Sophia Carvalho
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CURRICULUM VITAE

Address Email: scarvalho@tgen.org
Position Postdoctoral Research Fellow

Education

Undergraduate: B.S., 2011, Biology: Case Western Reserve University, Cleveland, OH

Graduate: Ph.D., 2017, Toxicology: University of Montana, Missoula, MT

Societies, Professional Activities, and Related Volunteer Work

Member, Society of Toxicology (2010-present)

Member, Air and Waste Management Association (2011-2018)

Member, American Association for the Advancement of Science (2017-present)

Secretary, AWMA UMT Student Chapter (2012-2014)

Planning Committee Member, AWMA International Specialty Conference "Strategies to Reduce Residential Woodsmoke." Missoula, MT (2014)

Mentor, Helios Scholars at TGen (2021)

Poster Judge, Helios Scholars at TGen Symposium (2021)

Judge, Montana State Science Fair (2015)

Presenter and Participant, Center for Environmental Health Sciences Toxicology Student Journal Club (2011-2017)

Teaching Experience

Lecturer:
Toxicants, Introduction to Toxicology, Online Course 2013-2016
Course Facilitator: Andrij Holian, Ph.D.
University of Montana, Center for Environmental Health Sciences

Teaching Assistant:
Physiological Systems I/ Physiological Systems I Laboratory 2012-2016
Course Facilitator: Jerry Smith, Ph.D.
University of Montana, Skaggs School of Pharmacy

Physiological Systems II/ Physiological Systems II Laboratory 2012-2016
Course Facilitator: Mark Pershouse, Ph.D.
University of Montana, Skaggs School of Pharmacy

Research Experience

Translational Genomics Research Institute (North)

"Tracing the origins of an antibody cross-reactivity to multiple human Coronaviruses at amino-acid resolution."
Postdoctoral research project. (2020-present)
Primary Investigator: John Altin, Ph.D.
Laboratory focus: Pathogen immunogenomics

Project Hypotheses:

- Individual HR2-reactive antibodies that arise/expand in COVID-19 convalescent subjects bind (cross-react with) both the SARS-CoV-2 and HCoV-OC43 antigen variants
- The breadth of cross-reactivity is determined by sequence features of the IgH/L
- Pre-existing antibodies against the OC43 antigen acquire IgH/L mutations following SARS-CoV-2 exposure that confer increased affinity for the SARS-CoV-2 variant

Northern Arizona University
"Investigating the anti-tumor cytotoxicity of reovirus-treated NK cells for cancer therapy."
Postdoctoral research project. (2018-2020).
Primary Investigator: Narendiran Rajasekaran, Ph.D.
Laboratory focus: Cancer immunobiology

Project Abstract:

Reovirus is a non-engineered, non-enveloped, double-stranded RNA virus which specifically targets tumor cells expressing activated Ras and is thus a promising foundation for new cancer therapies; indeed, preclinical studies have demonstrated broad anti-cancer activity of the wild-type Type 3 Dearing strain of reovirus. While reovirus is itself directly oncolytic, it has also been shown to enhance natural immune responses to oncogenesis. For instance, systemic delivery of reovirus to cancer patients has resulted in increased activation of their natural killer (NK) cells. NK cells are part of the anti-viral and anti-tumor immune response, able to recognize infected and aberrant cells and induce apoptosis in those cells via cytotoxic molecules. As the adoptive transfer of NK cells is already being tested in clinical trials, increasing the cytotoxic activity of these cells—and thus the efficacy of treatment—has become a priority.

