Ninaad Lasrado

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EDUCATION	
2015- 2018	BSc in Biotechnology, St. Aloysius College, Mangalore, India
2018- Present	Graduate Research Assistant (PhD), University of Nebraska-Lincoln (UNL), Lincoln, NE. GPA: 3.95/4
AWARDS AND FELLOWSHIPS	
2015	Best Outgoing Student – Ambika Pre-University College, Mangalore, India
2016	3 rd place in the State Level Bioquiz-2016 – DBT, Government of India
2017	Inspire Research Scholarship for Higher Education – DBT, Government of India
2018	Star student award – St. Aloysius College, Mangalore, India
2018	Distinguished seminar award – St. Aloysius College, Mangalore, India
2018	Distinguished project award – St. Aloysius College, Mangalore, India
2019	Travel for techniques award, American Association of Immunologists (AAI)
2019	Immune epitope database (IEDB) - NIH Travel award, La Jolla Institute for Allergy and Immunology, San Diego, CA
2019	Larrick-Whitmore Travel award, UNL
2019	CRWAD Travel award, SVMBS, UNL
2019	Laboratory travel grant, 104th Annual meeting, AAI, MD
2020	Widaman distinguished graduate fellowship award, ARD, UNL
2020	Susan Ann Smith Mills memorial fellowship, SVMBS, UNL
2021	Trainee Abstract Award, 104th Annual meeting, AAI, MD
2021	Milton-Mohr Fellowship, UNL
MENTORING AND TEACHING EXPERIENCE 2015 – 2018 Mentored underprivileged kids as a part of <i>Make a difference</i> , non-government organization (NGO), India	
2015 - 2018	Mentored High school students in life sciences
2019 - Present	Teaching assistant for Veterinary Immunology course (VMED-680)
a) Tutored DVM students, Professional Program in Veterinary Medicine (2019 - Present)	

- Kaylee Pothoff
- Sarah Schuelke

- Hannah Evans
- b) Mentored and trained undergraduate and graduate students
 - Allison Shelbourn (Undergraduate student)
 - Mahima Rasquinha (Graduate student)
 - Meghna Sur (Graduate student)
- c) Taught molecular biology techniques and disease inducing protocols to post-doctoral fellows
 - Dr. Sabarirajan Jayaraja (Dr. Jay Reddy's Laboratory)
 - Dr. Paul Velander (Dr. Amanda-Ramer-Tait's Laboratory)

PUBLICATIONS

- 1) Lasrado N, Gangaplara A, Arumugam R, Massilamany C, Pokal S, Zhou Y, Xiang S-H, Steffen D and Reddy J. "Identification of immunogenic epitopes that permit the detection of antigen-specific T cell responses in multiple serotypes of group B Coxsackievirus infections". *Viruses*. 2020 Mar 21. Doi: 10.3390/v12030347. PMID: 32245257.
- **2) Lasrado N**, Yalaka B and Reddy J. "Triggers of inflammatory heart disease". *Frontiers in Cell and Developmental Biology*. 2020 Mar 24. Doi: 10.3389/fcell.2020.00192. PMID: 32266270.
- **3) Lasrado N** and Reddy J. "An overview of the immune mechanisms of viral myocarditis". *Reviews in Medical Virology*. 2020 Jul 28. DOI: 10.1002/rmv.2131. PMID: 32720461.
- **4) Lasrado N**, Jia T, Massilamany C, Franco R, Illes Z and Reddy J. *Biology of Sex Differences*. "Mechanisms of sex hormones in autoimmunity: focus on EAE". 2020 September 10. DOI: 10.1186/s13293-020-00325-4. PMID: 32894183.
- 5) Gangaplara A, Massilamany C*, **Lasrado N***, Steffen D and Reddy J. "Evidence for anti-viral effects of complete Freund's adjuvant in the mouse model of enterovirus infection". *Vaccines*. 2020 Jul 7. Doi: 10.3390/vaccines8030364. PMID: 32645845. *equal contributors
- 6) Basavalingappa R*, Arumugam R*, **Lasrado N***, Yalaka B, Massilamany C, Gangaplara A, Xiang S, Steffen D and Reddy J. "Viral myocarditis involves the generation of autoreactive T cells with multiple antigen specificities that compartmentalize in lymphoid and non-lymphoid organs in the mouse model of CVB3 infection". *Molecular Immunology*. 2020 Jun 29. DOI: 10.1016/j.molimm.2020.06.017. PMID: 32615275. *equal contributors
- 7) Arumugam R, Yalaka B, Massilamany C, Haider Ali MS, **Lasrado N**, Riethoven JJ, Sun X and Reddy J. "An evidence for surface expression of an immunogenic epitope of sarcoplasmic/endoplasmic reticulum calcium-ATPase2a on antigen-presenting cells from naive mice and its significance in the induction of autoimmune myocarditis". *Immunobiology*. 2019 Dec 13. Doi: 10.1016/j.imbio.2019.12.005. PMID: 31870642
- 8) Lasrado N, Gangaplara A, Massilamany C, Arumugam R, Shelbourn A, Rasquinha M, Basavalingappa R, Delhon G, Xiang S-H, Pattnaik A, Steffen D and Reddy J. "Attenuated strain of CVB3 with a mutation in the CAR-interacting region protects against both myocarditis and pancreatitis". (Under review in *Scientific Reports*)
- 9) Lasrado N, Borcherding N, Arumugam R, Starr T, Reddy J. "Dissecting the Cellular Landscape and Transcriptome Network in Viral Myocarditis by Single-Cell RNA Sequencing". (In preparation)

