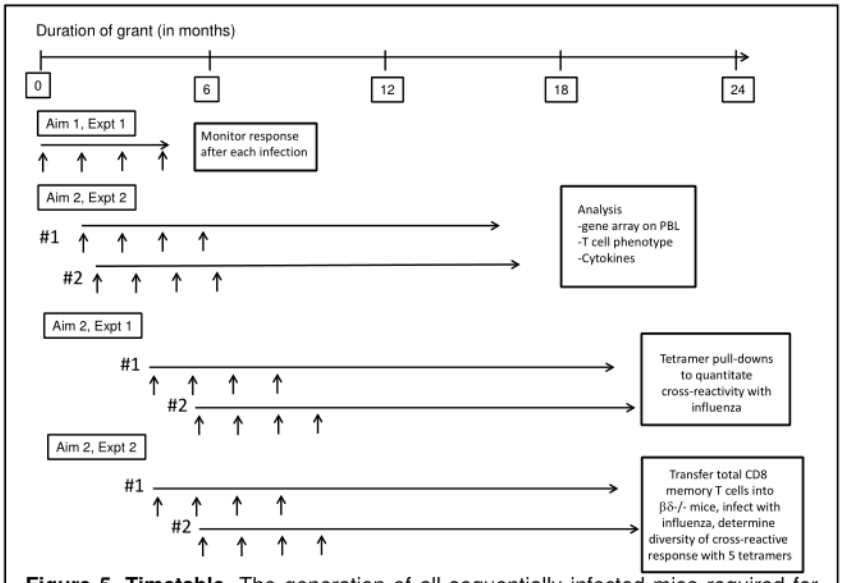


**Figure 5. Timetable.** The generation of all sequentially infected mice required for the proposal must be initiated in the first 6 months to allow time for aging and analysis within the 2 year limit of funding. We will set up two extra cohorts of mice to increase the "n" in caseof unexpected death of animals during aging and/or the development of TCE in greater than 50% of the aged mice.

**Summary and conclusions**. The proposed experiments test an important concept- that mice that have received carefully controlled infections prior to aging make better models for human aging. We acknowledge that the 4 pathogen infections included here does not cover the broad range of viral, bacterial and parasitic infections harbored by humans throughout their lifetime, but we believe it is an important first step. Confirmation of the role of fortuitously cross reactive memory cells in the response of aged individuals to new infections would be significant, and would support a program of increased vaccinations during adulthood into middle age to enhance immunity in the elderly. This proposal is well suited for the R21 mechanism, as the study is risky but would have high impact if successful. Trudeau Institute is the ideal place to carry out these experiments and Drs. Blackman and Reiley have the appropriate expertise to successfully complete the proposed experiments.

**VERTEBRATE ANIMALS:**

The proposed studies that use animals will be carried out at the Trudeau Institute (Animal Welfare Assurance number A-3075-01).

The following five points concern the use of mice:

(i) This study will use approximately 662 male and female mice (50 BALB/c mice, 516 C57BL/6 mice**,** and 96 B6.129P2-Tcrbtm1MomTcrdtm1Mom/j (βδ-/-) mice) as outlined below:

**Aim 1.**

**MCMV prep.** 50 BALB/c female mice to grow MCMV in the salivary glands.

60 C57BL/6 female mice will be needed to titer the new stock of MCMV.

**Experiment 1.** C57BL/6 mice will be infected sequentially with 4 pathogens (γHV68, MCMV, Sendai and *H. polygyrus*) as shown in **Figure 3**. Six mice will be sampled after each infection for the relevant tetramer (or worm titer for *H. Polygyrus*) to confirm successful infection x 2 (for SPF mock-infected or pathogen-infected) x 4 (different infections) x 2 (male and female) = 96 mice for Aim 1, experiment 1. Standard power calculations based on preliminary data indicate that with 6 young mice per group our experiments will have power = 0.8 to discover (with $\alpha$=0.05) whether a prior infection changes the frequency of tetramer positive cells in a subsequent infection with a different pathogen by 50% or more in comparison with infected control mice that have not been given prior infections.