Our recent experiments reveal that the viral surface protein Sigma-1, which is necessary for reovirus' binding to target cells, can be clearly identified by flow cytometry on the outer surfaces of PBMC-derived NK cells following only one hour of incubation with the virus; analysis of both fresh blood samples and frozen PBMC indicates that Sigma-1 is a reliable marker of reoviral attachment to primary NK cells, although it is most useful in fresh samples. Sigma-1 can also be detected inside NK cells after 24 hours using intracellular staining, which suggests internalization of the virus. Alterations in the expression of inhibitory and activating NK surface proteins (NKp46, NKG2D, CD94, CD69, CD56, CD48, CD44, and 2B4) are also being examined. Several of these, notably CD56 and NKG2D, have shown no change in expression following the introduction of virus, and may require signals from other cell types to do so. However, increased CD69 after 24 hours of incubation with virus was observed clearly in six fresh blood samples, and is suggestive of NK activation. Interestingly, some changes in expression appear to vary between patients. CD44 expression, for example, remained unchanged between 1 and 24 hours post reovirus

treatment in Donors 1 and 2 but decreased at 24 hours in Donor 3. NKp46 was increased after 1 hour in the presence of virus in Donor 3, decreased in Donor 2, and was unchanged in Donor 1; yet all three samples showed decreased NKp46 after 24 hours of reovirus treatment. CD48, which may act to inhibit or activate NK cytotoxicity based on other signals, was elevated in Donor 3 and lowered in Donor 1. Investigation of these phenomena is ongoing.

Attachment of reovirus to NKL (an aggressive NK leukemia line) and NK92 (an NK line which has been utilized in multiple clinical trials) has also been analyzed, with flow cytometry data thus far showing moderate internalization of the virus at the 24-hour timepoint. TEM analysis of these cell lines is currently in progress to confirm reovirus uptake.

Experiments assaying cytotoxicity of infected NKL and NK92 cells against the DLD-1 adenocarcinoma line are presently underway, as is analysis of IFNγ secretion. Preliminary results suggest that NK92 anti-tumor cytotoxicity may be increased by exposure to reovirus, while NKL cytotoxicity is unaffected. Both cell lines appear to suffer dampened interferon responses following reovirus incubation; as seen in flow cytometry examination of surface markers, primary NK cells display wide variability in cytokine production.

Interestingly, while NKL cells do not show most typical signs of activation, they do seem to be able to sequester reovirus within their cytoplasm. T3D at a concentration of 50 MOI consistently kills one million L929 murine epithelial cells within 24 to 48 hours; however, a majority of NKL cells incubated with 50 MOI reovirus under the same conditions survive for up to 72 hours. Additionally, when NKL are washed in clean media following a one-hour infection period, supernatant taken from those cultures 24 hours later can be used to infect and kill L929 cultures; this implies that not only are NKL capable of greater resilience against this obligate-lytic virus, but they may be able to release viable viral particles into the surrounding environment. Further repetitions of this experiment will be necessary to confirm this.

The data to date suggest successful binding of reovirus to primary NK cells and subsequent internalization; the degree of NK activation resulting from this is variable. Cell line responses are more consistent and—with the notable exception of CD69 increase in both NKL and NK92 and possible cytotoxicity amplification in NK92—most metrics of NK cell activation suggest that these lines are neither immune to reovirus infection nor activated by such infection in a traditional sense. Investigation of the NKL line in particular suggests that rather than being utilized as direct anti-tumor implements, these cells may instead have potential as a means of ferrying reovirus to tumor sites.

University of Montana:
"The Inflammatory Effects of Natural and Engineered Airborne Particulate Matter in the Lung, and Related Cellular Mechanisms of Immunity."
Doctoral thesis. (2011-2017)
Primary Investigator: Kevan Roberts, Ph.D.
Laboratory focus: Asthma and related immunotoxicology

Doctoral Thesis Abstract:

Asthma, defined as a complex, chronic inflammatory disease of the airways, affects approximately 300 million individuals worldwide and is the single most common chronic

disease among children. Airway inflammation is the defining characteristic of asthmatic pathophysiology; and as asthma increases in severity, the airways become more susceptible to environmental insults, including air pollutants. Wildfires and prescribed burns are significant sources of airborne particles, as well as gaseous pollution, which can temporarily increase the overall levels of air pollution over hundreds or thousands of square miles. Multi-walled carbon nanotubes (MWCNT) are increasingly used in a broad range of applications, including medical treatments, construction in the aerospace industry, and electronics manufacture.

