BIOGRAPHICAL SKETCH

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NAME: KRASILNIKOV, MARIA

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

POSITION TITLE: Graduate Student

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Boston University		06/2013	08/2014	General education
University of California Berkeley Berkeley, CA	B.A.	08/2015	05/2019	Molecular and Cell Biology, Immunology and Pathogenesis
Tufts University, Boston, MA	Ph.D.	09/2021	In progress	Molecular Microbiology

A. Personal Statement

Currently, I am a second-year Ph.D. candidate in the lab of Dr. Ekaterina Heldwein at the Tufts University School of medicine where I am studying the HCMV viral fusogen, glycoprotein B. Specifically, I am interested in characterizing it structurally and antigenically so that it may be used as the basis of new, effective anti-HCMV therapeutics. My interest in scientific research was kindled during my second year of college when I joined Dr. Daniel Portnoy's lab at UC Berkeley which culminated in a publication on immune responses to phagosome-confined Lm. My experience studying *Listeria monocytogenes* (Lm) in the Portnoy Lab, along with my position as an organic chemistry instructor, inspired me to pursue microbial biochemistry research in graduate school.

Following the completion of my project in the Portnoy Lab, I combined my love of mentorship and my technical expertise by teaching laboratory techniques at Makerere University in Kampala, Uganda with the Center for Emerging and Neglected Diseases. There, I designed and taught seven biomedical research workshops on topics ranging from bioinformatics and molecular cloning to tissue culture and microscopy. After working at Makerere University, I wanted to diversify my research experience by immersing myself in an unfamiliar field. This led me to Dr. Tien Peng's lab in the pulmonary division at UCSF. Starting in Dr. Peng's lab was challenging, not only because of the new set of techniques I had to learn, but also because I was exposed to an entirely foreign biological discipline. However, my efforts paid off and in my time in the Peng Lab I achieved a publication detailing the role of senescent cells in epithelial repair and developed a novel method for isolating senescent cells without a reporter – a previously uncharacterized technique.

In the first year of my PhD, I joined Dr. Ekaterina Heldwein's lab as it was a perfect match for me in terms of project and mentoring style. My project characterizing the structure and antigenicity of human cytomegalovirus glycoprotein B, upon which this proposal is based, allows me to combine my undergraduate immunology background, the microbiology techniques I gained in the Portnoy Lab, and the assay development methods I learned in Dr. Peng's lab. It additionally will allow me to add virology and structural biology to my repertoire. Although my experience has spanned various disciplines, it is centered around my passion for multidisciplinary science and reflects my interest in translational research. Thanks to the TP1 award, I will receive valuable training in cryo-EM which will allow me to begin independently and efficiently driving my project to completion.

B. Positions and Scientific Appointments

Positions and employment:

2022: Teaching Assistant, Graduate Biochemistry, Graduate School of Biomedical Sciences, Tufts University, Boston, MA
2019-2021: Research Associate, Peng Lab, University of California San Francisco, San Francisco, CA
2019: Alliance for Global Health and Science Intern, Center for Emerging and Neglected Diseases, Berkeley, CA
2017-2019: Undergraduate Researcher, Portnoy Lab, University of California Berkeley, Berkeley, CA
2017-2018: Undergraduate Student Instructor, Organic Chemistry, College of Chemistry, University of California Berkeley, Berkeley, CA

C. Contributions to Science

1. TLR2 and endosomal TLR-mediated secretion of IL-10 and immune suppression in response to phagosome-confined *Listeria monocytogenes*

As part of its life cycle, *Listeria monocytogenes* (Lm) relies on two canonical virulence factors, ActA and LLO, to successfully infect host cells. Lm utilizes ActA to for cell-cell spread, while LLO allows Lm to escape from the phagosome after phagocytosis into the host-cell. Previously in the Portnoy Lab, it was shown that Δ*actA* Lm can be used as a potent cancer vaccine vector strain due to its attenuated nature. Given this finding, we hypothesized that deletion of LLO (Δ*hly*) would result in a secondary or even safer vaccine strain due to Δ*hly* being phagosome confined. However, upon further investigation, it was shown that Δ*hly* Lm results in a minimally effective vaccine strain through its induction of cellular IL-10 secretion, a canonically immune suppressive cytokine. This was an unexpected phenotype as Lm had not previously been documented to induce IL-10, thus we sought to investigate the mechanism behind this phenomenon. We determined that a strain lacking both LLO and prolipoprotein diacylglyceryl transferase (Δ*hly*Δ*lgt*) vaccinated mice at an efficacy rate similar to Δ*actA* and did not incude IL-10. Lgt is responsible for anchoring Lm lipoproteins to the bacterial cell surface, so I decided to investigate whether all lipoproteins contributed equally to cellular secretion of IL-10. I concluded that all lipoproteins need to be removed from the bacterial cell wall in order to significantly affect IL-10 levels. My research contributed to a larger investigation of how phagosome-confined Lm mediates immune suppression, which is now published in *PLOS Pathogens*.