**Experiment 2.** C57BL/6 (B6) mice (males and females) will either be sequentially infected with 4 pathogens (30 mice) or mock infected (PBS)(30 mice) and then housed until 18-22 months of age, at which time 12 mice per group will be analyzed for transcriptional profile, T cell phenotyping and inflammatory cytokines as in **Figure 3.** The experiment will be repeated and will use a total of 120 mice. By starting with 15 mice per group and analyzing 12 mice per group, we have built in 3 extra mice per group to allow for attrition during sequential infection and aging. Standard power calculations using preliminary data indicate that with 12 aged mice per group, our experiments will have power = 0.8 to discover (with α=0.05) whether previous infections change the the phenotype of the mice. We have built in the repeat experiments to allow for a larger “n” because of potential complications due to TCE.

**Aim 2.**

**Experiment 1.**

Fifteen each male and female three month old C57BL/6J (B6) mice will be sequentially infected or mock infected (SPF) and aged to 18-22 months of age, as in Aim 1. They will be analyzed by tetramer pull-down assays to determine the frequency and absolute number of (cross-reactive) influenza-specific cells in the memory population. This will yield 60 total mice for analysis and the experiment will be repeated. The large number of individual mice to be analyzed is necessary to accommodate both TCE and non-TCE mice.

**Experiment 2.**

Fifteen each male and female three month old C57BL/6J (B6) mice will be sequentially infected or mock infected (SPF) and aged to 18-22 months of age, as in Aim 1, and will serve as donor mice. Donor $CD44^{high}$ (memory cells) from individual mice will be injected into individual T cell-deficient B6.129P2-Tcrbtm1MomTcrdtm1Mom/j (βδ-/-) recipients, and the mice will be infected with influenza virus. There will be twelve donor mice for each of 4 groups (male and female, sequentially-infected or SPF-housed mice) = 48 mice. The experiment will be repeated. There will be an equal number of recipient mice (βδ-/-). Standard power calculations using preliminary data indicate that our experiments will have power = 0.8 for determining (with α=0.05) changes in repertoire diversity. We have built in the repeat experiments in case we need a larger “n” because of potential complications due to TCE.

The βδ -/- mice are maintained at Trudeau Institute. Young (2-3 months) C57BL/6 and BALB/c female mice will be purchased from Jackson Laboratories. Infected mice will be housed in a designated SPF ABSL2/3 containment facility. All experimental protocols have been approved by both the Biosafety Committee and Animal Care and Use Committee of Trudeau Institute. All studies involving infected cells and tissues will be carried out in a dedicated BSL2 lab. Mice will be infected intranasally with γHV68, Sendai or influenza virus, intraperitoneally with MCMV, and by oral gavage with *H. polygyrus*, according to the doses in **Table 1**. Intranasally infected mice will be anesthetized using Avertin (2,2,2 tribromoethanol, 300 mg/kg) or isofluorane anesthesia. Under these conditions, the symptoms of the

infections are not severe and mice recover completely within 20 days. At 18-22 months of age mice will be sacrificed, and various organs collected for analysis or adoptive transfer. If mice unexpectedly become lethargic and/or lose >20% of their body weight, they will be immediately sacrificed for humane reasons. Mice will be humanely sacrificed to attain blood and tissues for analysis. Adoptive transfer of cells into T cell-deficient bd-/- mice is by i.v. injection.

We will use both male and female mice for our experiments. We are aware that many studies of the immune response to infection have demonstrated significant differences between male and female mice.

Our power calculations were carried out by Dr. Larry Johnson, an expert in statistics, based on our preliminary data. Dr. Johnson will continue to help us with the statistical analysis of our data as an "other significant contributor" (see letter of consultation).

(ii) Analysis of the immune response to virus and helminth infection must be studied *in vivo*, using animals. There are no suitable *in vitro* models that recapitulate the complex interactions involved in the response to viral infections (based on literature searches using the keywords "mouse, viral infection, alternatives-animal testing"). Mice are the appropriate animal model for these studies because we are using mouse or mouse-adapted pathogens. Relatively large numbers of mice are necessary to obtain sufficient cells for experiments and to obtain sufficient data for statistical analysis, and to account for a large proportion of TCEs.

(iii) The animals will not suffer unnecessary discomfort, pain or injury. The intranasal infections are done under anesthesia, and the mice show no signs of distress. All infections are with a sub-lethal dose of virus or larvae. Analgesics cannot be used, as these interfere with the immune response, and the interpretation of data. If mice become unexpectedly moribund as a consequence of sequential infections, they will be sacrificed and the protocol for sequential infection re-evaluated. We will work in consultation with Drs. Ray Welsh and Skip Virgin (see letters of consultation), who have extensive experience with sequential infections of mice.

