PI: **Contreras, Nico**	Title: The Immunological Consequences of Mouse Cytomegalovirus on Adipose Tissue	
Received: 08/12/2016	FOA: PA16-308	Council: 01/2017
Competition ID: FORMS-D	FOA Title: RUTH L. KIRSCHSTEIN NATIONAL RESEARCH SERVICE AWARD INDIVIDUAL PREDOCTORAL FELLOWSHIP TO PROMOTE DIVERSITY IN HEALTH-RELATED RESEARCH (PARENT F31 - DIVERSITY)	
1 F31 AI131622-01	Dual: DK	Accession Number: 3962809
IPF: 490201	Organization: UNIVERSITY OF ARIZONA	
Former Number:	Department:	
IRG/SRG: ZRG1 F07-T (20)L	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A)	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Nico Contreras	ABOR, UNIVERSITY OF ARIZONA	PD/PI
JANKO NIKOLICH-ZUGICH Ph.D	Arizona Board of Regents, University of Arizona	Other (Specify)-Sponsor

[redacted]

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

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ❍ Application ● Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number
Grant12231434

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

Legal Name*: **ABOR, UNIVERSITY OF ARIZONA**
Department:
Division:
Street1*: **UNIVERSITY OF ARIZONA**
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: First Name*: **Sherry** Middle Name: Last Name*: **Esham** Suffix:
Position/Title: **Director, Sponsored Projects Services**
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*:
Phone Number*: [redacted] Fax Number: [redacted] Email [redacted]

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

7. TYPE OF APPLICANT* **H: Public/State Controlled 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*
The Immunological Consequences of Mouse Cytomegalovirus on Adipose Tissue

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/2017 **03/31/2020**

13. CONGRESSIONAL DISTRICTS OF APPLICANT
AZ-003

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: **Mr.** First Name*: **Nico** Middle Name: Last Name*: **Contreras** Suffix:

Position/Title: **Graduate Research Assistant**

Organization Name*: **ABOR, UNIVERSITY OF ARIZONA**

Department:

Division:

Street1*: **UNIVERSITY OF ARIZONA**

Street2: [redacted]

City*: [redacted]

County:

State*: [redacted]

Province:

Country*: [redacted]

ZIP / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	[redacted]
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	[redacted]
d. Estimated Program Income*	$0.00

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

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

DATE:

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

❍ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

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

● I agree*

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

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: **Kimberly** Middle Name: Last Name*: **Andrews Espy** Suffix: **Ph.D**

Position/Title*: **Senior Vice President for Research**

Organization Name*: **Arizona Board of Regents, University of Arizona**

Department: **Immunobiology**

Division: **Medicine**

Street1*: [redacted]

Street2:

City*: [redacted]

County:

State*: [redacted]

Province:

Country*: [redacted]

ZIP / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: [redacted] Email*: [redacted]

Signature of Authorized Representative* [redacted]

Date Signed* [redacted]

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:**F31_Cover_letter.pdf**

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

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

Project/Performance Site Location(s)

Project/Performance Site Primary Location

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

Organization Name: **Arizona Board of Regents, University of Arizona**
Duns Number: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: **AZ-003**

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:

Animal Welfare Assurance Number [redacted]

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*	**F31_Summary_Abstract.pdf**
8. Project Narrative*	**F31_Project_Narrative.pdf**
9. Bibliography & References Cited	**References.pdf**
10.Facilities & Other Resources	**Facilities_and_Other_Equipment.pdf**
11.Equipment	
12. Other Attachments	**Certification_Letter_F31.pdf**

Tracking Number: GRANT12231644
Funding Opportunity Number: PA-16-308. Received Date: 2016-08-12T15:15:24.000-04:00

Abstract

Adipose tissue has long been thought to simply be a site of lipid synthesis and energy storage. However, it has become increasingly clear that the inflammatory state of adipose tissue has profound effects on host immunity and metabolism. Recent reports have demonstrated that both viruses and parasites are capable of directly infecting the adipocytes and cellular constituents of adipose tissue. Furthermore, Human Immunodeficiency Virus (HIV) is capable of becoming latent within T cells found in adipose. Cytomegalovirus (CMV), a ubiquitous betaherpesvirus, results in a persistent lifelong infection and the holy grail of CMV research has been to identify sites of latency, but no study has demonstrated the extent to which adipose tissue is infected or harbors latent and persistent virus. CMV has a broad cellular and tissue tropism, and susceptible cells are all represented within the adipose tissue. Thus, it is necessary to investigate the consequences, if any, of CMV infection within adipose. In order to understand the consequence(s) of CMV infection on adipose we will employ the C57BL/6 mouse CMV (mCMV) model of infection. The goal of this proposal is to understand the functional consequences and mechanism of spread during mCMV infection within adipose tissue. The overall hypothesis of this proposal is **that mCMV disseminates to adipose tissue, replicates, establishes latency, leading to an lifelong CD8 T cell response.** We will address the hypothesis and achieve the goals of this proposal by first, determining the cell type(s) that are infected within adipose tissue during infection by qPCR, plaque assay, and flow cytometry. We will also determine if mCMV is capable of becoming reactivated from within adipose tissue. Second, we will determine the kinetic expansion and contraction of mCMV specific CD8 T cells. Investigation into infection of and the role of adipose tissue during an immune response is a new and growing field, thus this work, when completed, will represent a significant advancement in our fundamental base of knowledge regarding mCMV cell tropism and persistence. The findings of this proposal will call for the consideration of adipose tissue in the context of infection, which has far reaching impact on vaccinology, immunology, virology, and endocrinology.

Project Narrative:

Cytomegalovirus (CMV) infects a majority of the world's population. There has been correlation between CMV infection and metabolic health decline, such as atherosclerosis. Our preliminary results expand this correlation and possibly mechanistically link CMV infection [redacted]

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116. Bayer, C. *et al.* Human cytomegalovirus infection of M1 and M2 macrophages triggers inflammation and autologous T-cell proliferation. *J. Virol.* **87,** 67–79 (2013).
117. Redman, T. K., Britt, W. J., Wilcox, C. M., Graham, M. F. & Smith, P. D. Human cytomegalovirus enhances chemokine production by lipopolysaccharide-stimulated lamina propria macrophages. *J. Infect. Dis.* **185,** 584–90 (2002).
118. Smith, P. D. *et al.* Cytomegalovirus enhances macrophage TLR expression and MyD88-mediated signal transduction to potentiate inducible inflammatory responses. *J. Immunol.* **193,** 5604–12 (2014).
119. Morris, D. L. *et al.* Adipose tissue macrophages function as antigen-presenting cells and regulate adipose tissue CD4+ T cells in mice. *Diabetes* **62,** 2762–72 (2013).
120. Wensveen, F. M. *et al.* NK cells link obesity-induced adipose stress to inflammation and insulin resistance. **16,** (2015).
121. Martinez, F. O. & Gordon, S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep.* **6,** 13 (2014).
122. Rudd, B. D. *et al.* 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,** 13694–13699 (2011).
123. Goldberg, E. L. *et al.* Lifespan-extending caloric restriction or mTOR inhibition impair adaptive immunity of old mice by distinct mechanisms. *Aging Cell* **14,** 130–138 (2015).
124. Metcalf, T. U. *et al.* Global analyses revealed age-related alterations in innate immune responses after stimulation of pathogen recognition receptors. *Aging Cell* (2015). doi:10.1111/acel.12320

Facilities and Other Equipment. During this proposed work I will have access to personal cubicle space in the Medical Research Building at the University of Arizona. In addition to the personal area I will also have access to the BSL-2 shared laboratory space (J. NIkolich-Zugich) 2,000 sq. ft. equipped with benches, sinks, water, pressurized air, vacuum, etc. in the Medical Research Building at the University of Arizona College of Medicine. Dedicated tissue culture space of 300 sq. ft. is adjacent to the lab. Equipment includes CO2 incubators, phase and inverted microscopes, spectrophotometers, centrifuges, PCR machines (including real time), a gel imaging system, a custom made 4-laser/26-color BD-Fortessa analyzer, DNA sequencers (ABI 3100), ultracentrifuges, tissue culture hoods, and freezers (-80 and -20 C).

Flow cytometry analysis. Data acquisition will be performed on a custom-made, four-laser BD Fortessa flow cytometer (Becton Dickinson, Sunnyvale, CA), and analyzed using FlowJo software (Tree Star, Inc., Ashland, OR). Further, rainbow calibration beads will be run within all experiments to allow for cross comparison of studies over time

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

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Mr. **First Name*:** Nico **Middle Name** **Last Name*:** Contreras **Suffix:**

Position/Title*: Graduate Research Assistant
Organization Name*: ABOR, UNIVERSITY OF ARIZONA
Department:
Division:
Street1*: UNIVERSITY OF ARIZONA
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]

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

Credential, e.g., agency login: [redacted]

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

Degree Type: **Degree Year:**

	File Name
Attach Biographical Sketch*:	Nico_Contreras_Biosketch_160809.pdf
Attach Current & Pending Support:	

Tracking Number: GRANT12231644
Funding Opportunity Number: PA-16-308 . Received Date: 2016-08-12T15:15:24.000-04:00

PROFILE - Senior/Key Person

Prefix: **First Name*:** JANKO **Middle Name** **Last Name*:** NIKOLICH-ZUGICH **Suffix:** Ph.D

Position/Title*: Prof and Head, Dept. of Immunobiology
Organization Name*: Arizona Board of Regents, University of Arizona
Department: Immunobiology
Division: Medicine
Street1*: [redacted]
Street2:
City*:
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*:

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

Credential, e.g., agency login: [redacted]

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

Degree Type: MD,PHD,MS **Degree Year:**

File Name

Attach Biographical Sketch*: JNZ_Biosketch.pdf

Attach Current & Pending Support:

Tracking Number: GRANT12231644 Funding Opportunity Number: PA-16-308 . Received Date: 2016-08-12T15:15:24.000-04:00

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

BIOGRAPHICAL SKETCH
DO NOT EXCEED FIVE PAGES.

NAME: Contreras, Nico Anthony

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

POSITION TITLE: Graduate Research Assistant

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 Arizona, Tucson, AZ	B.S.	05/2012	Physiological Sciences
University of Arizona, Tucson, AZ	M.S.	05/2014	Professional Sciences in Applied Biosciences
University of Arizona, Tucson, AZ	Ph.D.	projected 05/2019	Immunobiology

A. Personal Statement

During my time in graduate school, and most specifically in the Nikolich-Zugich laboratory I have developed my technical skills as a researcher in the use of multi-parameter flow cytometry, as well as other molecular and cellular biology tools. Furthermore, I have developed and improved upon my critical thinking and experimental design. The proposal described herein, we conceived independently during my time in the laboratory and I believe it adds a new layer to the work that is currently being performed within the Nikolich-Zugich lab.

Prior to joining Dr. Janko Nikolich-Zugich's laboratory for my Ph.D. thesis work I completed a Professional Science Master's degree with Dr. Linda Powers. This project was focused on developing a lateral flow assay to be used in remote and rural locations to determine if blood samples were contaminated with bloodborne pathogens, sponsored by the Office of Naval Research and the Department of Defense. This project was extremely collaborative and I was responsible for weekly progress briefings, as well as monthly written reports for an extremely interdisciplinary team. I learned a wide array of new techniques and was a successful member of the team. As a member in the Nikolich-Zugich laboratory I have been exposed to cutting edge immunologic methodologies and have demonstrated that I am capable of quickly learning necessary techniques in order to drive experimental progress.

I have demonstrated improved understanding and work within my field over time. As the first member of my family to complete a Bachelor's degree and obtain a position in graduate school, as well as being a member of several diversity development groups I believe that I would make a strong candidate to receive funding through an NIH/NIA research diversity supplement. Additionally, I have received NIH funding through the Initiative to Maximize Student Diversity Award and currently sit on the Department of Immunobiology Diversity Committees and these activities are firmly within the spirit of this program. I believe I clearly show an ability to learn necessary laboratory techniques to drive projects forward, as well as be responsible for communicating and defending the results.

B. Positions and Honors

List in chronological order previous positions, concluding with the present position. List any honors. Include present membership on any Federal Government public advisory committee.

Wildcat Excellence Award 2008-2012 (Tuition Scholarship)
Arizona Instrument to Measure Standards Award 2008-2012 (Tuition Scholarship) *Declined*
Dean's List Honorable Mention 2008
Roman DeSanctis Scholarship 2010-2011

Capital One All-American Mascot Challenge Award 2011-2012
University of Arizona Graduate College Dean's Tuition Award 2014
American Association for the Advancement of Science Member 2014-Current
American Aging Association Member 2014-Current
Initiative for Maximizing Student Development Grantee 2014-2015
ThymUS Maui NIH Under Represented Minority Travel Award 2016

C. Contributions to Science

Oral Presentations

Contreras, N.A. (2015) Your Immune System and Your Food. Immunobiology Seminar. The University of Arizona. Tucson, AZ.

Contreras, N.A. (2016) Fattening Up Your Immune System. Immunobiology and Cellular Molecular Medicine Joint Seminar. The University of Arizona. Tucson, AZ.

Poster Presentations

Contreras, N.A., Fontana, L., and Nikolich-Zugich J. (2016) Reversible Lymphopenia Induced By Calorie Restriction. Frontiers in Immunobiology Symposium. The University of Arizona. Tucson, AZ.