a) Nguyen BN*, Chávez-Arroyo A*, Cheng MI, **Krasilnikov M**, Louie A, Portnoy DA. TLR2 and endosomal TLR-mediated secretion of IL-10 and immune suppression in response to phagosome-confined Listeria monocytogenes. *PLoS Pathog*. 2020 Jul 7;16(7):e1008622. doi: 10.1371/journal.ppat.1008622. PMID: 32634175; PMCID: PMC7340287. (**Publication)**
   *Indicates co-first authors
b) **Krasilnikov M**, Nguyen BN, Chávez-Arroyo A, Portnoy DA. Surface lipoproteins mediate immune suppression in phagosome-confined Listeria monocytogenes. Summer Undergraduate Research Fellowship Symposium. Berkeley, CA, August 2018. **(Oral Presentation)**

2. Workshop-based learning and networking: a scalable model for research capacity strengthening in low- and middle-income countries.

As academic researchers, our main sources of funding are government agencies. In the US, we are fortunate enough to have an ample amount of such funding opportunities. Such is not the case in the scientific community of low- and middle-income countries. Traditionally, such laboratories must either suffer from limited productivity due to lack of funding and resources or rely on the sponsorship of a principal investigator from a high-income nation. While such sponsorships allow for increased resources and funding, they also result in the sponsor being last author on the lab's publications despite their lack of intellectual involvement. Given that the currency of the academic world is publications, this still depletes the low-income investigators of resources and prevents them from acquiring future funding. Additionally, the added racial discrepancy between the often-white sponsors and often-BIPOC sponsees further emphasize the critical need for developing a new method of uplifting international scientific research. Thus, myself and others at the Center for Emerging and Neglected Diseases developed a series of capacity-building workshops to mitigate this issue. We first tested this approach at Makerere University in Kampala, Uganda. Using a workshop-based approach we were able to fill the training and resource gap for many local trainees and investigators. I had the opportunity to communicate these findings and the workshop

design through a publication with all my collaborators in *Global Health Action* so that in the future these capacity-building workshops will be more common throughout international scientific community.

a) Perier C, Nasinghe E, Charles I, Ssetaba LJ, Ahyong V, Bangs D, Beatty PR, Czudnochowski N, Diallo A, Dugan E, Fabius JM, Fong Baker H, Gardner J, Isaacs S, Joanah B, Kalantar K, Kateete D, Knight M, **Krasilnikov M**, Krogan NJ, Langelier C, Lee E, Li LM, Licht D, Lien K, Lyons Z, Mboowa G, Mwebaza I, Mwesigwa S, Nalwadda G, Nichols R, Penaranda ME, Petnic S, Phelps M, Popper SJ, Rape M, Reingold A, Robbins R, Rosenberg OS, Savage DF, Schildhauer S, Settles ML, Sserwadda I, Stanley S, Tato CM, Tsitsiklis A, Van Dis E, Vanaerschot M, Vinden J, Cox JS, Joloba ML, Schaletzky J. Workshop-based learning and networking: a scalable model for research capacity strengthening in low- and middle-income countries. Glob Health Action. 2022 Dec 31;15(1):2062175. doi: 10.1080/16549716.2022.2062175. PMID: 35730550; PMCID: PMC9225690. **(Publication)**

b) **Krasilnikov M**, Nguyen BN, Stanley S. Introduction to Listeria monocytogenes virulence genes and immune response. Workshop on tissue culture and immunological techniques, Makerere University, Kampala, UG. **(Oral Presentation)**

3. Sentinel p16INK4a+ cells in the basement membrane form a reparative niche in the lung

Previous literature has hinted at a relationship between the expression $p16^{INK4a}$, a biomarker of cellular senescence, and idiopathic pulmonary fibrosis (IPF), but little is known about the involved mechanisms. This gap in knowledge is partially due to the fact that anti-$p16^{INK4a}$ antibodies and previous mouse models have had a very high threshold of $p16^{INK4a}$ expression detection, thereby missing a majority of the population. We engineered an ultrasensitive reporter of $p16^{INK4a}$ so that we could, for the first time, study all $p16^{INK4a}$+ cells. Our reporter detected $p16^{INK4a}$-expressing fibroblasts with certain senescent characteristics that appeared shortly after birth in the basement membrane adjacent to epithelial stem cells in the lung. Furthermore, these $p16^{INK4a+}$ fibroblasts had enhanced capacity to sense tissue inflammation and response through their increased secretory capacity to promote epithelial regeneration. In addition, $p16^{INK4a}$ expression was required in fibroblasts to enhance epithelial regeneration. Using this reporter, I established that fibrotic injury caused an increase in $p16^{INK4a}$ expression *in vivo* - both in the murine lung and liver. The increased $p16^{INK4a}$ expression upon injury drove fibroblasts into expressing smooth-muscle markers, helping to explain how sentinel cells contribute to the fibrosis phenotype. This study highlights a role for $p16^{INK4a+}$ fibroblasts as tissue-resident sentinels in the stem cell niche that monitor barrier integrity and rapidly respond to inflammation to promote tissue regeneration.