## SELECT AGENTS

This proposal does not involve select agents. All pathogens used are ABSL-2 pathogens and infected mice will be housed under ABSL-2 containment.

## Project Leadership Plan

### Rationale for choosing a multi-PI approach

The distinct areas of expertise of the PIs on this project are essential for its successful completion. Dr. Blackman has an extensive track record in studying the impact of aging on CD8 T cells and the T cell response to influenza virus. She is a viral immunologist with expertise in CD8 T cell immunity to respiratory viruses and γHV68, a mouse γ-herpesvirus. Dr. Reiley is an infectious disease immunologist with expertise in CD4 T cell responses during acute and chronic infections and head of TICRO BioServices (Trudeau Institute Contract Research Organization), which focuses on providing *in vivo* preclinical efficacy studies for industry and academic labs involving over 20 pathogens. In addition, Dr. Reiley is Manager of the Trudeau Institute Molecular Biology Core and has expertise in molecular techniques such as the generation of MHC class I and class II tetramers and tetramer pull-down assays, which will be valuable for the completion of the experimental Aims. Drs. Blackman and Reiley have initiated a close working relationship and routinely hold joint lab meetings.

### Roles and Responsibilities of the PIs

Drs. Blackman and Reiley will work together on all Aims of the proposal. Dr. Blackman will be responsible for setting up the sequential infections, tetramer analysis and adoptive transfer studies for analyzing the contribution of memory T cells to the *de novo* response to influenza virus infection. She will work directly with the Gene Expression and Genomics Unit of the Laboratory of Genetics for the microarray analysis. Her lab has expertise in the influenza virus, Sendai virus and γHV68 mouse infection models. Although we will obtain our initial viral stocks from our collaborator Ray Welsh (see letter of collaboration), Dr. Reiley will assist in the generation and tittering of future stocks of MCMV, as well as *Heligmosomoides polygyrus*, as his work with the CRO has provided him with extensive experience on the growth and characterization of a variety of mouse pathogens. Dr. Reiley will also be responsible for the production of all tetramer reagents and the pull-down assays for the proposed study. Both PIs will be responsible for directing the experiments, analysis and interpretation of data, and publication. All publications will be jointly authored, with corresponding authorship assigned appropriately. Drs. Blackman and Reiley will share responsibility for matters related to institutional oversight, including the use of animals and biosafety issues.

### Communication plans

Drs. Blackman and Reiley hold joint weekly lab meetings and have demonstrated that they can work together efficiently, each contributing their specific expertise to the design and interpretation of experiments.

### Process for decision making on scientific questions

Results and data interpretation will be discussed on a weekly basis. Decisions regarding long-term scientific goals will be made by consensus between Drs. Blackman and Reiley.

### Procedures for resolving conflicts

Conflicts that cannot be resolved by discussion between Drs. Blackman and Reiley will be referred to an arbitration committee at Trudeau Institute consisting of Dr. Atsuo Kuki (President and CEO) and William Chapin (COO) and a third impartial faculty member from Trudeau Institute, Dr. Jr-Shiuan Lin. No members of the arbitration committee will be directly involved in the research funded by this grant.

### Budget allocation

All funds will be allocated to Trudeau Institute, as outlined in the budget justification.

### Change in PI location

If a PI moves to a new institution, efforts will be made to transfer the relevant portion of the grant to that institution. In the event that a PI can no longer fulfill their duties, a new PI will be recruited from Trudeau institute as a replacement.

### Intellectual Property

The Technology Transfer office at Trudeau Institute will be responsible for preparing and negotiating an agreement for intellectual property sharing.

LITERATURE CITED:


1. Virgin HW, Wherry EJ, Ahmed R. 2009. Redefining chronic viral infection. *Cell* 138: 30-50.
2. Davis MM. 2012. Immunology taught by humans. *Science translational medicine* 4: 117fs2.
3. Goronzy JJ, Weyand CM. 2013. Understanding immunosenescence to improve responses to vaccines. *Nature Immunol* 14: 428-36.
4. Reese TA, Bi K, Kambal A, Filali-Mouhim A, Beura LK, Burger MC, Pulendran B, Sekaly RP, Jameson SC, Masopust D, Haining WN, Virgin HW. 2016. Sequential Infection with Common Pathogens Promotes Human-like Immune Gene Expression and Altered Vaccine Response. *Cell Host & Microbe* 19: 713-9.
5. Beura LK, Hamilton SE, Bi K, Schenkel JM, Odumade OA, Casey KA, Thompson EA, Fraser KA, Rosato PC, Filali-Mouhim A, Sekaly RP, Jenkins MK, Vezys V, Haining WN, Jameson SC, Masopust D. 2016. Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature* 532: 512-6.
6. Yewdell JW, Haeryfar SM. 2005. Understanding presentation of viral antigens to CD8(+) T cells in vivo: The Key to Rational Vaccine Design *. *Annu Rev Immunol* 23: 651-82.
7. Rudd BD, Venturi V, Li G, Samadder P, Ertelt JM, Way SS, Davenport MP, Nikolich-Zugich J. 2011. Nonrandom attrition of the naive CD8+ T-cell pool with aging governed by T-cell receptor:pMHC interactions. *Proc Natl Acad Sci U S A* 108: 13694-9.
8. Sempowski GD, Gooding ME, Liao HX, Le PT, Haynes BF. 2002. T cell receptor excision circle assessment of thymopoiesis in aging mice. *Mol Immunol* 38: 841-8.
9. Effros RB, Cai Z, Linton PJ. 2003. CD8 T cells and aging. *Crit Rev Immunol* 23: 45-64.
10. Naylor K, Li G, Vallejo AN, Lee WW, Koetz K, Bryl E, Witkowski J, Fulbright J, Weyand CM, Goronzy JJ. 2005. The influence of age on T cell generation and TCR diversity. *J Immunol* 174: 7446-52.
11. Lerner A, Yamada T, Miller RA. 1989. Pgp-1hi T lymphocytes accumulate with age in mice and respond poorly to concanavalin A. *Eur J Immunol* 19: 977-82.
12. Callahan JE, Kappler JW, Marrack P. 1993. Unexpected expansions of CD8-bearing cells in old mice. *J Immunol* 151: 6657-69.
13. Posnett DN, Sinha R, Kabak S, Russo C. 1994. Clonal populations of T cells in normal elderly humans: the T cell equivalent to "benign monoclonal gammapathy". *J Exp Med* 179: 609-18.
14. Smithey MJ, Li G, Venturi V, Davenport MP, Nikolich-Zugich J. 2012. Lifelong persistent viral infection alters the naive T cell pool, impairing CD8 T cell immunity in late life. *J Immunol* 189: 5356-66.
15. Britanova OV, Putintseva EV, Shugay M, Merzlyak EM, Turchaninova MA, Staroverov DB, Bolotin DA, Lukyanov S, Bogdanova EA, Mamedov IZ, Lebedev YB, Chudakov DM. 2014. Age-related decrease in TCR repertoire diversity measured with deep and normalized sequence profiling. *J Immunol* 192: 2689-98.
16. Lee JB, Oelke M, Ramachandra L, Canaday DH, Schneck JP. 2011. Decline of influenza-specific CD8+ T cell repertoire in healthy geriatric donors. *Immun Ageing* 8: 6.
17. Messaoudi I, Guevara Patino JA, Dyall R, LeMaoult J, Nikolich-Zugich J. 2002. Direct link between MHC polymorphism, T cell avidity, and diversity in immune defense. *Science* 298: 1797-800.
18. Messaoudi I, Lemaoult J, Guevara-Patino JA, Metzner BM, Nikolich-Zugich J. 2004. Age-related CD8 T cell clonal expansions constrict CD8 T cell repertoire and have the potential to impair immune defense. *J Exp Med* 200: 1347-58.
19. Nikolich-Zugich J, Slifka MK, Messaoudi I. 2004. The many important facets of T-cell repertoire diversity. *Nat Rev Immunol* 4: 123-32.
20. Woodland DL, Blackman MA. 2006. Immunity and age: living in the past? *Trends Immunol* 27: 303-7.
21. Yager EJ, Ahmed M, Lanzer K, Randall TD, Woodland DL, Blackman MA. 2008. Age-associated decline in T cell repertoire diversity leads to holes in the repertoire and impaired immunity to influenza virus. *J Exp Med* 205: 711-23, PMC2275391.
22. Mason D. 1998. A very high level of crossreactivity is an essential feature of the T-cell receptor. *Immunol Today* 19: 395-404.
23. Brehm MA, Pinto AK, Daniels KA, Schneck JP, Welsh RM, Selin LK. 2002. T cell immunodominance and maintenance of memory regulated by unexpectedly cross-reactive pathogens. *Nat Immunol* 3: 627-34.
24. Shimojo N, Cowan EP, Engelhard VH, Maloy WL, Coligan JE, Biddison WE. 1989. A single amino acid substitution in HLA-A2 can alter the selection of the cytotoxic T lymphocyte repertoire that responds to influenza virus matrix peptide 55-73. *J Immunol* 143: 558-64.
25. Selin LK, Cornberg M, Brehm MA, Kim SK, Calcagno C, Ghersi D, Puzone R, Celada F, Welsh RM. 2004. CD8 memory T cells: cross-reactivity and heterologous immunity. *Semin Immunol* 16: 335-47.