PhD Rotations

To date I have been part of a study involving the characterization of the immune response following stroke, within Kristian Doyle's PhD lab, in the context of both acute and chronic strokes. Specifically my aims were to determine cytokine and chemokine profiles in the brain with first incidence of stroke as well as recurrent strokes by using two mouse models to account for heterogeneity. Additionally, I was able to use fresh frozen tissue from human subjects and analyze their cytokine and chemokine profiles across an aged cohort. Finally, I quantified T- and B-cell counts in the stroke lesion as well as within the brain to demonstrate the leakiness of the glial scar that forms following stroke. These studies will lead to the development of novel therapeutics for better outcomes following stroke.

During a second rotation study I was a member of the Anity Koshy, MD lab where I worked to develop a genetic knockout of *Toxoplasma gondii* using the CRISPR/Cas9 system. Using this novel genetic tool I attempted to knockout a sugar transporter in this parasite in order to prevent the formation of cysts in the central nervous system to determine what pathways this cyst interrupts, in hopes that these pathways can be used for a better understanding of neuroinflammation in the brain that is present in several diseases such as Alzheimer's and MS.

My final obligatory research rotation was performed in the Nikolich-Zugich laboratory, where I continue to conduct research. My project focus was on peptide stimulation of banked human peripheral blood mononuclear cells (PBMCs) with epitopes of the human cytomegalovirus. After stimulation I was to assay cytokine production by CD8 T cells. In parallel I was tasked with determining the neutralizing antibody titers of the same people using banked serum. The intent of this project was to determine if there was a correlation with amount of cytokine produced by T cells and neutralizing antibody from serum across the lifespan of patients infected with cytomegalovirus.

Outreach

The University of Arizona Joint Biology Retreat Committee Member 2015

The University of Arizona Department of Immunobiology Diversity Committee Member 2015 - Current
BASIS School Oro Valley Science Outreach Volunteer:
I volunteered at a charter school to teach children basic scientific concepts including surface tension, acid/base differences, and viscosity and density.

Masters Thesis

As a member of an interdisciplinary team I helped develop a rapid lateral flow assay for the detection of blood borne pathogens. Specifically I was involved in the development of an affinity peptide assay for use to detect Hepatitis C virus, Human Immunodeficiency Virus, and Plasmodium *sp.* The team was successfully able to

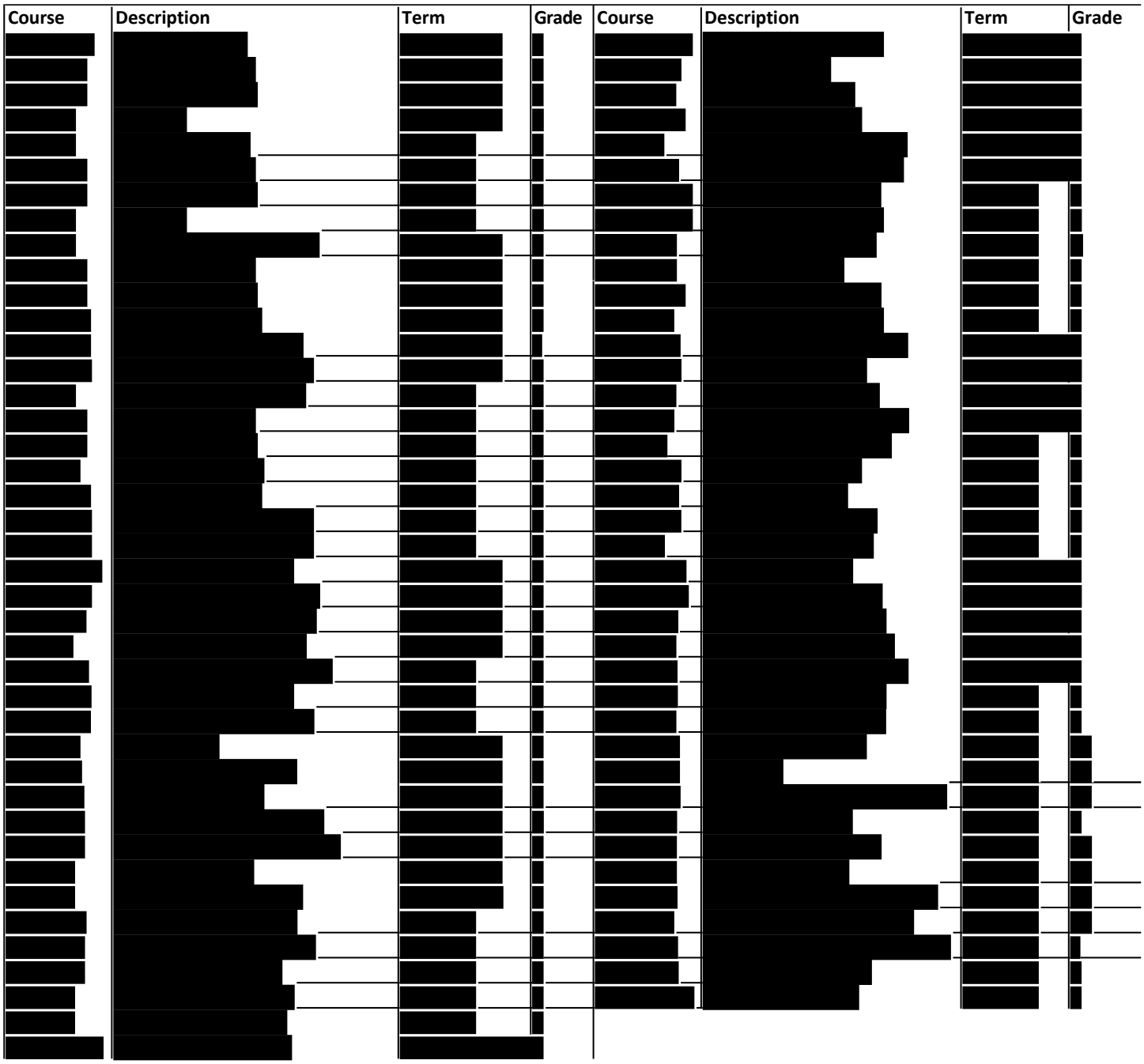
develop a test that is currently undergoing military specification qualifications for use in the field and has led to a publication in press. These studies will lead to a military grade point-of-care device to be used for rural medicine needs as well as blood screenings for use in military operations.

D. Scholastic Performance

Graduate Record Exam Scores:

[redacted]

The University of Arizona grades on a Regular Grading Scale: A, B, C, D, E. With D being a passing grade. Transfer Credits are denoted with a T prior to the Regular Grade. S = Superior and is not calculated in the GPA. K = course in progress that spans multiple semesters. P = pass in a Pass/Fail course and is not calculated in the GPA.



OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

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: NIKOLICH-ZUGICH, JANKO

eRA COMMONS USER NAME (agency login): [redacted]

POSITION TITLE: Prof and Head, Dept. of Immunobiology

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

A. Personal Statement

My principal interests are to understand (i) basic mechanisms of immunity and how these mechanisms decline and deteriorate with age to erode protective immunity against infection, including persistent infections such as those with the cytomegalovirus (CMV); and (ii) how we can devise methods to correct or ameliorate immune dysfunction by means of new vaccines, immunomodulatory and metabolic intervention and/or immune rejuvenation. We use infectious disease models, including biodefense and emerging infection (cat. A-C) agents to probe immune defense, homeostasis, T-cell repertoire and memory in the mouse, non-human primate and human models.

My other interest is longevity and Healthspan modulation by nutritional and metabolic intervention. The promise of healthspan extension via dietary and pharmacologic intervention raises the possibility of delaying many organ-specific diseases of aging simultaneously with longevity extension. However, that mandates careful investigation into benefits, as well as possible costs, of different treatments to truly achieve healthspan extension. Immune system is particularly sensitive to nutrient deprivation if faced with acute infection, and we are studying whether and how different longevity extension treatments can improve function of the immune system while still providing longevity/healthspan benefit.

With regard to this I have trained and mentored 11 doctoral students (10 graduated with Ph.D., one with M.Sc) and 20 postdoctoral trainees, and am currently training/mentoring one doctoral student, 2 postdoctoral trainees and 5 junior faculty/clinical fellows with K or R awards. Of these, 11 former postdoctoral trainees hold faculty or senior industry positions, and of 10 former Ph.D. students, two are in faculty positions and 6 have continued postdoctoral education.

1. Messaoudi I, Guevara Patiño JA, Dyall R, LeMaoult J, Nikolich-Zugich J. Direct link between mhc polymorphism, T cell avidity, and diversity in immune defense. Science. 2002 Nov 29;298(5599):1797-800. PubMed PMID: 12459592.
2. Brien JD, Uhrlaub JL, Hirsch A, Wiley CA, Nikolich-Zugich J. Key role of T cell defects in age-related vulnerability to West Nile virus. J Exp Med. 2009 Nov 23;206(12):2735-45. PubMed PMID: 19901080; PubMed Central PMCID: PMC2806630.
3. Uhrlaub JL, Brien JD, Widman DG, Mason PW, Nikolich-Zugich J. Repeated in vivo stimulation of T and B cell responses in old mice generates protective immunity against lethal West Nile virus encephalitis. J Immunol. 2011 Apr 1;186(7):3882-91. PubMed PMID: 21339368; PubMed Central PMCID: PMC3501996.
4. Li G, Smithey MJ, Rudd BD, Nikolich-Žugich J. Age-associated alterations in CD8α+ dendritic cells impair CD8 T-cell expansion in response to an intracellular bacterium. Aging Cell. 2012 Dec;11(6):968-77. PubMed PMID: 22862959; PubMed Central PMCID: PMC3533767.

B. Positions and Honors

Positions and Employment

1987 - 1990	Research Associate, Scripps Clinic and Research Foundation, La Jolla, CA
1990 - 2001	Assistant/Associate Member, MEMORIAL SLOAN-KETTERING CANCER CENTER, New York, NY
2001 - 2008	Professor and Senior Scientist, OREGON HEALTH & SCIENCE UNIVERSITY, Portland, OR
2008 -	Prof and Head, Dept. of Immunobiology, UNIVERSITY OF ARIZONA, Tucson, AZ

Other Experience and Professional Memberships

Honors

1989	AAI Travel Assistance Award, 7th International Congress of Immunologists, American Association of Immunologists
1991	Pew Scholar in the Biomedical Sciences,, Pew Charitable Trust
1998	Boyer Young Investigator Award, Memorial Sloan-Kettering Cancer Center
2003	Visiting Professor, School of Medicine, University of Belgrade
2008	Bowman Professor in Medical Research, University of Arizona College of Medicine
2009	President, American Aging Association

C. Contribution to Science

1. T cell development: In the course of my final training and early independent career (1987-1998, I contributed several seminal studies that have mapped precursor-product relationship in the main TCR alpha/beta lineage development in the thymus. This work provided a roadmap to dissect phenotypic and functional stages in T cell development.

    a. Nikolić-Zugić J, Bevan MJ. Thymocytes expressing CD8 differentiate into CD4+ cells following intrathymic injection. Proc Natl Acad Sci U S A. 1988 Nov;85(22):8633-7. PubMed PMID: 3141930; PubMed Central PMCID: PMC282513.

    b. Nikolić-Zugić J, Moore MW, Bevan MJ. Characterization of the subset of immature thymocytes which can undergo rapid in vitro differentiation. Eur J Immunol. 1989 Apr;19(4):649-53. PubMed PMID: 2567243.

    c. Andjelić S, Jain N, Nikolić-Zugić J. Immature thymocytes become sensitive to calcium-mediated apoptosis with the onset of CD8, CD4, and the T cell receptor expression: a role for bcl-2?. J Exp Med. 1993 Nov 1;178(5):1745-51. PubMed PMID: 8228820; PubMed Central PMCID: PMC2191237.

    d. Dyall R, Nikolić-Zugić J. The majority of postselection CD4+ single-positive thymocytes requires the thymus to produce long-lived, functional T cells. J Exp Med. 1995 Jan 1;181(1):235-45. PubMed PMID: 7528769; PubMed Central PMCID: PMC2191814.

2. Intrathymic positive selection: During the same period, together with Dr Michael Bevan, I provided the first evidence that in the course of intrathymic poistive selection TCR recognizes a complex of a self peptide and a self MHC molecule. Subsequently, I published several key manuscripts dissecting molecular details of that interaction and its impact for T cell repertoire formation and defense against cancer.

    a. Nikolić-Zugić J, Bevan MJ. Role of self-peptides in positively selecting the T-cell repertoire. Nature. 1990 Mar 1;344(6261):65-7. PubMed PMID: 2304556.

    b. Nikolić-Zugić J, Carbone FR. The effect of mutations in the MHC class I peptide binding groove on the cytotoxic T lymphocyte recognition of the Kb-restricted ovalbumin determinant. Eur J Immunol. 1990 Nov;20(11):2431-7. PubMed PMID: 2253683.

    c. Dyall R, Fremont DH, Jameson SC, Nikolić-Zugić J. T cell receptor (TCR) recognition of MHC class I variants: intermolecular second-site reversion provides evidence for peptide/MHC conformational variation. J Exp Med. 1996 Jul 1;184(1):253-8. PubMed PMID: 8691139; PubMed Central PMCID: PMC2192691.

d. Dyall R, Bowne WB, Weber LW, LeMaoult J, Szabo P, Moroi Y, Piskun G, Lewis JJ, Houghton AN, Nikolić-Zugić J. Heteroclitic immunization induces tumor immunity. J Exp Med. 1998 Nov 2;188(9):1553-61. PubMed PMID: 9802967; PubMed Central PMCID: PMC2212523.