Accordingly, the following studies were conducted to narrow the gaps in existing knowledge of the health hazards posed by wood smoke (WS) and inhaled MWCNT. To model allergic asthma, the most common form of the disease, we introduced house dust mite (HDM) allergen, an allergy trigger for almost 85% of asthmatics. We found that adult C57BL/6 mice previously sensitized to HDM displayed significantly exacerbated lung inflammation following oropharyngeal MWCNT instillation. This was characterized by elevated levels of EPO and correspondingly increased eosinophil populations, as measured by flow cytometry, in the BALF. Th2-associated cytokines traditionally implicated in allergic asthma, such as IL-13 and IL-22, were notably lacking; likewise, no clear alteration in CD4+ or CD8+ T cells was found. Instead, a significant increase in levels of cysteinyl leukotrienes (cys-LT) was detected, which correlated to increases in eosinophilia.

This coincidence of augmented eosinophil recruitment and increased cys-LT production was again observed in our study of WS exposure. Adult female mice exposed to WS while pregnant displayed markedly increased eosinophilia when later sensitized to HDM, as evidenced by EPO levels and quantification via flow cytometry, as well as elevated cys-LT levels. Interestingly, the offspring of those WS-exposed mice, when sensitized to HDM in adulthood, responded with dramatically exacerbated inflammation in the same mode. H&E and PAS staining revealed mucus deposition and marked cellular infiltration in the lungs of prenatally exposed offspring following HDM challenge. Pups displayed highly significant differences in EPO levels between WS/HDM and Air/HDM groups at 8 weeks of age; eosinophilia decreased over time, with these differences remaining significant at 16 weeks and being lost by 24 weeks. The numbers of CD4+ and CD8+ T cells were also affected: in dams, WS inflated these populations and HDM decreased them; in pups, the opposite was true. These findings indicate a direct relationship between the biosynthesis of cys-LT and the recruitment of eosinophils in response to MWCNT inhalation, and suggest that a similar mechanism—likely with a greater T cell component—may be responsible for the inflammation arising from exposure to WS.

Case Western Reserve University:
"Comparison of *In Silico* and *In Vitro* Screening of Potential Small-Molecule Duffy Binding Protein Inhibitors."
Undergraduate research capstone. (2010-2011)
Primary Investigator: Brian Grimberg, Ph.D.
Laboratory focus: Malaria

Project Abstract:

Malaria is a devastating, blood-borne disease resulting from a victim's infection by members of the protozoan *Plasmodium* parasite family, which commonly use humans, apes,

and other vertebrates as hosts. *P. vivax* is known to cause roughly 70 to 80 million new cases of malaria infection each year, and its recurring paroxysms place heavy economic and physical burdens on people who are often already at a financial and medical disadvantage.

P. vivax infection in humans is mediated by a membrane-bound ligand on the parasite—the Duffy binding protein, or DBP—and its matching antigen on the host erythrocyte. The antigen is a transmembrane glycoprotein chemokine receptor and is therefore also known as the Duffy antigen receptor for chemokines, or DARC. Unlike *P. falciparum*, which has displayed alternative invasion strategies, *P. vivax* relies exclusively on the DBP-DARC interface; this weakness provides an ideal target for new medications. To lay the groundwork for development of new antimalarial drugs, we cultured samples of the *P. vivax* Salvador 1 strain derived from the blood of infected *Aotus* monkeys. Once husbanded to experimentally viable levels, parasites were incubated with a variety of small-molecule compounds identified via virtual screening. Initial identification of potential compounds was the result of collaboration with Dr. Menachem Shoham and powered by the CWRU High Performance Computing Cluster. Further examination of DBP-DARC binding inhibition by selected compounds was conducted using a BD LSRII flow cytometer. The goal of this ongoing project is the isolation of numerous small-molecule compounds able to occupy the Duffy protein's antigen-binding pocket, at minimal doses, and thereby interrupt or prevent the parasitic invasion of patient erythrocytes.

Undergraduate volunteer work (2010)
Primary Investigator: Menachem Shoham, Ph.D.
Laboratory focus: Methicillin-resistant *Staphylococcus aureus*

This summer internship focused on the creation and maintenance of a database detailing all chemical compounds owned and used by the laboratory. This work not only supported the ongoing efforts of the full-time lab personnel but also became part of the foundation for the Grimberg Lab's research into small-molecule inhibitors of DBP.