26. Selin LK, Welsh RM. 2004. Plasticity of T cell memory responses to viruses. *Immunity* 20: 5-16.
27. Welsh RM, Selin LK, Szomolanyi-Tsuda E. 2004. Immunological memory to viral infections. *Annu Rev Immunol* 22: 711-43.
28. Kim SK, Cornberg M, Wang XZ, Chen HD, Selin LK, Welsh RM. 2005. Private specificities of CD8 T cell responses control patterns of heterologous immunity. *J Exp Med* 201: 523-33.
29. Welsh RM, Che JW, Brehm MA, Selin LK. 2010. Heterologous immunity between viruses. *Immunol Rev* 235: 244-66.
30. Su LF, Kidd BA, Han A, Kotzin JJ, Davis MM. 2013. Virus-specific CD4(+) memory-phenotype T cells are abundant in unexposed adults. *Immunity* 38: 373-83.
31. Che JW, Selin LK, Welsh RM. 2015. Evaluation of non-reciprocal heterologous immunity between unrelated viruses. *Virology* 482: 89-97.
32. Ahmed M, Lanzer KG, Yager EJ, Adams PS, Johnson LL, Blackman MA. 2009. Clonal expansions and loss of receptor diversity in the naive CD8 T cell repertoire of aged mice. *J Immunol* 182: 784-92.
33. Goronzy JJ, Lee WW, Weyand CM. 2007. Aging and T-cell diversity. *Exp Gerontol* 42: 400-6.
34. Cicin-Sain L, Smyk-Pearson S, Currier N, Byrd L, Koudelka C, Robinson T, Swarbrick G, Tackitt S, Legasse A, Fischer M, Nikolich-Zugich D, Park B, Hobbs T, Doane CJ, Mori M, Axthelm MK, Lewinsohn DA, Nikolich-Zugich J. 2010. Loss of naive T cells and repertoire constriction predict poor response to vaccination in old primates. *J Immunol* 184: 6739-45.
35. Brune W, Hengel H, Koszinowski UH. 2001. A mouse model for cytomegalovirus infection. *Current protocols in immunology / edited by John E. Coligan, et al.* Chapter 19: Unit 19 7.
36. King IL, Mohrs K, Mohrs M. 2010. A nonredundant role for IL-21 receptor signaling in plasma cell differentiation and protective type 2 immunity against gastrointestinal helminth infection. *J Immunol* 185: 6138-45.
37. Lanzer KG, Johnson LL, Woodland DL, Blackman MA. 2014. Impact of ageing on the response and repertoire of influenza virus-specific CD4 T cells. *Immunity & Ageing* 11: 9, PMC 4082670.
38. Brincks EL, Roberts AD, Cookenham T, Sell S, Kohlmeier JE, Blackman MA, Woodland DL. 2013. Antigen-specific memory regulatory CD4+Foxp3+ T cells control memory responses to influenza virus infection. *J Immunol* 190: 3438-46, PMC608733.
39. Kohlmeier JE, Reiley WW, Perona-Wright G, Freeman ML, Yager EJ, Connor LM, Brincks EL, Cookenham T, Roberts AD, Burkum CE, Sell S, Winslow GM, Blackman MA, Mohrs M, Woodland DL. 2011. Inflammatory chemokine receptors regulate CD8(+) T cell contraction and memory generation following infection. *J Exp Med* 208: 1621-34, PMC3149221.