3. MHC polymorphism, TCR repertoire formation and immune defense against infection: How is TCR reperoire shaped by MHC polymorphism, what is its capacity to recognize foreign invaders and how well can it protect against infection? My next series of contributions focused on these functional implications of positive intrathymic selection. My group discovered that MHC polymprphism is directly linked to TCR diversity, with subtle shifts in TCR repertoire occurring as a consequence of subtle MHC polymorphism changes. We further showed that this directly impacted immune defense against infection.

   a. Molano A, Erdjument-Bromage H, Fremont DH, Messaoudi I, Tempst P, Nikolić-Zugić J. Peptide selection by an MHC H-2Kb class I molecule devoid of the central anchor ("C") pocket. J Immunol. 1998 Mar 15;160(6):2815-23. PubMed PMID: 9510184.
   b. Dyall R, Messaoudi I, Janetzki S, Nikolic-Zugić J. MHC polymorphism can enrich the T cell repertoire of the species by shifts in intrathymic selection. J Immunol. 2000 Feb 15;164(4):1695-8. PubMed PMID: 10657612.
   c. Janković V, Remus K, Molano A, Nikolich-Zugich J. T cell recognition of an engineered MHC class I molecule: implications for peptide-independent alloreactivity. J Immunol. 2002 Aug 15;169(4):1887-92. PubMed PMID: 12165513.
   d. Messaoudi I, Guevara Patiño JA, Dyall R, LeMaoult J, Nikolich-Zugich J. Direct link between mhc polymorphism, T cell avidity, and diversity in immune defense. Science. 2002 Nov 29;298(5599):1797-800. PubMed PMID: 12459592.

4. Immune homeostasis and aging: In a series of innovative experiments we have probed the boundaries of naive and memory T cell homeostasis and maintenance in the course of aging. We discovered several novel compensatory homeostatic mechanisms in mice and non-human primates that attempt to conserve T cells during aging, but eventually fail due to exhaustion. We showed that this leads to selection of a small subset of surviving naive T cells cells that exhibit high avidity towards both self and foreign MHC, and that tend to dominate immune responses in an oligoclonal fashion, potentially favoring escape of microbial variants.

   a. Messaoudi I, Lemaoult J, Guevara-Patino JA, Metzner BM, Nikolich-Zugich J. Age-related CD8 T cell clonal expansions constrict CD8 T cell repertoire and have the potential to impair immune defense. J Exp Med. 2004 Nov 15;200(10):1347-58. PubMed PMID: 15545358; PubMed Central PMCID: PMC2211915.
   b. Cicin-Sain L, Messaoudi I, Park B, Currier N, Planer S, Fischer M, Tackitt S, Nikolich-Zugich D, Legasse A, Axthelm MK, Picker LJ, Mori M, Nikolich-Zugich J. Dramatic increase in naive T cell turnover is linked to loss of naive T cells from old primates. Proc Natl Acad Sci U S A. 2007 Dec 11;104(50):19960-5. PubMed PMID: 18056811; PubMed Central PMCID: PMC2148405.
   c. Rudd BD, Venturi V, Davenport MP, Nikolich-Zugich J. Evolution of the antigen-specific CD8+ TCR repertoire across the life span: evidence for clonal homogenization of the old TCR repertoire. J Immunol. 2011 Feb 15;186(4):2056-64. PubMed PMID: 21248263; PubMed Central PMCID: PMC4119821.
   d. Rudd BD, Venturi V, Li G, Samadder P, Ertelt JM, Way SS, Davenport MP, Nikolich-Žugich J. 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. 2011 Aug 16;108(33):13694-9. PubMed PMID: 21813761; PubMed Central PMCID: PMC3158207.

5. Infection and immunity with aging: how does an older immune system deal with new infection, reinfection and persistent infection? We have filled critical gaps in our knowledge in this regard, studying acute (West Nile virus, Listeria monocytogenes, Poxviruses, Chikungunya virus) and persistent (HSV-1, cytomegalovirus) microbial pathogens in mice, non-human primates and humans. We found profound, stereotypical lesions in adaptive immunity to acute infection, and a less well defined set of innate immune defects. With regard to persistent viruses, we have discovered that while these infections clearly have a

negative impact on immune responses to third-party antigens, this impact is probably not fatal and can also have potential benefits.

a. Brien JD, Uhrlaub JL, Hirsch A, Wiley CA, Nikolich-Zugich J. Key role of T cell defects in age-related vulnerability to West Nile virus. J Exp Med. 2009 Nov 23;206(12):2735-45. PubMed PMID: 19901080; PubMed Central PMCID: PMC2806630.

b. 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. Loss of naive T cells and repertoire constriction predict poor response to vaccination in old primates. J Immunol. 2010 Jun 15;184(12):6739-45. PubMed PMID: 20483749; PubMed Central PMCID: PMC3504654.

c. Smithey MJ, Li G, Venturi V, Davenport MP, Nikolich-Žugich J. Lifelong persistent viral infection alters the naive T cell pool, impairing CD8 T cell immunity in late life. J Immunol. 2012 Dec 1;189(11):5356-66. PubMed PMID: 23087407; PubMed Central PMCID: PMC3504138.

d. Wertheimer AM, Bennett MS, Park B, Uhrlaub JL, Martinez C, Pulko V, Currier NL, Nikolich-Žugich D, Kaye J, Nikolich-Žugich J. Aging and cytomegalovirus infection differentially and jointly affect distinct circulating T cell subsets in humans. J Immunol. 2014 Mar 1;192(5):2143-55. PubMed PMID: 24501199; PubMed Central PMCID: PMC3989163.

D. Research Support

Ongoing Research Support

2014/07/15-2019/04/30
R01 AG048021-02, National Institute on Aging (NIA)
NIKOLICH-ZUGICH, JANKO (PI)
IMPACT OF CMV UPON T-CELL AGING AND IMMUNE DEFENSE
The goal of this project is to dissect how CMV modulates T-cell receptor repertoire and function in mice and humans, and how virus activity interplays with T cell function and immunity in aging.
Role: PI

2014/05/01-2017/04/30 (no cost extension)
R21 AG045734-02, National Institute on Aging (NIA)
NIKOLICH-ZUGICH, JANKO (PI)
Longevity extension and immune function in aging (R21)
The goal of this project is to examine whether the most potent longevity extension interventions may adversely affect innate and adaptive immunity.
Role: PI

Completed Research Support

2011/05/16-2016/05/15
HHSN 272201100017C , NIAID/NIH
NIKOLICH-ZUGICH, JANKO (PI)
Immune protection in Special Populations:
To use a combination of rodent and human models to elucidate critical age-related defects in innate and adaptive responses to West Nile Virus and other bioterror and emerging infections.
Role: PI

2001/08/01-2014/07/31
R01 AG020719-10, National Institute on Aging (NIA)
NIKOLICH-ZUGICH, JANKO (PI)
T cell Homeostasis and Function in Immune Senescence
Role: PI

2009/04/15-2014/03/31
R01 AI082529-04, National Institute of Allergy and Infectious Diseases (NIAID)
NIKOLICH-ZUGICH, JANKO (PI)
Rejuvenation of the T-cell compartment in aging primates
Role: MPI

2009/04/20-2014/02/28
U54 AI081680-01, National Institute of Allergy and Infectious Diseases (NIAID)
NIKOLICH-ZUGICH, JANKO (PI)
Broad based intervention to improve CD8-mediated protection
Role: PI

2009/09/30-2013/08/31
R01 AG035309-03, National Institute on Aging (NIA)
NIKOLICH-ZUGICH, JANKO (PI)
MECHANISMS OF REDUCED T-CELL IMMUNITY IN OLDER ADULTS
Role: PI

PHS Fellowship Supplemental Form

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

Introduction

1. Introduction
(RESUBMISSION)

Fellowship Applicant Section

2. Applicant's Background and Goals for Fellowship Training*	**Goals_for_F31.pdf**

Research Training Plan Section

3. Specific Aims*	**F31_Specific_Aims.pdf**
4. Research Strategy*	**F31_Research_Strategy.pdf**
5. Respective Contributions*	**Respective_Contributions.pdf**
6. Selection of Sponsor and Institution*	**SELECTION_OF_SPONSOR_AND_INSTITUTION.pdf**
7. Progress Report Publication List (RENEWAL)	
8. Training in the Responsible Conduct of Research*	**Responsible_Conduct_Research.pdf**

Sponsor(s), Collaborator(s) and Consultant(s) Section

9. Sponsor and Co-Sponsor Statements	**F31_2016_Janko_Sponsor_Section-jnz.pdf**
10. Letters of Support from Collaborators, Contributors and Consultants	

Institutional Environment and Commitment to Training Section

11. Description of Institutional Environment and Commitment to Training	**Description_Institutional_Environment_F31_jnz.pdf**

Other Research Training Plan Section

Human Subjects

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

Are Human Subjects Involved? ☐ Yes ☑ No

12. Human Subjects Involvement Indefinite?

13. Clinical Trial?

14. Agency-Defined Phase III Clinical Trial?

15. Protection of Human Subjects

16. Data Safety Monitoring Plan

17. Inclusion of Women and Minorities

18. Inclusion of Children

Vertebrate Animals

The following item is taken from the Research & Related Other Project Information form and repeated here for your reference. Any change to this item must be made on the Research & Related Other Project Information form.

Are Vertebrate Animals Used? ☑ Yes ☐ No

19. Vertebrate Animals Use Indefinite? ☑ Yes ☐ No

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20. Are vertebrate animals euthanized?	☑ Yes ☐ No
If "Yes" to euthanasia Is method consistent with American Veterinary Medical Association (AVMA) guidelines?	☑ Yes ☐ No
If "No" to AVMA guidelines, describe method and provide scientific justification	
21. Vertebrate Animals	**Vertebrate_Animals.pdf**

Other Research Training Plan Information

22. Select Agent Research

23. Resource Sharing Plan

24. Authentication of Key Biological and/or Chemical Resources

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Additional Information Section

25. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ☐ Yes ☑ No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s), using the registry information provided within the agency instructions. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

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

Cell Line(s):

26. Alternate Phone Number:

27. Degree Sought During Proposed Award:

Degree:	If "other", please indicate degree type:	Expected Completion Date (month/year):
PHD: Doctor of Philosophy		**05/2019**

28. Field of Training for Current Proposal*: 151 Immunology

29. Current Or Prior Kirschstein-NRSA Support?* ☐ Yes ☑ No

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

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

30. Applications for Concurrent Support?* ☐ Yes ☑ No

If yes, please describe in an attached file:

31. Citizenship*

U.S. Citizen — U.S. Citizen or Non-Citizen National? ☑ Yes ☐ No

Non-U.S. Citizen
- ☐ With a Permanent U.S. Resident Visa
- ☐ With a Temporary U.S. Visa

If you are a non-U.S. citizen with a temporary visa who has applied for permanent resident status and expect to hold a permanent resident visa by the earliest possible start date of the award, please also check here. ☐

Name of Former Institution:*

32. Change of Sponsoring Institution

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Budget Section

All Fellowship Applicants:

1. Tuition and Fees*:

☐ None Requested ☑ Funds Requested

Year 1	[redacted]
Year 2	[redacted]
Year 3	[redacted]
Year 4	
Year 5	
Year 6 (when applicable)	
Total Funds Requested:	[redacted]

Senior Fellowship Applicants Only:

2. Present Institutional Base Salary:

Amount	Academic Period	Number of Months

3. Stipends/Salary During First Year of Proposed Fellowship:

a. Federal Stipend Requested:

Amount	Number of Months
$0.00	0.0

b. Supplementation from other sources:

Amount	Number of Months

Type (sabbatical leave, salary, etc.)

Source

Appendix

Tracking Number: GRANT12231644

Funding Opportunity Number: PA-16-308. Received Date: 2016-08-12T15:15:24.000-04:00

GOALS FOR FELLOWSHIP AND TRAINING CAREER

To this point of my training I have fulfilled all required coursework as well as passed my qualifying exams to continue to PhD Candidacy. However, I will continue to be an active participant in journal clubs to discuss primary research, our research seminars to present my own research, as well as attend invited speakers from other institutions to continue to broaden my knowledge of my scientific field. In addition to these activities I will also attend domestic and international conferences, as will be outlined in the timeline below, and participate our department's local Frontiers in Immunobiology Symposium as well as my laboratories own sponsored International Cytomegalovirus Meeting. The main focus of my training will be the further development of the project herein described under the supervision of Dr. Janko Nikolich-Zugich, MD, PhD. In conjunction with the scientific training provided by Dr. Nikolich-Zugich, his network of colleagues and collaborators provides an unparalleled level of interaction with leaders of my field. To date I have been involved in several aspects of Program Projects Grant meetings, development of a second Program Project, and one-on-one interaction with Vishwa Deep Dixit, Charlie Surh, Michael Diamond, Richard A. Miller, and others. The beneficial impact that these interactions have to my career are immeasurable, as they provide interactions that will undoubtedly help me reach my primary goal of a post-doctoral training position following my PhD completion. After obtaining my PhD in Immunobiology, I aim to continue a career in biomedical and scientific research focused on the interface of infectious disease and adipose tissue immunology.