ABSTRACTS

- **1. Lasrado N**, Arumugam, R, Massilamany C, Krishnan B, and Jay Reddy. MHC Dextramers: versatile tools for assessing antigen-specific T cell responses. 17th International Congress of Immunology IUIS 2019. Beijing, October 19-23, 2019.
- **2. Lasrado N**, Gangaplara A, Arumugam R, Massilamany C, Steffen D and Reddy J. Identification of immunogenic epitopes that permit the detection of antigen-specific T cell responses in multiple serotypes of group B Coxsackievirus infections. CRWAD annual meeting, Chicago, IL, November 3-5, 2019.
- **3.** Arumugam R, Yalaka B, Massilamany C, **Lasrado N**, Haider Ali MS, Riethoven JJ, Sun X and Reddy J. Antigen-presenting cells from naive mice constitutively present the T cell epitope of SERCA2a and induce inflammatory cytokine production in antigen-specific T cells. 106th Annual meeting of the American Association of Immunologists, May 9-13, 2019, San Diego, CA.
- **4. Lasrado N**, Arumugam R, Jia T, Krishnan B, Xiang S and Reddy J. Evidence for cross-reactive self-antigen in the mediation of post-infectious myocarditis induced by Coxsackie virus B3 infection. FLYSWAT annual meeting, March 17-18, Nebraska City, NE.
- **5. Lasrado N**, Arumugam R, Jayaraja S, Thibivilliers S, Libault M, Starr T and Reddy J. Single cell RNA sequence analysis reveals novel clusters unique to post-infectious myocarditis in the mouse model of Coxsackievirus infection. 104th Annual meeting of the American Association of Immunologists, May 8-12, 2020, Honolulu, HI. (canceled due to COVID-19)
- **6.** Massilamany C, Arumugam R, **Lasrado N**, Gangaplara A, Jayaraja S, Steffen D, Gurumurthy C, and Reddy J. A unique model of cardiac myosin-specific TCR transgenic mice for dilated cardiomyopathy. 104th Annual meeting of the American Association of Immunologists, May 8-12, 2020, Honolulu, HI. (canceled due to COVID-19)
- 7. Won T, Kalinoski H, Wood M, Hughes D, Talor M, Lasrado N, Reddy J and Cihakova D. Autoimmunity against cardiac antigen drives immune checkpoint inhibitor-associated myocarditis development. Meeting the Challenges of Myocarditis Workshop, May 3-6, 2021, NHLBI.
- **8.** Lasrado N, Borcherding N, Arumugam R, Starr T, and Reddy J. Dissecting the complexity of heart infiltrates in post-infectious myocarditis induced with CVB3 infection by single-cell RNA sequencing analysis. 104th Virtual Annual meeting of the American Association of Immunologists, May 10-15, 2021.
- **9.** Rasquinha M, **Lasrado N**, Larman B, and Reddy J. PhIP-Seq analysis reveals autoantibodies for novel antigens in the mouse model of Coxsackievirus B3 infection. 104th Virtual Annual meeting of the American Association of Immunologists, May 10-15, 2021.
- **10.** Reddy J, Arumugam R, **Lasrado N**, Renu S and Gourapura R. Antigen-specific T cell tolerance in the prevention of post-infectious myocarditis induced with Coxsackievirus B3. 104th Virtual Annual meeting of the American Association of Immunologists, May 10-15, 2021.
- **11. Lasrado N**, D'Silva P and Lasrado L. Evaluation of the phytochemical, antioxidant, antihemolytic and anti-cancer activities of the solvent extracts of *Imperata cylindrica*: an *in-vitro* study. February 2-3, 2018, Mangalore, India.