While characterizing the role of $p16^{INK4a}$ in IPF, I realized that research in the senescence field had long been hindered by a lack of tools, specifically the inability to isolate senescent cells without a reporter. To address this issue, I developed a novel method for isolating human and murine senescent cells without the use of a reporter – a previously unpublished technique. My technique also isolated pathological cells involved in murine IPF. My research is currently the basis of multiple projects in the Peng Lab, and two of these projects currently have manuscripts in preparation, of which I am co-author.

a) Reyes N, **Krasilnikov M**, Allen NC, Lee JY, Hyams B, Zhou M, Ravishankar S, Cassandras M, Wang C, Khan I, Matatia P, Johmura Y, Molofsky A, Matthay M, Nakanishi M, Sheppard D, Campisi J, Peng T. Sentinel p16INK4a+ cells in the basement membrane form a reparative niche in the lung. *Science*, 2022, In Press. **(Publication)**

b) **Krasilnikov M**, Reyes N, Peng T. p16INK4A+ cells modulate myofibroblast transformation in idiopathic pulmonary fibrosis. Meeting of the Stem Cell. San Francisco, CA, August 2020. **(Oral Presentation)**

4. Group 2 innate lymphoid cells suppress innate type 3/17 responses in hepatic fibrosis

Type 2 immunity can promote physiologic tissue remodeling, yet excessive activation can also drive fibrotic disease. Group 2 innate lymphoid cells (ILC2s) are a dominant organizer of type 2 immunity, but how ILC2 topography and local interactions dictate these responses are unknown. As an expert on development of murine fibrosis models, I collaborated with the primary author on this project to develop a hepatic liver fibrosis model and read-out. Using these models, we used quantitative 3D imaging to define fibrosis-associated portal and periductal ILC2 accumulation in proximity to an expanded IL-33-producing fibroblast subset. However, ablation of IL-33 or IL-4/IL-13 had no impact on hepatic fibrosis. Unexpectedly, I found that constitutive or inducible loss of ILC2s worsened carbon tetrachloride (CCl4)- or bile duct ligation-induced liver fibrosis. Mechanistically this occurred in part via suppression of innate IL-17A-producing lymphocytes, which also accumulated in periportal regions during fibrosis. Collectively, these data identify a novel role for ILC2s in the liver portal tracts as a

negative regulator of the innate type3/17 immune response to hepatic damage and suggests resident lymphocyte topographic crosstalk may be a critical determinant of liver health and disease. A manuscript for this project has just been submitted to *Nature Immunology*, with me as a co-author. These findings are critical for the development of hepatic disease diagnostics, prognosis determinants, and therapeutics.

a) Sbierski-Kind J, **Krasilnikov M**, Cautivo M, Wagner J, Mroz N, Lu Gan A, Dahlgren M, Matatia P, Taruselli M, Chang A, O'Leary C, Kotas M, Caryotakis S, Mattis A, Locksley R, Peng T, Molofsky AB. Group 2 innate lymphoid cells suppress innate type 3/17 responses in hepatic fibrosis. Cytokines 2022: 4th International Conference on Innate Lymphoid Cells, September, 2022. **(Oral Presentation)**

5. Development of a Dual-Fluorescent-Reporter System in Clostridioides difficile Reveals a Division of Labor between Virulence and Transmission Gene Expression.

The bacterial pathogen *Clostridioides difficile* (Cd) causes gastroenteritis by producing toxins and transmits disease by making resistant spores. Toxin and spore production are energy-expensive processes that are regulated by multiple transcription factors in response to many environmental inputs. While toxin and sporulation genes are both induced in only a subset of Cd cells, the relationship between these two subpopulations remains unclear. To address whether Cd coordinates the generation of these subpopulations, I aided in developing and characterizing a dual-transcriptional-reporter system that allows toxin and sporulation gene expression to be simultaneously visualized at the single-cell level using chromosomally encoded mScarlet and mNeonGreen fluorescent transcriptional reporters. I then helped adapt an automated image analysis pipeline to quantify toxin and sporulation gene expression in thousands of individual cells under different medium conditions and in different genetic backgrounds. These analyses revealed that toxin and sporulation gene expression rarely overlap during growth on agar plates, whereas broth culture increases this overlap. The results suggest that certain growth conditions promote a "division of labor" between transmission and virulence gene expression, highlighting how environmental inputs influence these subpopulations. Our data further suggest that the RstA transcriptional regulator skews the population to activate sporulation genes rather than toxin genes. Given that recent work has revealed population-wide heterogeneity for numerous cellular processes in Cd, we anticipate that our dual-reporter system will be broadly useful for determining the overlap between these subpopulations. My results helped contribute to the completion of this project, now published in *mSphere*.

a) Donnelly ML, Shrestha S, Ribis JW, Kuhn P, **Krasilnikov M**, Alves Feliciano C, Shen A. Development of a Dual-Fluorescent-Reporter System in Clostridioides difficile Reveals a Division of Labor between Virulence and Transmission Gene Expression. mSphere. 2022 Jun 29;7(3):e0013222. doi: 10.1128/msphere.00132-22. Epub 2022 May 31. PMID: 35638354; PMCID: PMC9241537. **(Publication)**