Post-doctoral training following completion of my PhD studies will help me to achieve the penultimate goal of an academic position where I am capable of having an impact not only in the biomedical research community and to also be involved in the development and training of students who are under represented minorities, such as myself. In line with the latter goal, I sit as a student-member of the Department of Immunobiology Diversity Committee and contribute to increasing visibility of a career in scientific research to non-traditional and under represented students. To this point in my career I have been given numerous opportunities to develop my grant writing skills, through a grant writing course proctored by several faculty members, I have been provided ample opportunity to speak in both scientific and non-scientific settings, I have been provided leadership positions as a planning member of a joint biological sciences program retreat at the University of Arizona. I will continue developing these skills during my remaining years as a PhD candidate in Dr. Nikolich-Zugich's laboratory. Finally, I will continue developing increased independence and autonomy as a researcher. Adipose tissue immunology in Dr. Nikolich-Zugich's laboratory was a completely unexplored field prior to my arrival and as such it has provided myself a level of independence and freedom that I believe would be difficult to come by in other laboratories. All of these various opportunities, I believe, will allow for my continued success in the sciences as my training progresses.

The Ruth L. Kirschstein NRSA Diversity Fellowship presents a unique opportunity to bolster many of the skills that I have been developing during my training. The identification of a specific unanswered and unexplored question in the field of immunobiology, and the generation of ways to address the question through the development and drafting of this proposal will pay dividends in my ability to apply for and obtain grant funding. Continuation of the funding will require the development, execution, and analysis of experiments and data. These activities will expand upon my critical thinking skills, the drafting of reports for both a scientific and non-scientific community, and ability to defend my work and thought process. As this is a diversity fellowship, I certainly understand and appreciate what that means for not only myself but also those who have applied before and will apply after me. The development of under represented minority students is crucially important to me, as it has provided a world that otherwise I would never have experienced. I have been funded by the NIH Initiative to Maximize Student Development and now am currently funded by an R01 Diversity Supplement. During my career I hope to continue to be provided by these programs and encourage my trainees to apply as well. Finally, the funding provided by this fellowship would help accomplish my research projects in a timely fashion whereby I can continue onto the future stages of my training.

ACTIVITIES PLANNED UNDER THIS AWARD

Year 1 (2017): Nikolich-Zugich Laboratory (The University of Arizona)

Research: 80%, Seminars/Journal Club: 10%, Conferences/Career Development: 10%

Research: Complete Aim 1.

Conferences/Career Development: Attend and present at the 6th International Workshop on CMV and Immunosenescence in Tucson, AZ. Attend and present at The American Association of Immunologists in Washington, DC.

Year 2 (2018): Nikolich-Zugich Laboratory (The University of Arizona)

Research: 80%, Seminars/Journal Club: 10%, Conferences/Career Development: 10%

Research: Submit manuscript on Aim 1. Complete Aim 2. Being writing thesis/dissertation.

Conferences/Career Development: Attend and present at the Keystone Symposium Integrating Metabolism and Immunity in Dublin, Ireland. Attend and present at The American Association of Immunologists in Austin, Texas.

Year 3 (2019): Nikolich-Zugich Laboratory (The University of Arizona)

Research: 80%, Seminars/Journal Club: 10%, Conferences/Career Development: 10%

The beginning of year 3 is the proposed year of thesis/dissertation defense.

Research: Submit manuscript for Aim 2. Defend thesis/dissertation.

Conferences/Career Development: Attend and present at relevant international meeting. Attend and present at The American Association of Immunologists in San Diego, California.

Attend relevant international conference.

RESEARCH EXPERIENCE

My research experience was delayed compared to most of my peers. I was the first person in my family to attend and graduate from university and as can be imagined I didn't really have much of a plan or understanding of what volunteering and academic research even was. I worked for most of my undergrad until I was fortunate to get a scholarship [redacted] The only laboratory experience that I had during my undergraduate studies had been the obligatory curriculum associated labs of chemistry, organic chemistry, physiology, biology, and organic chemistry. These were obviously not focused on answering unsolved questions but more focused on immersion of students into basic laboratory techniques. It was not until after I graduated that I first experienced basic scientific research.

I was accepted into a Professional Science Masters program, which, in my mind, can best be described as a mixture of MBA level business courses and first year PhD sciences courses. It was here that I first began conducting scientific research. As part of my Master's thesis I worked in Dr. Linda Powers' laboratory where the primary task was the development of a lateral flow assay for the detection of blood borne pathogens. This device was funded by a Office of Naval Research contract, and the assay was intended be used as a point of care device to determine if blood was suitable for transfusions. Specifically, my objectives were to use phage display peptide library to determine peptide interactions between Hepatitis B and C viruses, as well as HIV. The fundamental concept behind this project was that peptides identified to interact with these viruses would be conjugated to fluorescent markers and these markers would be detected and inform the operator whether blood was infected or not. During my time in the Powers lab I learned chemical techniques such as the sanitation of glassware by 'piranha etch,' which is a mixture of sulfuric acid and hydrogen peroxide. I learned how to perform peptide library biopanning with phage display technology and to crosslink peptides to glass surfaces. I learned proper techniques in handling select-agents. I successfully defended my Master's thesis after 1.5 years in the program.

Shortly before completing my Master's I was accepted into the University of Arizona biological sciences umbrella program. At the beginning of my studies I initially identified Dr. Nikolich-Zugich's laboratory as where I wished to conduct my thesis research, but as required by the program I had to perform research rotations. Fortunately for me I was funded by the NIHs Initiative to Maximize Student Development program and began research earlier than my cohort in the summer. I began my first rotation in the laboratory of Dr. Kirstian Doyle who investigates the long-term cognitive effects of stroke, and the immune response within stroke lesions. In this laboratory I was involved in setting up the workflow of a Magpix Luminex Assay, which allows for the detection of up to 25 different cytokines. We used this assay to investigate the inflammatory environment of the stroke lesions in the brain following stroke. From this work I gave an oral presentation to my cohort and faculty members.

My second research rotation was in the laboratory of Dr. Anita Koshy. Her laboratory focuses in the lifelong chronic infection of a parasite named *Toxoplasma gondii*. My responsibilities were two-fold, I helped quantify the number of macrophages and T cells in the brain following infection by using immunohistochemistry and I was responsible for engineering a mutant *Toxoplasma* with Crispr/Cas9 technology. Once again I presented my resulting data in an oral presentation to faculty and students of my cohort.

My third and final rotation was in the Nikolich-Zugich laboratory, where I currently am working on several projects. I am investigating if caloric restriction can be used to rejuvenate the aged immune system and, as seen in this research proposal, the contribution of adipose tissue immune cells to lifelong infection of cytomegalovirus. Since joining the lab I have given several oral and poster presentation on my work. Most, notably I was awarded an under represented minority travel award to attend the International ThymUS Meeting in Maui, Hawaii to present my work on calorie restriction. It is these experiences that I believe will allow me success in completing the goals stated within this proposal.

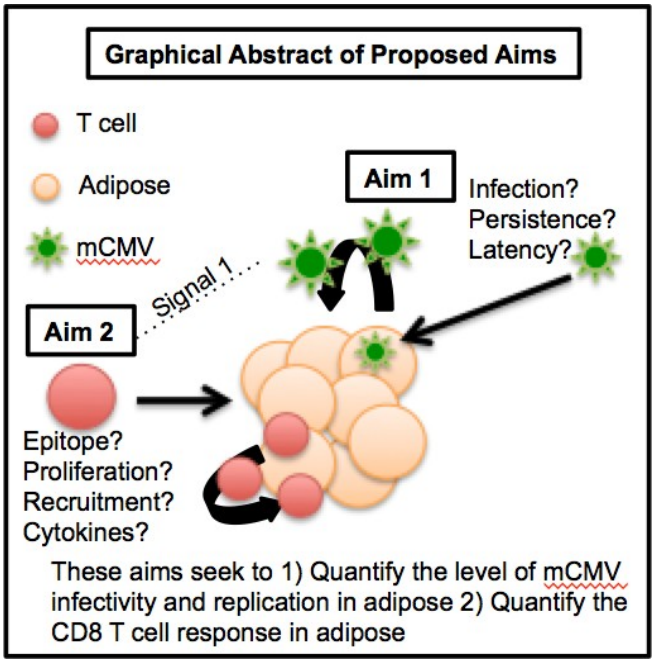
A. SPECIFIC AIMS

Adipose tissue consists of adipocytes that are crucial in lipid synthesis and energy storage, and a smaller population of cells of the stromal vascular fraction (SVF). The SVF represents a heterogeneous mixture of endothelial, stem, and immune cells, including T cells, macrophages, B cells, and NK cells. It has become increasingly clear that the immune responses within adipose tissue, such as cytokine secretion and tissue remodeling, influence host health and metabolism. Much emphasis has been placed on the activation of T cells and macrophages and their role in the chronic low-grade inflammation seen in obesity. However, inflammation in response to infections has been less thoroughly investigated. Adipocytes infected by adenovirus display an increased size and density, and *Trypanosoma cruzi* directly infects adipocytes resulting in adipose tissue inflammation. Recently, adipose CD4 T cells were shown to provide a site of latent viral infection in Human Immunodeficiency Virus (HIV) and Simian Immunodeficiency Virus (SIV).

Another virus capable of persistence and latency is cytomegalovirus (CMV), and it has been long implicated in low-level, systemic inflammation. The primary site of persistence for CMV is believed to be the salivary gland, but primary sites of latency have been difficult to conclusively identify. CMV infection results in a strong T cell response; in an acute infection ~5% of mouse, and up to 40% in some human patients, peripheral T cells are specific for CMV antigen. In our hands, we find that ~10% of adipose CD8 T cells in a mouse CMV (mCMV) infection are specific to mCMV at early comparable acute infection time points. This suggests that adipose tissue is an underappreciated site of viral infection and immune activity during CMV infection. **The primary hypothesis of this proposal is that mCMV disseminates to adipose tissue, replicates, establishes latency, leading to an lifelong CD8 T cell response.** Our long-term research objectives are to identify the lifelong immunological consequences of CMV infection. *The objective of this proposal* is to determine if mCMV establishes a productive infection within adipose tissue, and the consequences, if any, of that infection. As CMV is highly specific for host species, we will employ the mCMV model of infection to achieve the primary objective of this proposal through the following specific aims:

AIM 1: Evaluate adipose tissue as a reservoir for replicative and persistent virus. We hypothesize that adipose tissue is a location of active mCMV replication. To that end we will quantify viral load by plaque assay and qPCR in adipose compared to peripheral blood mononuclear cells (PBMCs) and salivary glands. We will determine the extent to which mCMV persists in adipose in a chronically infected mouse using multiple modes of reactivation. We will also identify infected cells within adipose. These experiments will determine what cells within adipose tissue can harbor replicative and persistent infection.

AIM 2: Determine the response of adipose tissue CD8 T cells during mCMV infection. We hypothesize that mCMV specific T cells expand in adipose tissue. We have observed a significant expansion of mCMV specific CD8 T cells following infection. As the immune response to mCMV in adipose tissue has never been fully characterized we will determine the kinetics of mCMV specific CD8 T cell expansion and proliferation. We will determine if local T cells clonally expand or naïve cells are constantly recruited to adipose. These experiments will provide, for the first time, an understanding of adipose tissue mCMV immune response.



See Graphical Abstract for Summary of Aims

IMPACT: Upon the completion of these studies, we will have significantly advanced the understanding of mCMV cell tropism. We will have identified adipose tissue as a site of replicative and persistent virus that is capable of reactivation. The functional response of CD8 T cells and their mechanism of recruitment to adipose will have been identified. We will also have revealed the mechanism of viral spread into adipose tissue. These findings will have far reaching implications on the consideration of adipose tissue during vaccine design.

B. SIGNIFICANCE. Cytomegalovirus (CMV) is a ubiquitous betaherpesvirus that infects a large percentage of people worldwide[1,2–4]. Infection progresses from an acute replicative cycle leading to a latent and lifelong infection[5,6]. can be damaging in the immune compromised such as immune suppressed transplant patients, Human Immunodeficiency Virus (HIV) patients, Acquired Immunodeficiency Syndrome (AIDs) patients, and unborn fetuses[6–9]. The hallmarks of CMV disease progression are not seen in immune competent patients, and this is a result of the significant amount of resources that the adaptive immune system dedicates to control CMV infections. In fact, 5-10% of CD8 T cells during a primary CMV response can be specific for an antigen generated by CMV[12–15]. The magnitude of this response is largely unparalleled in any other infection, and due to this, CMV has been investigated for its role in age-related T cell memory inflation[3,5,16–18]. Studies of CMV T cell inflation and viral dissemination throughout the host have largely been focused upon spleen, lung, liver, blood, and salivary glands[13,19–22]. These studies demonstrate that cell-free virus is spread in fluids that come into contact with mucosal barriers, such as saliva and breast milk[20,23]. However, the contribution of T cells and monocytes to the immune response, dissemination, and control of persistence *within* adipose tissue has never been interrogated. Many cells involved in the control and spread of CMV are all represented within adipose tissue (Figure 1), and recent reports have demonstrated the viral and parasitic effects on adipose tissue[28–30].