40. Freeman ML, Lanzer KG, Cookenham T, Peters B, Sidney J, Wu TT, Sun R, Woodland DL, Sette A, Blackman MA. 2010. Two Kinetic Patterns of Epitope-Specific CD8 T-Cell Responses following Murine Gammaherpesvirus 68 Infection. *J Virol* 84: 2881-92, PMC2826075.
41. Flano E, Kim IJ, Woodland DL, Blackman MA. 2002. gamma-Herpesvirus Latency Is Preferentially Maintained in Splenic Germinal Center and Memory B Cells. *J Exp Med* 196: 1363-72.
42. Saito F, Ito T, Connett JM, Schaller MA, Carson WFt, Hogaboam CM, Rochford R, Kunkel SL. 2013. MHV68 latency modulates the host immune response to influenza A virus. *Inflammation* 36: 1295-303.
43. Furman D, Jojic V, Sharma S, Shen-Orr SS, Angel CJ, Onengut-Gumuscu S, Kidd BA, Maecker HT, Concannon P, Dekker CL, Thomas PG, Davis MM. 2015. Cytomegalovirus infection enhances the immune response to influenza. *Science translational medicine* 7: 281ra43.
44. Cicin-Sain L, Brien JD, Uhrlaub JL, Drabig A, Marandu TF, Nikolich-Zugich J. 2012. Cytomegalovirus infection impairs immune responses and accentuates T-cell pool changes observed in mice with aging. *PLoS Pathog* 8: e1002849.
45. Mekker A, Tchang VS, Haeberli L, Oxenius A, Trkola A, Karrer U. 2012. Immune senescence: relative contributions of age and cytomegalovirus infection. *PLoS Pathog* 8: e1002850.
46. Selin LK, Varga SM, Wong IC, Welsh RM. 1998. Protective heterologous antiviral immunity and enhanced immunopathogenesis mediated by memory T cell populations. *J Exp Med* 188: 1705-15.
47. Chen HD, Fraire AE, Joris I, Welsh RM, Selin LK. 2003. Specific history of heterologous virus infections determines anti-viral immunity and immunopathology in the lung. *Am J Pathol* 163: 1341-55.
48. Khan N, Shariff N, Cobbold M, Bruton R, Ainsworth JA, Sinclair AJ, Nayak L, Moss PA. 2002. Cytomegalovirus seropositivity drives the CD8 T cell repertoire toward greater clonality in healthy elderly individuals. *J Immunol* 169: 1984-92.
49. Karrer U, Sierro S, Wagner M, Oxenius A, Hengel H, Koszinowski UH, Phillips RE, Klenerman P. 2003. Memory inflation: continuous accumulation of antiviral CD8+ T cells over time. *J Immunol* 170: 2022-9.
50. Khan N, Hislop A, Gudgeon N, Cobbold M, Khanna R, Nayak L, Rickinson AB, Moss PA. 2004. Herpesvirus-specific CD8 T cell immunity in old age: cytomegalovirus impairs the response to a coresident EBV infection. *J Immunol* 173: 7481-9.