Poster Presentations

"Comparison of *In Silico* and *In Vitro* Screening of Potential Small-Molecule Duffy Binding Protein Inhibitors." *Intersections,* Case Western Reserve University faculty, students, and public. (December 2010; updated April 2011)

"Inhaled Nanoparticles Prompt Inflammatory Responses in the Lung." *Graduate Student Research Conference*, University of Montana faculty and students. (April 2015)

"Pulmonary Exposure to Multi-Walled Carbon Nanotubes Exacerbates Asthmatic Inflammation: a Role for Cysteinyl Leukotrienes." *External Advisory Committee Conference*, University of Montana, Center for Environmental Health Sciences faculty and students. (September 2015)

"Prenatal Exposure to Wood Smoke Exacerbates Allergic Responses in Adulthood." *Graduate Student Research Conference*, University of Montana faculty and students. (April 2016)

"Investigating the anti-tumor potential of reovirus-treated natural killer cells." *Southwest Health Equity Research Collaborative Poster Session*, Northern Arizona University faculty, staff, and students. (November 2018).

"Effects of Reovirus on NK Cells." *Southwest Health Equity Research Collaborative Advisory Committee Meeting*, Northern Arizona University, SHERC faculty and students. (March 2019).

Presentations

"Malaria, *P. vivax*, and Exploiting the Immune Response." *Toxicology Journal Club lecture*. University of Montana, Center for Environmental Health Sciences students and doctoral committee. (November 2012)

"Cellular Assassins: the Lives and Times of Natural Killer Cells." *Graduate student seminar*. University of Montana faculty and students. (October 2014)

"A Study of the Inflammatory Effects of Multi-Walled Carbon Nanotubes in the Lung." *Graduate research update seminar*. University of Montana faculty, students, and public. (December 2016)

"The Inflammatory Effects of Natural and Engineered Airborne Particulate Matter in the Lung." *Doctorate defense seminar*. University of Montana faculty, students, and public. (May 2017)

Publications

Prenatal wood smoke exposure predisposes mice to exacerbated allergic lung inflammation. Sophia Carvalho, Maria Ferrini, Britten Postma, Kevan Roberts, Zeina Jaffar. *In preparation.*

Type-3 Dearing reovirus does not stimulate antiviral NK cell responses. Sophia Carvalho, Aubrey Funke, Taylor Arehart, Moises Ceja, Carina Magdaleno, Emyly Fernandez, Narendiran Rajasekaran. *In preparation.*

Fibronectin assembly regulates lumen formation in breast acini. Carina Magdaleno, Trenton House, Jogendra Pawar, Sophia Carvalho, Narendiran Rajasekaran, Archana Varadaraj. 2020. Journal of Cellular Biochemistry 122(5):524-537.

Multi-walled carbon nanotubes augment allergic airway eosinophilic inflammation by promoting cysteinyl leukotriene production. Sophia Carvalho, Maria Ferrini, Lou Herritt, Andrij Holian, Zeina Jaffar, Kevan Roberts. 2018. Frontiers in Pharmacology 9:585.

Prenatal tobacco smoke exposure predisposes offspring mice to exacerbated house dust mite-elicited allergic airway inflammation associated with altered innate effector function. Maria Ferrini, Sophia Carvalho, Yoon Hee Cho, Britten Postma, Lucas Miranda Marques, Kent Pinkerton, Kevan Roberts, Zeina Jaffar. 2017. Particle and Fibre Toxicology 14:30.

PGI_2 controls pulmonary NK cells that prevent airway sensitization to house dust mite allergen. Bryan Simons, Maria Ferrini, Sophia Carvalho, David Bassett, Zeina Jaffar, Kevan Roberts. 2017. Journal of Immunology 198:461-471.

Discovering Duffy binding protein inhibitors using *in silico* screening of small-molecule inhibitors. Sophia Carvalho, D'Arbra Blankenship, Lenore Carias, Alex Popko, Menachem Shoham, Christopher King, Emmitt Jolly, Brian Grimberg. 2011. Midwest Neglected Infectious Diseases Conference Abstracts.

Professional References

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