Adipose tissue is a heterogeneous tissue found at a variety of anatomical locations[31,32]. Visceral adipose found on the trunks of humans and mice is home to a large proportion of cells of the innate and adaptive immune systems[33–37]. Long thought to simply be a site of lipid synthesis and energy storage[31,32,38], adipose tissue is now regarded as the crossroads between host metabolism and immune system communications[39–42]. Indeed, adipose tissue is resident to T cells, B cells, macrophages, dendritic cells, NK cells, among others[33,35,36,43]. Investigation of infections and their effect on adipocytes have also increased interest in adipose tissue as an immunological site. [redacted] Futhermore, adipose tissue of HIV and SIV infected humans and monkeys harbored latent virus[51]. Given these observations, as well as our own preliminary data demonstrating a murine CMV (mCMV) specific CD8 T cell expansion in adipose tissue at 7 days post infection with mCMV (Figure 3), in comparison to uninfected mice, *we propose that adipose tissue is a location of productive mCMV infection and can possibly represent a site for persistent mCMV as well.*

This proposal is of significant clinical and biological importance as CMV is a high vaccine priority[52] and there have been no studies demonstrating the immune response within adipose tissue specific to mCMV. An understanding of the immune response to mCMV within adipose tissue and potential identification of a latent reservoir for mCMV will allow for more pointed future therapeutic and vaccine designs.

Figure 1: Immune Cells in Adipose

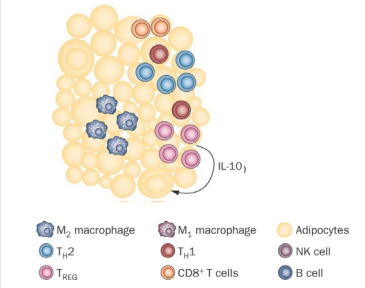


Fig 1. The immune system is represented in adipose tissue. During homeostasis anti-inflammatory macrophages and Tregs predominate. Adapted from Sell, H. *et al* (2012) *Nat. Rev. Endocrinol.*

Figure 2: Waist vs CMV Index Grouped

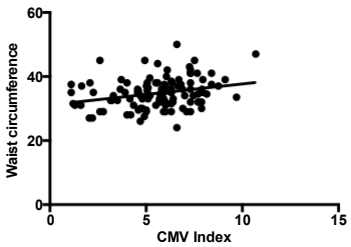


Fig 2. Waist Circumference as a function of CMV Index as quantified by Gold Standard CMV kit. Data gathered from Maecker et al. ImmPort Database. Pearson Correlation test p-value =0.0042

Figure 3: %M38+ of CD8+ T cells

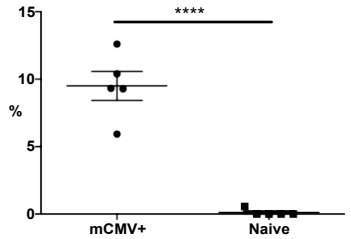


Fig 3. Increased frequency of mCMV m38 specific CD8 T cells in the adipose of infected C57BL/6 mice at 7 dpi. Significance determined by two-tailed unpaired t test. p-value < 0.0001

C. INNOVATION. CMV infection and the immunological consequences within adipose tissue have never been described. This proposal will provide clear evidence that adipose tissue is a site of active viral replication and a location for a productive immune response against mCMV during infection. This work represents a significant conceptual advancement in our understanding of host-pathogen interactions, and mCMV cellular tropism. Furthermore, the work described in this proposal will advance the development of vaccines against CMV by demonstrating the need for considering adipose tissue specific immune responses.

Development of new technologies for the detection of virus study is crucial for understanding host-pathogen interactions. While not the primary objective, the technical innovation of this proposal stems from the development of next generation RNA Prime Flow Probes specific for viral gene transcripts to be used in flow cytometry analyses.

D. APPROACH. Preliminary Data. Analysis of data[17] uploaded by Maecker et al. to the Immunology Database and Analysis Portal (ImmPort) revealed a correlation between adiposity as indicted by waist circumference[53,54] (Figure 2) and CMV Index as assayed by Gold Standard Biotechnologies CMV ELISA. These results indicated to us that adipose tissue could play a role in CMV infection, either serving as a primary site of replication or

allowing for increased viral load, or both. Lending more weight to these hypotheses are published reports demonstrating that increasing levels of adipose tissue are also correlated with increased pathogen burden[55,56].

These observations led us to explore what occurs within adipose tissue during acute mCMV infection of C57BL/6 mice. Mice were infected intraperitoneal (IP) with $3x10^5$ pfu of mCMV and sacrificed at 7 days post infection. Abdominal (pre-epididymal) adipose tissue depots were harvested and stained for analysis by flow cytometry. We employed a very simple phenotype and tetramer panel (Viability, CD4, CD8, m38 tetramer) to determine if there are an increased number or frequency of virus specific CD8 T cells in adipose tissue during acute mCMV infections. As can be seen in Figure 3 the CD8 T cells harvested from infected adipose tissue are significantly increased in comparison to uninfected mice. We fully expected this to be the result, however this data has not been previously reported. These results would suggest mCMV is infecting a component of the cellular constituents within adipose tissue and driving an adaptive immune response. Alternatively, mCMV antigen could be being presented within adipose regardless of productive replication or virus specific CD8 T cells are searching for their cognate antigen in this location following infection.

The long-term goal of our research is to identify the lifelong immunological consequences of CMV infection. The objective of this proposal is to determine if mCMV establishes a productive infection within adipose tissue. Additionally, we seek to determine mechanisms by which mCMV spreads to adipose tissue, if it does, and how antigen specific CD8 T cells arrive to adipose. **The primary hypothesis of this proposal is that mCMV disseminates to adipose tissue, replicates, establishes latency, leading to an lifelong CD8 T cell response.**

All experiments will use male mice 6-12 weeks of age from the C57BL/6 background. We use males to control for sex hormones, and microbiota differences[57]. We also use this background to take advantage of several established transgenic mice. Cohort size and design will be described within the following aims and sub-aims and are based upon power requirement analysis. By power analysis group sizes of 8 experimental and 8 control animals will allow for detection of >50% effect size between groups at $\beta=0.80$ and $\alpha=0.05$. All experiments will be carried out, independently, at least twice. All mice will be maintained in the specific pathogen free (SPF) animal facilities at The University of Arizona. Procedures will be performed in strict accordance with The University of Arizona Animal Care and Use Committee requirements. Adipose tissue described herein will refer to the abdominal fat of mice found within the peritoneum and surrounding the intestines. This fat pad has been published as pre-epididymal, epididymal, perigonadal, and visceral adipose.

AIM 1: Evaluate adipose tissue as a reservoir for replicative and persistent virus. mCMV reaches replicative latency following acute infection. Salivary glands release virion long after initial infection in both mice and humans[20,58,59]. It has been difficult to identify the cell that is most responsible for maintenance of persistent viral genome as many cells are susceptible to infection, with several studies implicating cell of the myeloid lineage[60–62]. mCMV research has shown many cells of the adipose tissue, in different contexts, as being important for spread and control of mCMV. It is necessary to investigate adipose tissue as a possible site of mCMV persistence. The *objective* of this aim is to determine the extent that adipose tissue serves as a reservoir for both replicative and persistent mCMV. We will test the *working hypothesis* that the cellular constituents of adipose tissue can serve as a site for active viral replication and following acute infection are sites of viral latency and persistence. We will test our working hypothesis by the *approach* of quantifying viral load within adipose tissue in comparison to previously identified replication and persistent sites. We will determine the extent that adipose tissue cellular constituents harbor latent virus by pharmacological, radioactive, and tissue explant reactivation of virus. Finally, we will determine the viral load of individually sorted cells of both the stromal vascular fraction and adipocyte populations. The *rationale* for this aim is that successful completion of the proposed research will contribute to the identification of persistent mCMV replication sites, which to this point have been difficult to identify. The identification of these latent sites of infection will provide a targeted location for the development of novel therapeutics designed to completely clear CMV. When the proposed studies of Aim 1 have been completed, it is our *expectation* that adipose tissue will have been identified as a site of persistent viral replication.

During infection, does mCMV replicate within adipose tissue? Currently it is unknown what consequence, if any, mCMV has within abdominal adipose tissue. We have observed inflationary[12,19,65] m38 specific CD8 T cells in acutely mCMV-infected adipose tissue from mice. While it is not surprising to observe an immune response in this location, we have found no reports demonstrating mCMV specificity. The expansion of CD8 T cells in adipose tissue in response to mCMV leads us to *the hypothesis that mCMV is actively replicating within adipose tissue*. Alternatively, it could be that mCMV specific CD8 T cells are searching for cognate antigen within adipose tissue rather than being activated. This, sub-aim will determine if adipose tissue serves as a site of viral replication during acute mCMV infection. To assess adipose tissue as a virally replicating site during acute infection we will infect 24 adult C57BL/6 mice, and compare viral load to 24 uninfected C57BL/6 mice. We will use the well-characterized Smith strain of mCMV[66–69] and infect with $3x10^5$ plaque forming units (pfu)

by intraperitoneal (IP) injection. At days 3, 7, and 14 post-infection we will sacrifice mice by isoflurane euthanasia. At each time point we will sacrifice 8 mice from the infected and uninfected groups. Adipose tissue, PBMCs, spleen, and salivary glands will be taken for viral quantification at each time point. We will determine viral load by plaque assay as previously described[58,70,71] and by quantitative (qPCR) against DNA, to determine viral load, and reverse transcribed cDNA to determine level of activation, of mCMV Immediate-Early 1 gene (IE-1) as well as Late (L) gene gB[72–75]. Tissues will be weighed and cut in half to quantify viral load per gram. Half of the tissue will be used for plaque assay on the 3T3 mouse embryonic fibroblast (MEF) line as previously described[76]. The remaining half of tissues will be taken for DNA/RNA extraction, as previously described[77–79], and copy number of IE-1 and gB will be determined. As a control in qPCR we will determine copy number of GAPDH. The data will be analyzed using GraphPad Prism using a Two-sample Student's T test between the same tissues and products (I.E. uninfected adipose vs. infected adipose). A p-value < 0.05 will be considered statistically significant.

Anticipated Results. It is expected that the viral load of adipose tissue to be comparable to that of spleen at day three and seven post-infection. At day fourteen post-infection we expect to see a reduction in viral load in adipose tissue and an increase in salivary gland as has been reported[58,69,80]. We anticipate that plaque assays at these time points will be sufficient to detect replicating virus. From these experiments we will have established adipose tissue and its cellular constituents as a site for productive mCMV replication.

Pitfalls and Alternatives. Our laboratory has experience with cell culture and it is not anticipated that these experiments will be technically challenging. The gold standard for mCMV plaque assays has been the use of MEFs but these cells are not immortalized and therefore must be repeatedly produced and stocks can vary from lot to lot. If these cells do not yield interpretable data or do not form plaques we will switch to a newly described protocol that allows for greater viral spreading during assay[70]. It is entirely possible that mCMV does not replicate within adipose tissue. This is not our expectation based upon the increased presence of mCMV specific CD8 T cells in comparison to uninfected adipose. If we find no virus, this would suggest that CD8 T cells are either searching for their cognate antigen or were trafficking through adipose at the time point we looked. Even if this null result occurs the presence of CD8 T cells within adipose tissue even without detectable actively replicating virus raises the following questions (i) what signals are these mCMV specific T cells receiving to traffic to adipose tissue and (ii) is antigen being presented in the absence of viral replication within adipose tissue? The sub-aims to follow this will seek to address these questions regardless of viral replication or not and therefore this is not a hindrance to this proposal.

Does the stromal vascular fraction (SVF) or buoyant adipose fraction of adipose tissue contribute higher viral load? In order to study the stromal vascular fraction of adipose tissue by flow cytometry it is necessary to separate SVF cells from adipocytes[81,82]. During processing a centrifugation step separates the buoyant adipocytes and macrophages and leaves the pelletable SVF. The floating fraction and the pelleted fraction are a heterogeneous mixture of cells found within adipose tissue. Both fractions are potential targets for mCMV infection[83–85]. We will infect 16 C57BL/6 mice with $3x10^5$ pfu of Smith mCMV by IP injection and at days 3 and 7 post infection we will sacrifice 8 mice each and harvest adipose tissue. We will process adipose tissue as previously described[81,82,86], but once we reach the fractionation step we will culture the SVF and the buoyant fraction, separately, on a monolayer of MEFs to determine what component of adipose tissue contains infectious virus. Cells from SVF and buoyant layer will be quantified by hemacytometer, serially diluted, and added to a monolayer of MEFs in RPMI media with 10% fetal bovine serum and broad-spectrum antibiotics. Plates will be incubated at 37 C with 5 % carbon dioxide. We will monitor plates for plaque formation or cytopathic effect (CPE) on MEFs. Plaques will be quantified and compared against uninfected fractions of equivalent cohort sizes. The data will be analyzed in GraphPad Prism. Statistical significance will be determined between SVF and buoyant fraction by Two-sample Student's T test. A p-value < 0.05 will be considered significant.

Anticipated Results. There are four possible outcomes following this experiment (i) the SVF results in viral plaque formation, (ii) the buoyant fraction results in viral plaque formation, (iii) both result in plaques, or (iv) neither SVF nor buoyant fraction result in plaques. If the first outcome is true then the adipocytes can be excluded from the following aims. It is not expected that our second described outcome be observed alone, as the SVF contains the canonically infected cells. The third possibility is our expectation and would indicate that both the adipocytes and the heterogeneous vascular fraction are susceptible to viral infection. We do not expect the fourth outcome to occur based upon antigen specific CD8 T cells being harvested from infected fat.