51. Munks MW, Cho KS, Pinto AK, Sierro S, Klenerman P, Hill AB. 2006. Four distinct patterns of memory CD8 T cell responses to chronic murine cytomegalovirus infection. *J Immunol* 177: 450-8.
52. Reese TA, Wakeman BS, Choi HS, Hufford MM, Huang SC, Zhang X, Buck MD, Jezewski A, Kambal A, Liu CY, Goel G, Murray PJ, Xavier RJ, Kaplan MH, Renne R, Speck SH, Artyomov MN, Pearce EJ, Virgin HW. 2014. Coinfection. Helminth infection reactivates latent gamma-herpesvirus via cytokine competition at a viral promoter. *Science* 345: 573-7.
53. Minciullo PL, Catalano A, Mandraffino G, Casciaro M, Crucitti A, Maltese G, Morabito N, Lasco A, Gangemi S, Basile G. 2016. Inflammaging and Anti-Inflammaging: The Role of Cytokines in Extreme Longevity. *Archivum immunologiae et therapiae experimentalis* 64: 111-26.
54. Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G. 2000. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci* 908: 244-54.
55. Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panourgia MP, Invidia L, Celani L, Scurti M, Cevenini E, Castellani GC, Salvioli S. 2007. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mechanisms of Ageing and Development* 128: 92-105.
56. Fagiolo U, Cossarizza A, Scala E, Fanales-Belasio E, Ortolani C, Cozzi E, Monti D, Franceschi C, Paganelli R. 1993. Increased cytokine production in mononuclear cells of healthy elderly people. *Eur J Immunol* 23: 2375-8.
57. Kapasi ZF, Murali-Krishna K, McRae ML, Ahmed R. 2002. Defective generation but normal maintenance of memory T cells in old mice. *Eur. J. Immunol.* 32: 1567-73.
58. Valkenburg SA, Venturi V, Dang TH, Bird NL, Doherty PC, Turner SJ, Davenport MP, Kedzierska K. 2012. Early priming minimizes the age-related immune compromise of CD8(+) T cell diversity and function. *PLoS Pathog* 8: e1002544.
59. Moon JJ, Chu HH, Pepper M, McSorley SJ, Jameson SC, Kedl RM, Jenkins MK. 2007. Naive CD4(+) T cell frequency varies for different epitopes and predicts repertoire diversity and response magnitude. *Immunity* 27: 203-13.
60. Nelson RW, Rajpal MN, Jenkins MK. 2015. The Neonatal CD4+ T Cell Response to a Single Epitope Varies in Genetically Identical Mice. *J Immunol* 195: 2115-21.
61. Lang A, Brien JD, Messaoudi I, Nikolich-Zugich J. 2008. Age-related dysregulation of CD8+ T cell memory specific for a persistent virus is independent of viral replication. *J Immunol* 180: 4848-57.
62. Ely KH, Ahmed M, Kohlmeier JE, Roberts AD, Wittmer ST, Blackman MA, Woodland DL. 2007. Antigen-specific CD8+ T cell clonal expansions develop from memory T cell pools established by acute respiratory virus infections. *J Immunol* 179: 3535-42.
63. Kohlmeier JE, Connor LM, Roberts AD, Cookenham T, Martin K, Woodland DL. 2010. Nonmalignant clonal expansions of memory CD8+ T cells that arise with age vary in their capacity to mount recall responses to infection. *J Immunol* 185: 3456-62.
64. Connor LM, Kohlmeier JE, Ryan L, Roberts AD, Cookenham T, Blackman MA, Woodland DL. 2012. Early dysregulation of the memory CD8+ T cell repertoire leads to compromised immune responses to secondary viral infection in the aged. *Immun Ageing* 9: 28, PMC3564731.
65. Goldrath AW. 2002. Maintaining the status quo: T-cell homeostasis. *Microbes and infection / Institut Pasteur* 4: 539-45.
66. Haluszczak C, Akue AD, Hamilton SE, Johnson LD, Pujanauski L, Teodorovic L, Jameson SC, Kedl RM. 2009. The antigen-specific CD8+ T cell repertoire in unimmunized mice includes memory phenotype cells bearing markers of homeostatic expansion. *J Exp Med* 206: 435-48.
67. Akue AD, Lee JY, Jameson SC. 2012. Derivation and maintenance of virtual memory CD8 T cells. *J Immunol* 188: 2516-23.
68. Lee JY, Hamilton SE, Akue AD, Hogquist KA, Jameson SC. 2013. Virtual memory CD8 T cells display unique functional properties. *Proc Nat Acad Sci USA* 110: 13498-503.
69. Jameson SC, Lee YJ, Hogquist KA. 2015. Innate memory T cells. *Advances Immunol* 126: 173-213.
70. Chiu BC, Martin BE, Stolberg VR, Chensue SW. 2013. Cutting edge: Central memory CD8 T cells in aged mice are virtual memory cells. *J Immunol* 191: 5793-6.
71. Jacomet F, Cayssials E, Basbous S, Levescot A, Piccirilli N, Desmier D, Robin A, Barra A, Giraud C, Guilhot F, Roy L, Herbelin A, Gombert JM. 2015. Evidence for eomesodermin-expressing innate-like CD8(+) KIR/NKG2A(+) T cells in human adults and cord blood samples. *European J Immunol* 45: 1926-33.
72. Van Kaer L. 2015. Innate and virtual memory T cells in man. *European J Immunol* 45: 1916-20.
73. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. 1999. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401: 708-12.