Pitfalls and Alternatives. We do not anticipate this experiment to be technically challenging and there exist several alternative methods to harvest and separate the cellular constituents of adipose tissue[81,82,87]. We have also identified alternative methods within this proposal to use if we have technical challenges using 3T3 MEFs[70]. If plaques do not form on the cell monolayers then we will bulk assay both the buoyant and SVF of

adipose tissue and carry out whole cell qPCR for viral DNA of IE-1 and L gene gB. If plaques do not form and we do not detect viral products by qPCR then we will conclude that neither adipose nor SVF are infected.

What cells harbor virus within adipose tissue? We will identify the cell types that contribute to mCMV infection *in vivo*. To do this we will make use of a mCMV mutant that has an eGFP within the m157 ORF and expresses the immunodominant SIINFEKL peptide. This virus will make any cell infected with mCMV be fluorescent by eGFP. We will infect 16 C57BL/6 mice with $3x10^5$ pfu of mCMV-eGFP-SL8 by IP injection[88]. At day 3 and 7 post infection we will harvest the adipose tissue and spleens of 8 mice each day. Spleen will be used as a positive control. We will make comparisons against uninfected littermates. By flow cytometry we will determine the infected cell types of the SVF using specific phenotyping markers for differentiation of macrophages, monocytes, neutrophils, T cells, NK cells, B cells, and endothelial cells. If we find that the buoyant fraction of adipose tissue is also infected as determined by plaque assay we will include adipocyte markers within our flow cytometry panel, as previously described[81,82,86]. We will identify individual cells that co-express eGFP. Flow cytometry data will be collected on our custom-made BD Fortessa flow cytometer (Becton Dickinson, Sunnyvale, CA). Data will then be generated for analysis in FlowJo software and analyzed for significant differences by using GraphPad Prism using a Two-sample Student's T test. A p-value < 0.05 will be considered statistically significant.

Anticipated Results It is our expectation that macrophages at either end of their polarization will be infected with mCMV, as determined by eGFP+ signal. We also expect that virus will infect endothelial cells found within adipose tissue. These results would suggest that mCMV is capable of a broad cellular tropism and there are multiple cells within adipose tissue that are capable of becoming infected and contributing to viral burden.

Pitfalls and Alternatives. It is possible that the adipose tissue does not serve as a site of primary infection or persistence; we absolutely do not expect this to be the case. However, if adipose tissue is not a site of viral replication as determined from the above proposed work then we can conclude that there is/are signal(s) that attract CD8 T cells to adipose tissue. We will determine these signals by adipose tissue culture explants. This experiment is also described below in Aim 2. If we are unable to identify individually infected cells by flow cytometry with our fall back of signal amplification with anti-eGFP antibody we will then use a next generation RNA-Prime Flow Assay (eBiosciences). We have had probes generated that are specific for Immediate Early gene-1 and -2 (IE-1 and IE-2) that are conjugated to a fluorophore. By flow cytometry we will use these probes to identify infected cells and then determine infected cells by this method.

Does adipose tissue serve as a site for mCMV reactivation? If we find that adipose tissue serves as a site of viral replication as determined by plaque assay and qPCR then *we hypothesize that this location can become a reservoir for latent virus.* mCMV reactivation has been demonstrated to occur stochastically[71,88–90], but reactivation can also occur during immunosuppression and infection, as seen in HIV infection and during transplantation surgery[9]. We will test our hypothesis by infecting 10 mice per three different treatments (30 total) C57BL/6 mice with $3x10^5$ pfu of Smith mCMV by IP and allow infection to continue for at least three months. We will use three methods of viral reactivation; (i) pharmacological, (ii) radiation, and (iii) tissue culture explants. For pharmacological reactivation mice that were infected will be treated with cyclophosphamide (CY) as previously described[59,71,91]. Mice to be analyzed for radiation reactivation will be total-body gamma irradiation with a single, sub-lethal dose of 6 Gy from a ^{137}Cs gamma radiation source[89]. The following 8 days, mice will be treated with the antibiotic cotrimoxazole, 240 mg/L of drinking water. Finally, we will harvest the adipose tissue of infected mice and culture by adaptation of a previously described protocol[22,92,93]. Controls will compare equivalent numbers of uninfected mice and mice infected for greater than three months without intervention. Positive controls will be infected analyze lungs[89,92,93]. All methods will be quantified by plaque assay and qPCR quantification of viral DNA and reverse transcribed cDNA, for viral state of reactivation, at 7-8 days post reactivation treatment. The data will be analyzed using GraphPad Prism. Significance will be determined by a one-way ANOVA. A p-value < 0.05 will be considered statistically significant.

Expected Results. It is expected that adipose tissue at greater than 3 months post infection will provide infectious virus following reactivation by drug, radiation, or tissue culture explants. Furthermore, we anticipate that we'll be able to detect virus from adipose of non-reactivation infected mice. We do, however, expect reactivation to have a significantly higher viral load than the untreated infected littermates. This result will indicate to us that adipose tissue is a site of persistence and a reservoir for viral latency. If we do not find virus following reactivation then we will conclude that adipose tissue is not a site of mCMV latency and persistence.

Pitfalls and Alternatives. We expect that CY will be sufficient to reactivate viral replication in

Figure 4: Experimental Progression of Aim 1

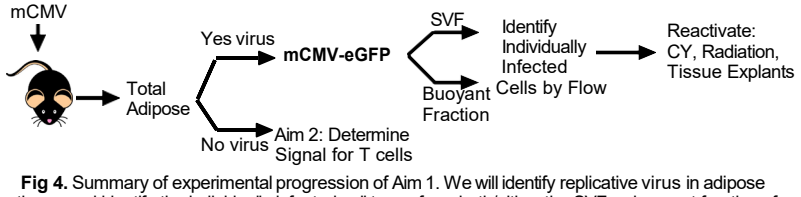


Fig 4. Summary of experimental progression of Aim 1. We will identify replicative virus in adipose tissue and identify the individually infected cell types from both/either the SVF or buoyant fraction of adipose.

adipose tissue due to its immune suppressant properties. However, if we are unable to find replicating virus in mice treated with CY we will then try a coupled immune suppression approach. We will use CY as well as depleting antibodies for CD8, CD4, and NK cells[94–98]. If following this coupled approach we do not find reactivated virus in adipose tissue, as well as through the other methods, then we will conclude that through this method virus is not reactivated. It is possible, but also highly unexpected, that adipose tissue does not serve as a site of viral replication in the chronic time point that can be reactivated by any of these three means. This would make our observation of an increase in m38 mCMV specific CD8 T cells even more interesting as it would suggest that adipose tissue serves as a site of antigen scanning and/or cytokine signaling changes.

Summary. From these above described experiments we have placed fallbacks and contingencies to determine if adipose tissue serves as a site of viral replication and latency, as well as identified the cells harboring virus through three separate methods. If we are unable to find reproducing virus we will determine (Aim 2) the signaling molecule(s), cytokine, chemokines, or adipokines, that are responsible for the trafficking of CD8 T cells to adipose tissue (Figure 4). This aim will provide the first definitive evidence of mCMV replication within adipose tissue. Furthermore, we will have evaluated adipose tissue as a reservoir of latent mCMV infection. Therefore, these experiments provide a significant advancement in our understanding of the contribution of adipose tissue to the overall viral load of an organism.

AIM 2: Determine the response of adipose tissue CD8 T cells during mCMV infection. The immune response to mCMV during acute or chronic infection *within adipose tissue* has never been reported. The *objective* of this aim is to determine what phenotypic and functional changes occur to CD8 T cells within, or trafficking to, adipose tissue during mCMV infection. We will identify these functional changes by testing the *working hypothesis* that mCMV establishes productive replication within adipose tissue that leads to a CD8 T cell response. We will test our working hypothesis by infecting C57BL/6 mice with the Smith strain mCMV and determine the kinetics of mCMV specific CD8 T cell expansion. We will carry out a phenotypic and intracellular cytokine staining of CD8 T cells by flow cytometry to determine the epitope specific T cells that expand and if these cells contribute to the phenomenon known as memory inflation[12,19,65,88]. The *rationale* for this aim is that we have observed an increased frequency of mCMV specific CD8 T cells in the adipose tissue of infected mice. This data would suggest that mCMV is either replicating within adipose or CD8 T cells specific to mCMV traffic to adipose following infection. When the proposed studies of Aim 2 have been completed, it is our *expectation* that adipose tissue will be a necessary site for investigation during infections. Such a finding would be of importance, because this work represents the first time the adipose tissue CD8 T cell response to mCMV infection has been characterized. Insight into adipose tissue immunological response is of importance as its impact on controlling infectious disease is currently incomplete.

Are antigen specific CD8 T cells in the adipose tissue during infection? From the above experiments we will have determined viral load of mCMV during acute infection in adipose tissue compared to previously described sites of infection. In this sub-aim we will determine the extent to which T cells found in adipose tissue contribute to memory inflation seen in the periphery during mCMV infections[19,65,88]. CMV infection, in mice and humans, leads to a robust CD8 T cell response. In the C57BL/6 mouse model of infection the CD8 T cell response is characterized by distinct memory CD8 T cell patterns[12]. During the acute infection most epitope specific cells contract however there are identified epitopes that continue to expand and have the short-lived effector cell phenotype ($CD62L^{low}$, IL-7Rα^{-}, IL-15Rβ^{-}). This phenomenon is termed memory inflation and due to the phenotype of these cells it is believed they are actively responding to antigen[99–101]. This sub-aim will determine the extent to which adipose tissue mCMV specific CD8 T cells follow the known epitope contraction and expansion, as well as the phenotypic and functional properties of T cells during mCMV infection. We hypothesize that CD8 T cells that are specific for an epitope of mCMV are recruited to adipose tissue during the course of infection. Furthermore, we hypothesize that these cells will possess the short-lived effector cell phenotype. To test this hypothesis we will infect 56 C57BL/6 mice with $3x10^5$ pfu of Smith strain mCMV by IP injection. At days 7, 14, 21, 30, 90, 150, and 300 post-infection we will sacrifice 8 mice and analyze tetramer specific CD8 T cells. These time points are selected to consider the acute epitope contraction as well as memory inflation. We will include in our analysis markers for CD4 T cells, as previous studies in the context of obesity have demonstrated a loss of regulatory CD4 T cells[44,102,103]. We will collect peripheral blood as a positive control for T cell expansion as this has been recently described in the blood[12]. Adipose and blood will be processed and stained for flow cytometry. The data that we collect will be analyzed in GraphPad Prism. Significant differences between the number and frequency of tetramer specific CD8 T cells in adipose and blood will be determined by a Two-sample Student's T test, comparisons made against uninfected mice of equivalent cohort size. A p-value < 0.05 will be considered statistically significant.

Anticipated Results. It is our expectation that adipose tissue specific CD8 T cells will mirror the reported expansion and contraction kinetics seen in blood, with contraction of m45 specific cells after 7 days of infection and a continued expansion of m38 and m139 tetramer specific cells. Based on our hypothesis that adipose

tissue is a site of latent viral replication we do expect that m38 memory inflation will occur faster within adipose tissue when compared to blood. We anticipate that the number and frequency of CD8 T cells that have the short-lived effector memory phenotype will be greater in adipose than the population found in blood.

Pitfalls and Alternatives. We fully expect that mCMV CD8 T cell expansion and contraction within adipose tissue will follow the same pattern as seen in the periphery and spleen of infected mice. However, it's possible that the expansion and contraction of certain viral epitopes would vary. Therefore, if we do not observe the same antigen specific CD8 T cell expansion and following memory inflation we will determine the epitopes that adipose tissue CD8 T cells are specific for by stimulating CD8 lymphocytes *ex vivo* from adipose tissue of infected mice. This technique has been described elsewhere[12], but briefly; K41 cells, an SV-40 transformed H-2^b fibroblast cell line will be plated at 4000 cells per well in a 96-well plate and transfected with plasmid DNA from ORF library. CD8 T cells from adipose tissue of infected mice will be sorted and plated at a density of $1x10^4$ per well in the presence of brefeldin A (BFA) and incubated for 7 hours at 37 C. Cells from wells will then be stained for CD8a, CD44, CD62L, and intracellular stained for IFNγ and quantified by flow cytometry. The percentage of T cells producing IFNγ will be quantified for each epitope. While it is unexpected that this will be required, this experiment will provide insight into any differences in mCMV response of adipose tissue CD8 T cells, as well as differences of cells that traffic to adipose tissue.

What signal(s) recruits T cells to adipose tissue during acute and chronic time points? In obese adipose tissue a myriad of cytokine, chemokine, and adipokines are secreted that leads to the recruitment of leukocytes resulting in chronic low-grade inflammation largely believed to lead to insulin resistance and glucose intolerance[35,44]. As mentioned in Aim 1, we seek to determine what signals are calling for the recruitment of T lymphocytes to adipose tissue during mCMV infections. We will infect 40 C57BL/6 mice with $3x10^5$ pfu of Smith mCMV by IP. At days 1, 2, 3, 7, and 100 days post infection we will harvest adipose tissue from 8 mice per time point and culture half of the adipose and SVF, freezing the other half in order to validate chemokines by qPCR following initial assay. Supernatants of adipose tissue and SVF will be collected one day post culture and then by Luminex Bead-based Multiplex Assay (R&D Systems) we will determine what cytokine, chemokine, and/or adipokines are upregulated at these time points using commercially available kit ProcartaPlex Mouse Cytokine & Chemokine Panel 1 (26 plex) (eBioscience). Data will be collected on a MAGPIX (Luminex Corp.) instrument from the Kristian Doyle Lab (University of Arizona). Data will be analyzed in GraphPad Prism and significance will be determined by one-way ANOVA comparisons between uninfected littermates. We will then validate any significantly increased molecules by qPCR. These experiments will be used for future study to deplete and/or knockout the signaling molecules we find to be upregulated.

Anticipated Results. [redacted] We expect that IL-6, TNFα, RANTES, CXCL12 will all be upregulated in comparison to uninfected adipose tissue. These chemokines and cytokines have been studied for their effects in T cell migration. If these molecules are indeed upregulated, then they will be correlated with the increase in T cells we find in infected adipose tissue. For future study we will attempt to disrupt the receptors, CCR5 and CCR6, to determine the extent to which T cell migration is ablated during infection.

Pitfalls and Alternatives. [redacted]

Summary. The experiments proposed in Aim 2 will characterize the CD8 T cell immune response within adipose tissue. We will have defined the kinetic response of mCMV specific CD8 T cell expansion and contraction, as well as the contribution, if any, of adipose tissue T cells to memory inflation seen in the periphery during mCMV infections. We will have determined the crucial signaling molecules necessary for T cell response to mCMV within adipose tissue.

Overall Summary and Conclusions

The Nikolich-Zugich laboratory is uniquely positioned to carry out the above-described work. We have demonstrated proficiency in the use of flow cytometry for analysis of single cells and whole cell populations[3,122–124]. The contributions of our laboratory to an understanding of lifelong CMV infection[5] will now address a completely unexplored area in the context of adipose tissue. *The above-proposed work represents a significant advancement in our understanding of mCMV infection and the contribution of adipose tissue to an immunological response.* Through these experiments we will have determined if mCMV is capable of replicating and persisting in adipose tissue and the consequential CD8 T cell immune response. These findings would increase our understanding of mCMV tropism and consequential immune response.

Respective Contributions:

The mentor plan was designed in collaboration by the Supervisor (Nikolich-Zugich) and Principal Investigator (PI, Contreras) based upon curriculum completed prior to joining the laboratory and work occurring within the laboratory. The research strategy and proposal was drafted and submitted to the PI's qualifying exam committee. Minor edits based upon feedback of the committee were made.

SELECTION OF SPONSOR AND INSTITUTION

Sponsor: Dr. Nikolich-Zugich is a Professor and Department head of the University of Arizona Department of Immunobiology and Co-direction of the Arizona Center on Aging. He sits as editor of several journals, members of various committees, and organizer of a variety of conferences. His laboratory is focused on the age related decline of the immune system and specifically that of the T cells. The long-term focuses of the lab are the basic mechanisms of T cell function, immunity to infection in older adults, immune rejuvenation, chronic infection, and the impact of inflammation and nutritional intervention in aging, among others. I selected Dr. Nikolich-Zugich's laboratory because of my interest in age related changes to biology and his work in calorie restriction and immune function. Dr. Nikolich-Zugich's expertise in the effect of lifelong infection with cytomegalovirus and immunological parameters provides crucial support to the aims of this proposal. Furthermore, the size and facilities available to his laboratory are a tremendous boost to experimental work and design.

Institution: I chose to attend the University of Arizona for several reasons. I acknowledge that movement from institutions are the norm and generally supported to provide a wide array of view points teaching styles, however being in a military family I moved every three years and the opportunity to live for an extended period of time in one location was very appealing to me. Second, and most important, the facilities, collaboration, level of openness, and the energy of the principal investigators in the Department of Immunobiology are contagious. The facilities are some of the best I've seen and new facilities and buildings are being constantly built and upgraded. Furthermore, Dr. Nikolich-Zugich has created an environment that, I believe, has provided unparalleled opportunity for success. For these reasons and more the University of Arizona has provided me so much room for personal and academic success and growth.

Additionally, our laboratory has collaborations with several top immunologists and virologists that have contributed greatly to the development of these and other projects within our laboratory. As mentioned above, the collaboration, openness, and energy has allowed for the free movement of ideas. Several lab groups in the department study cytomegalovirus, the focus of this proposal, and the investigation of one pathogen from multiple different angles will provide extraordinary support in the development of this proposal and future work.

Plan for Instruction in the Responsible Conduct of Research

The University of Arizona offers a certificate in RCR education designed to meet NIH guidelines. The certificate requires a minimum of nine (9) hours of RCR instruction, comprised primarily of live workshops, presentations, academic coursework and/or face-to-face discussions led and facilitated by faculty, compliance officers, and mentors. The NIH RCR Certificate may be earned by taking an approved course for academic credit or by completing the following workshop-based curriculum:

Core Workshop: 1.5 hrs

- Introduction to the Responsible Conduct of Research (choose one of the following): Introduction to RCR (face-to-face workshop) or Online Introduction to RCR (CITI)

Five Elective Workshops: 7.5 hrs

- Select from RCR Workshop Series (see 2016-2017 schedule below)
- Up to 3 hours of supplemental instruction from faculty mentor(s) may count toward the elective requirement.

Sample 2016-2017 RCR Workshop Schedule (Visit rgw.arizona.edu/rcr for most up to date schedule)

Fall 2016:

September 9 or 16	Introduction to the Responsible Conduct of Research
September 30	Ethics of Research with Human Subjects
October 14	Ethics and Practice of Mentoring
October 21	Animal Research: Ethical and Regulatory Considerations
October 28	Data Management, Acquisition, and Ownership
November 11	Ethics, Integrity, and the Handling of Research Misconduct
November 18	Ethics of Authorship and Publication

Spring 2017 - Dates TBD: Conflict of Interest, Ethics and Practice of Peer Review, Ethics of Research with Human Subjects, Ethics of Collaborative Research and Working with Industry, Introduction to Safe Laboratory Practices, Ethical Considerations in Biomedical Research, and Ethics of Overlapping Publications.

Training Completion Deadline: At the University of Arizona the NIH RCR Certificate program must be initiated within 30 days of the post-award date and completed within one calendar year after the post-award date.

Training Topics: RCR educational topic areas include but are not limited to: Animal Subjects Protection**; Collaborative Science; Conflict of Interest; Data Acquisition, Management, Sharing and Ownership; Human Subjects Protection**; Mentor/Trainee Relationships; Peer Review; Publication Practices and Responsible Authorship; and Research Misconduct. ***Special Note**: At the University of Arizona, research with human subjects (orcr.arizona.edu/hspp) and animals (orcr.arizona.edu/iacuc) require specific training requirements that are additional to and are not satisfied by the RCR training requirements described in this Institutional Plan for NIH-required RCR training.

Contact Hours Required: The University of Arizona requires a minimum of 9 hours of RCR instruction to earn the NIH RCR Certificate. However, more hours are encouraged.

Instructional Format: At least 7.5 hours must be comprised of live instructional formats such as workshops, academic course hours, and face-to-face discussions with faculty mentor(s) and peers. The University of Arizona encourages NIH-funded scholars, trainees, and fellows to participate in as much live instruction as possible. No more than 1.5 hours of supplemental online instruction in RCR may count toward RCR Certificate.

Tracking and Verification of Hours: Principal Investigators conducting NIH-supported research are responsible for ensuring that all students and postdoctoral researchers associated with the award complete the RCR training requirements as described above. Individuals should keep documentation of the RCR training they receive, including all workshops and face-to-face discussions with faculty mentors.

Monitoring Compliance: The University of Arizona, through the Office for Research and Discovery, is responsible for certifying that the RCR training plan is in place and verifying certificate completion. UAccess Learning will be the primary tool for this verification.

Consequences of Noncompliance: Noncompliance with the NIH requirements for RCR training may result in the forfeiture of research funds and sanctions against future NIH or other federal agency research funding, in addition to any institutional sanctions pursuant to relevant UA personnel or other policies.

The Value of RCR: Fulfillment of NIH requirements in Responsible Conduct of Research (RCR) training is just one, albeit very useful, component of training generations of University of Arizona researchers in the highest professional standards. Professional excellence in research includes a dedication to integrity. Education in the responsible conduct of research meets more than just regulatory standards and requirements. It also promotes ethical professional values and behaviors.

Sponsor Section and Statement

1. **Research Support Available**

Source	Application ID	Project Number	Title of Program	Principal Investigator	FY Start	FY End	Amount
NIA	8842072	5R21AG045734-02	LONGEVITY EXTENSION AND IMMUNE FUNCTION IN AGING (R21)	Nikolich-Zugich, Janko	2015	2017	177,449
NIA	9060874	5R01AG048021-03	IMPACT OF CMV UPON T-CELL AGING AND IMMUNE DEFENSE	Nikolich-Zugich, Janko	2016	2019	456,185

2. **Sponsor's Previous Fellows/Trainees**

With regard to this I have trained and mentored 11 doctoral students (10 graduated with Ph.D., one with M.Sc) and 20 postdoctoral trainees, and am currently training/mentoring one doctoral student, 2 postdoctoral trainees and 5 junior faculty/clinical fellows with K or R awards. Of these, 11 former postdoctoral trainees hold faculty or senior industry positions, and of 10 former Ph.D. students, two are in faculty positions and 6 have continued postdoctoral education. Below is a sample of five representative former trainees:

Name	Position in Nikolich Laboratory	Current Position and Institution
Kristin Renkema	Predoctoral	Postdoctoral Fellow, University of Minnesota
Nicholas Fox, M.Sc.	Predoctoral	Research Associate, Columbia University, New York, NY
Jason L. Pugh	Predoctoral	Postdoctoral Fellow, Stanford University
Emily Goldberg	Predoctoral	Postdoctoral Fellow, Yale University
Vesna Pulko, Ph.D.	Postdoctoral	Senior Scientist, Roche, Zurich, Switzerland

3. **Training Plan, Environment, Research Facilities.**

Training Plan

Formal coursework at the University of Arizona for Nico will encompass his required and elective courses towards his Ph.D. in Immunobiology. These courses include general cell and molecular biology courses: MCB 595E Topics in Research, IMSD (2 units); MCB 528L Microbial Genetics Lab (2 units); MCB 528R Microbial Genetics (3 units); MCB 568 Nucleic Acids (4 units); MCB 516A Statistics Bioinformatics and Genomic Analysis (3 units), and IMB 565 Principles and Molecular Mechanisms of Microbial Diseases (3 units), all of which are in progress and will be over by the end of Spring, 2015; and IMB 564 Advanced Topics (3 units); IMB 521 Scientific Writing (2 units); MCB 695E Science, Society and Ethics (1 unit) that he will take in

the Fall 2015; and the Seminar Series Course IMB 595A (1 unit) / IMB 695A (1 unit) that run throughout his studies until graduation. Nico has already completed with high marks two courses: MCB 577 Principles of Cell Biology (4 units) and IMB 548 Basic Immunological Concepts (3 units),
To complement this with specific knowledge in the Biology of Aging, he will be attending the ArizonaMed (Medical Student Curriculum at the University of Arizona) classes devoted to aging within the Life Cycle block, including the Biology of Aging; Aging and Cancer; and a series of translational and clinical lectures on delirium, dementia and other pertinent issues in gerontology and geriatrics.
Nico participates in the Seminar Series course (IMB595/695), which, amongst other activities, requires him to present his work once a year to an audience composed of faculty and trainees from the Department of Immunobiology, the Arizona Center on Aging and other departments and centers with interest in immunobiology and aging, including, but not limited to, Departments of Molecular & Cellular Biology, Cellular & Molecular Medicine, Pharmacology, Physiology, Bioengineering and others. Critical thinking, clarity of presentation, presentation style, content and density are all evaluated by his peers and teachers. Moreover, that same venue brings about top immunologists, many of whom are interested in aging. In the past year alone, we have had Drs. Charles Surh (Immune homeostasis in aging); Michael Diamond (Immunity to flaviviruses with aging), Elias Haddad (Innate immune defects with aging). This past spring semester, we also focused on proteins in the aging process and together with Departments of Molecular and Cell Biology and Cellular and Molecular Medicine, we hosted Drs. Ana Maria Cuervo (proteostasis and aging) and Rick Morimoto (Protein integrity during the aging process). We plan to continue this in the upcoming season with visits by Dr Vojo Deretic (Autophagy in immunity and aging); Shannon Turley (Genentech, Inc.; aging of secondary lymphoid organs) and Derek Huffman (Einstein; aging and metabolism).

Environment.

The “Advances in Aging” lecture series at the University of Arizona, as well as the “Frontiers in Medical Research” are two of the main venues that bring extramural researchers working on biology of aging to Tucson. Nico will continue to be active in attending the seminars and meeting the speakers, which, over the past few years, included Drs David Hammerman, John Burton, Kevin High, Jeremy Walston, Daniel Goldstein, Randy Strong, Richard Besdine, Richard A. Miller, William Hall, Vishwa Deep Dixit and others.
Furthermore, Nico is now a member of the American Aging Association and will be attending the Annual Meeting in June 2017, and yearly thereafter; he will further be encouraged to attend the annual GSA meeting. He will be expected to present his work either in a Poster or Oral Presentation format for every meeting he attends.

Nico will be applying to attend the NIA Advanced Course on the Biology of Aging, held by the Nathan Shock Centers, probably slightly later in his graduate career (4th year). This course would be supremely useful to his development, albeit we realize that the preference for attendance is usually given to advanced postdoctoral fellows and junior faculty. In addition, if the Molecular Biology of Aging course is repeated during his Ph.D. training (MBL, Woods Hole, MA), he will apply to attend.