74. Masopust D, Vezys V, Marzo AL, Lefrancois L. 2001. Preferential localization of effector memory cells in nonlymphoid tissue. *Science* 291: 2413-7.
75. Sallusto F, Geginat J, Lanzavecchia A. 2004. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annual Rev Immunol.* 22: 745-63.
76. Gerlach C, Loughhead SM, von Andrian UH. 2015. Figuring fact from fiction: unbiased polling of memory T cells. *Cell* 161: 702-4.

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Letters of Support

of the Freedom of Information and Privacy Act

**RESOURCE SHARING.**

As for our plan to share materials and our management of intellectual property, we will adhere to the NIH policy on sharing of research resources developed with NIH funding including the Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources issued in December, 1999 at http://grants.nih.gov/grants/intell-property_64FR72090.pdf, the NIH Policy on the Sharing of Model Organisms for Biomedical Research at http://grants.nih.gov/grants/policy/model_organism/model_organism_brochure.pdf and the NIH Public Access Policy at http://publicaccess.nih.gov/policy.htm.

**1. Plans to share research resources.** All "model organisms", including vectors for transgenic production and mouse strains generated, generated by this project will be distributed freely to the broader academic community or deposited into a repository/stock center, either before or immediately on publication. If we assume responsibility for distributing the newly generated model organisms, we will fill requests in a timely fashion. Requestees should receive the desired reagents within two weeks to two months of their request, depending on their chronological position in the queue. In addition, we will provide relevant protocols and published genetic and phenotypic data upon request. The infrastructure for this rapid sharing of newly developed reagents (both vectors and mice) is in place in my lab, and is supported by the Chief Operating Officer of Trudeau Institute.

**2. Intellectual property rights.** Consistent with Trudeau Institute's policy on intellectual property rights, we will make available any and all strains of transgenic mice produced under this grant for use at other academic or not-for-profit institutions at no cost except for standard maintenance and transportation expenses. Trudeau Institute will reserve the right to use these reagents for educational, research, or other non-business purposes. Trudeau Institute may establish a non-exclusive commercial license granting Trudeau Institute's rights to use such animals at specific for-profit entities; in these cases, Trudeau Institute will maintain the right to grant non-exclusive licenses for use of these materials by academic or not-for-profit institutions.

Material transfers to not-for-profit entities will be made with no more restrictive terms than in the Simple Letter Agreement (SLA) or the Uniform Biological Materials Transfer Agreement (UBMTA), published at http://www.ott.nih.gov/forms-model-agreements, and without reach through requirements. Transfer of materials to for-profit entities will be mediated through Trudeau Institute's Chief Operating Officer, and typically involves a simple license agreement with execution or annual fees as deemed appropriate, but in no way prohibitive to the ready distribution of these reagents. 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.

Authentication of Key Resources Plan

Biological reagents used in the proposal that need to be authenticated include viral stocks, cell lines for growing or titering viruses, *Heligmosomoides polygyrus stocks* and tetramer reagents.

**Viruses and cell lines:**

The present proposal will require the use of 4 different viruses; Influenza virus (A/PR/8/34), Sendai virus (Enders strain), γHV68 (WUMS), and MCMV (Smith ATCC VR194).

The influenza virus and Sendai virus was obtained from Dr. Peter Doherty's lab at St. Jude Children's Research Hospital. Mother stocks of both viruses were aliquoted and frozen. New stocks have all been generated directly from an aliquot of the mother stock and the virus. In this way the virus has not been sequentially passaged, to minimize the risk of genetic variation. A mother stock of γHV68 (murine gammaherpesvirus 68) was grown from virus obtained from ATCC (VR1465) on NIH 3T3 cells (ATCC CRL-1658), and subsequent stocks have been prepared from aliquots of the mother stock. Stocks of MCMV Smith (ATCC VR194, now VR1399) will be obtained from ATCC and grown according to information provided by the National Center for Biotechnology. MCMV will be grown in salivary glands of BALB/c mice according to Current Protocols in Immunology and titered on 3T12 murine embryonic fibroblasts (ATCC CCL-164). Biological effects of this stock will be compared with viral stocks obtained from Dr. Ray Welsh at the University of Massachusetts.

All virus stocks have also been IMPACT tested by IDEXX BioResearch before they are used to infect mice to avoid introduction of unknown pathogenic agent.

The strict adherence to mother stocks as the inoculum for growing new viral stocks, careful comparison of the biological effects of a new stock with the previous stock before using for experiments, and the use wherever possible of cell lines from ATCC ensure the authenticity and reproducibility of biological reagents.

***Heligmosomoides polygyrus*:** *Heligmosomoides polygyrus (H. polygyrus)* is an intestinal helminth that is continuously passaged in BALB/c mice at the Trudeau Institute and the stage 3 larvae are used to orally infect mice. Serum from *H. polygyrus* source mice have been IMPACT tested by IDEXX BioResearch to confirm the absence of unknown pathogenic agents.

**Tetramers**:

Trudeau Institute has a tetramer facility that has and will generate all tetramers used in the proposed studies. Tetramers are very defined reagents and, although the titer can vary between batches and institutions, the molecular biological techniques used to make the reagents are well established. All tetramer reagents are purified over FPLC size exclusion columns and quality control tests are carried out on each batch to ensuring reproducibility of these reagents.