Nico is a graduate student in my laboratory and is part of our regular laboratory meetings that are entirely devoted to the immunology of aging, every Friday for 1.5h; moreover, we meet regularly to review his direct progress and results every other week for one hour at minimum. During the period of manuscript preparation, preparation for committee meetings or presentations (departmental Research-In-Progress, University, College or extra mural poster or oral presentation) that frequency is increased to up to several times a week, until we both feel that Nico is ready to present his work externally. Over and above that in Spring 2015 we established Nico’s Thesis Committee. There will be regular meetings (2-3/year) of Nico’s committee, which will oversee his progress toward the Ph.D. thesis.

To further illustrate the environment, I would like to provide a brief overview: I am Professor and Head, Department of Immunobiology, and co-Director of the Arizona Center on Aging. I am leading or am part several large collaborative studies on immune aging, using rodent, Rhesus macaques (RM) and/or human models –a N01 contract to evaluate vulnerability to the West Nile virus in elderly; a N01 contract, based in Hiroshima, Japan, involving five US groups and 12 Japanese groups to evaluate the impact of radiation upon immune aging; and an immediately past collaborative transatlantic grant to connect immune aging studies between human and rodent model (with Prof. Arne Akbar). Furthermore, I have just finished a collaborative grant to

investigate rebalancing of T-cell pools by immune modulation and rejuvenation in aging primates (with Prof. Louis Picker), and am awaiting review of a P01 application submitted to NIA, to understand and correct molecular basis of thymic involution and peripheral T cell maintenance failure in aging (with Profs. Ellen Richie – MD Anderson; Lauren Ehrlich – Univ. of Texas, Austin; Nancy Manley – Univ. of Georgia; Marcel van den Brink – Sloan-Kettering and Charles Surh, Postech Institute). My entire group is heavily involved in studies of immune senescence, and we are engaged in several multidisciplinary studies with colleagues investigating cellular stress mTOR and aging (with Drs. Andrew Capaldi, UA Dept of. Molecular and Cellular Biology), nuclear 3-D organization in the course of aging and CMV infection (Drs Giovanni Bosco, Dartmouth Univ., and Felicia Goodrum, the UA BIO5 Institute), mTOR inhibition in metabolic, neurosensory and immunological aging (Drs. Theodore Price – UT Southwestern, Heddwen Brooks-UA, Sourav Ghosh - Yale, Kirsten Limesand – UA, John Konhilas – UA - Depts. Of Pharmacology, Physiology, Cell Biol. and Anatomy), and the ability of thermography to predict propensity for pressure ulcer in frail and resilient older adults (Drs. David Armstrong, Manish Bharara, Mindy Fain and Jane Mohler, Dept. of Surgery and the Arizona Center on Aging). Nico will be thoroughly exposed to all these scientific influences and contents. There are initiatives in microbiome and aging centered in our department and the Arizona Center on Aging, and we have a Biology of Aging Research Interest Group, encompassing about 35 scientists across campus. Nico will attend quarterly meetings of that group too.

Research Facilities

Laboratory space of 2,000 sq. ft. equipped with benches, desks, sinks, water, pressurized air, vacuum, etc. on the second floor of the Medical Research Building (MRB) of the University of Arizona College of Medicine. Additional dedicated tissue culture space of 300 sq. ft. is available adjacent to the laboratory space. Space for 3,500 mice and 30 rats is available to the investigator in the main vivarium (Building 201), as well as in the shared basement between the MRB and Keating buildings. That includes dedicated ABSL-2 and ABSL-3 areas for containment work with pathogens. All facilities are AALAAC accredited and staffed with full-time veterinarians and animal support staff. Core facilities: Transmission and scanning electron microscopes are available in the 4th floor of LSN building on a fee-for-service-basis. A facility for automated DNA sequencing and oligonucleotide synthesis is available on a fee-for-service-basis in the Keating building adjacent to MRB. Protein sequencing and mass spectrophotometry analysis are available at the College of Pharmacy. Microarray and proteomic state-of-the-art facilities within the Arizona Research Labs are located in the adjacent Keating building and are available at a fee-for-service basis. Flow cytometry sorting core facilities are available both in the MRB building and at the Arizona Cancer Center across the street. Arizona Cancer Center also houses the Cobalt source animal and cell irradiator. Other state-of-the-art core facilities including, but not limited to, the Transgenic Animal Immunohistochemistry and Animal Health Core are available on campus. In addition, the following equipment is available directly in the laboratory: CO2 incubators, phase microscopes, inverted microscopes, centrifuges, PCR machines (including real time), a gel imaging system, tissue culture hoods and freezers (-80 C and -20 C) are present in the laboratory. Shared gamma and beta counters, DNA sequencers (ABI 3100 is available in our lab) and ultracentrifuges are on our floor. Available in this building are spectrophotometers, HPLC

4. Number of Fellows/Trainees to be Supervised During the Fellowship

Two, in addition to Nico:

Heather Thompson, Postdoctoral Fellow
Mladen Jergovic, Postdoctoral Fellow

5. Applicant's Qualifications and Potential for a Research Career

Nico Contreras is an exceptional candidate for this Fellowship. He came to my laboratory following successful undergraduate and masters studies, the latter accomplished in a bacteriology/engineering laboratory, under the tutelage of Dr Linda Powers. He was therefore more mature than an average, out-of-undergrad Ph.D. student. He distinguished himself quickly, both by the ability to assimilate complex concepts

and experimental constructs and by the ability to put that knowledge to work when thinking about and designing experiments.

Moreover, he quickly came up with a well-defined set of experimental interests, that were unusually mature and shaped for an early-stage graduate student. He focused on the intersection of immunity, metabolism and aging, with a specific aim to address obesity in the context of these three themes. He started working on our collaborative projects with Drs Luigi Fontana (Washington University) and Valter Longo (University of Southern California) addressing effects of calorie restriction or its modifications upon the function of immune system and the overall physiology of the organism. He also bridged the mouse and human experimental models very nicely in the course of his initial training in my lab, providing him a perspective for translational work.

However, the most telling predictor of Nico's potential and future success is the fact that he came up with a research project that required little to no input from me. His ideas were highly original and he synthesized them nicely into the present proposal. He assembled the reagents, methods and support to address a question whether and to what extent direct infection of the adipose tissue with a persistent virus (cytomegalovirus, CMV) can impact inflammation, immunity and metabolic dysfunction, particularly if evaluated over the lifespan and into aging. I have just presented his preliminary data at the FASEB Science Research Conference in Big Sky, Montana, on Aug. 3, 2016, and was greeted by significant and strong enthusiasm of the audience, that lauded this new line of investigation.

Nico is a good experimentalist already, and has already grown in that regard by leaps and bounds every semester. He is an adept organizer, highly resourceful and able to mobilize his laboratory colleagues to help him when needed with complex harvests and organ processing. He is also eager to lend a hand himself and help others. He covers the literature well and explores novel approaches appropriately. He is not shy about introducing new techniques, and already possesses an impressive skill set. Finally, he wrote the application with only cursory input from myself, demonstrating considerable thought and writing skills.

I therefore view Nico as a future leader in academic medicine and research, addressing highly pertinent health care issue (obesity, metabolic syndrome and type 2 diabetes) in the context of aging. This area remains woefully underexplored and poorly understood and his original ideas and the ability to perform well controlled experiments will undoubtedly be yielding high returns in the immediate, intermediate and long-term future. He is an exceptionally well qualified and well suited candidate for the individual predoctoral research fellowship (F31).

DESCRIPTION OF INSTITUTIONAL ENVIRONMENT

Nico A. Contreras is situated in an ideal clinical research environment, replete with resources to facilitate training and help him develop into a successful investigator in immunobiology and virology.

The University of Arizona (UA) is the leading public research university in the American Southwest. UA provides an unusually interactive interdisciplinary academic community, highly conducive to the proposed project. UA is a land grant public university and has a 387- acre campus in Tucson, Arizona. The campus includes 159 buildings on the main campus and 25 buildings at the adjacent Health Sciences Center. With close to 40,000 students, 20 Colleges, and over 300 academic programs, the UA provides a rich environment for interacting with students, faculty and researchers from many diverse disciplines. The University's commitment to research was recognized by its ranking from the National Science Foundation as 19th among public universities in the US for research. The UA is one of 63 members of the prestigious Association of American Universities and has over $625 million in annual research funding.

The university is comprised of 13 colleges, one branch campus in Sierra Vista, and has expanded over the last few years in its colleges of Medicine, Pharmacy and Public Health to downtown Phoenix. The UA also has two supporting colleges—Honors and Outreach—and 76 research centers. More than 345 undergraduate, graduate and professional degree programs are offered on a semester schedule. Educational programs designed to meet the demand for virtual, hybrid, and distance offerings, are added, coordinated, and managed through the Outreach College. The UA offers a range of courses that cover fundamental and advanced concepts and analytic methods pertaining to the design, analysis and interpretation of research studies. Furthermore, the University of Arizona is strongly committed to diversity, with greater than 25% of graduate and professional students being of an ethnically diverse background.

The University of Arizona's Department of Immunobiology has a diverse array of faculty members involved in numerous research foci, providing broad and deep technical training on multiple platforms. Research in the department are centered in various areas, including neuroimmunology, adaptive, innate and microbial signaling (cellular and subcellular), autoimmunity, bacteriology, parasitology, aging, and virology. The Department promotes a collaborative environment, bolstered by the open laboratory space design within the Medical Research Building. Further collaboration is encouraged during weekly joint laboratory meetings of the Nikolich-Zugich, Schenten, Frelinger, Kuhns, and Wu laboratories, of which Nico is currently the organizer. Equipment is often shared between multiple laboratories including the flow cytometers, PCR machines, microscopes, and AutoMACS.

The Department also holds weekly joint student seminars with the Department of Cellular and Molecular Medicine to develop cross-disciplinary collaboration and communication. Furthermore, there is a weekly Immunobiology Journal Club that critically discuss current original basic, clinical and health services research articles linked to immunobiology; Nico is also the student organizer for this course. As alluded to in the Sponsor's Section, the Department holds the annual Frontiers in Immunobiology Symposium and has access to several cross-departmental seminars. Previously invited speakers include Drs. Charles Surh (Immune homeostasis in aging); Michael Diamond (Immunity to flaviviruses with aging), Ellias Haddad (Innate immune defects with aging) and others.

By attending these symposia and seminars, Nico has ample opportunities for intellectual interactions with immunobiologists and scientists with a variety of focuses. Opportunities for intellectual interactions with viologists, biostatisticians, immunologists, and other scientists are also available for Nico at UA and its community.

The University of Arizona's Department of Immunobiology PhD program is on average a 5-year graduation cycle. Coursework is completed within 1.5 years into the program, with a continuation of Journal Club, Joint Student Seminars, and Departmental Seminars until graduation. The qualifying exam is to be completed by the end of the 2nd year and includes an R01 style grant submission followed by an oral defense using the written submission as a framework for discussion, questions, and future experimental designs. Formal progress is followed by submission of several documents to the University's online UAccess portal, where committees, announcements, and results are submitted and available.

Vertebrate Animals

Use of animals

Male or female mice of varying ages (dominantly 3 months to 21 months) on the C57BL/6 genetic background (congenic, transgenic and targeted mutant derivatives) will be used for Specific Aims 1 and 2. As to date, we have found no sex-associated difference in immune responses between old male and female mice in response to cytomegalovirus (CMV), we will use males dominantly for this study.

Animals will be used for: (i) lymphoid organ harvest and adipose harvest for analysis of cytokine/chemokine and cellular (primarily T cell, macrophages, and NK cell) immune responses; (ii) blood and/or serum collection by retro-orbital bleeding or cardiac puncture (>200 ul); (iii) subcutaneous or intraperitoneal immunization for infection with live CMV (Smith strain and mutant derivaties). A number of animals could also be used for adoptive cell transfer followed by immunization and/or infection.

Justification for use

Mice are a logical and necessary choice for investigation of lifelong CMV infection and the effects on adipose tissue due to the similarity of CMV infection in both humans and mice, the existenece of congenic and recombinant strains of mice, defined genetics, availability of cell surface markers for flow cytometry, specific molecular probes, and monoclonal antibodies. Furthermore, a lifelong investigation on CMV infection and the consequences on human adipose tissue would not be feasible under the scope of this fellowship. The number of animals required provides statistical power needed for each experiment. For most experiments using 8 animals/groups allows us to observe >30% difference between groups with 80% power at $p<0.05$. Power calculations were based on preliminary data.

Veterinary care

A full-time veterinarian is in charge of the animal care program at the University of Arizona. The facility is accredited by AAALAC and in full compliance with NIH, as well as other federal, state, and local regulations and guidelines. All of the studies are covered by the IACUC UA protocol 08-102.

Procedures to minimize discomfort

Discomfort and injury to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research. Analgesia, anesthetic, and tranquilizing drugs will be used as determined by our veterinarian. Any discomfort or distress during bleeding will be minimized with the use of inhalation anesthetics such as isoflurane. If the mice experience distress (manifested by agitation), we will immediately halt the procedure. Mice will be observed until active after bleeding, injections, and surgery. All procedures have been approved under IACUC #08-102.

All injectables will be purchased as a pharmaceutical grade solution. All of the saline is purchased as sterile USP grade saline.

Euthanasia

Animals will be sacrificed by isoflurane overdose.