WORKSHOPS

- **1. AAI Travel for techniques training, August 2019, University of Minnesota, MN:** Learnt the use of Cellranger and Seurat R Package, important tools for extracting, analyzing and visualizing the single cell RNA sequencing data at the laboratory of Dr.Tim Starr.
- 2 HCC Summer Workshop series, June 2019, UNL: Learnt the Linux command line environment, the revision control software Git, and an overview of best practices for high performance computing including how to submit and manage jobs with the SLURM scheduler, the Lmod environment module system and transferring data to and from remote resources with Globus.
- 3 10x Genomics- What's beneath the Surface? Deep profiling of Adaptive immunity, AAI 2019: Learnt the use of the groundbreaking 10x genomics technology to unlock cell repertoires and understand T cell specificity in Tumors vs healthy tissue using single cell analysis.
- **4** Microbubbles- The next generation of cell Isolation, AAI 2019: Learnt the use of revolutionary microbubble technology for the isolation of mouse B cells, RBC cleanup of flow sorting samples, and an improved method for NK cell isolation.
- 5. Microteaching workshop, July 2019, Graduate studies office, UNL: Learnt new techniques to engage students while teaching and received valuable feedback in terms of improving my teaching skills and techniques.
- **6. NIH-IEDB workshop, November 2019, NIH:** Learnt the use of tools and techniques available in the IEDB website to efficiently identify T-cell and B-cell epitopes and their binding predictions
- 7. Grant writing workshop, January 2021, UNL: Learnt the dos and don'ts of the art of grant writing and received valuable feedback for improving my grant-writing skills.
- **8 StrengthsFinder workshop, March 2021, UNL:** Attended this workshop as a part of our lab activity to know one's strengths and utilize them for one's own benefit. The workshop also aimed at finding the strengths of all teammates and improving the communication for effective work.

OUTREACH ACTIVITIES

2019 – Present Graduate student representative, GSA, UNL

2019 Poster Judge for Undergraduate Science literacy (SCIL) posters, UNL

PROFESSIONAL MEMBERSHIPS

2015 – 2018 Member, Make a Difference (NGO) -India

2018 - Present Member, American Association of Immunologists

2020 – Present Member, American Association for the Advancement of Science

2020 – Present Member, American Heart Association

ADDITIONAL INFORMATION

a) Research Experience

Undergraduate Research

• Evaluation of medicinal properties in the weeds: as a part of my undergraduate project, I evaluated the phytochemical, antioxidant, anti-hemolytic and anti-cancer activities of the root, stems extracts of *Imperata cylindrica*, and showed that these weeds indeed have a medicinal significance.

Graduate Research

- Creation of CVB Vaccine for preventing viral myocarditis and diabetes: We are currently developing a monovalent CVB vaccine that can offer cross protection against multiple serotypes of Coxsackievirus. We identified three viral epitopes that can induce differential T cell responses and used antigen specific T cell hybridomas to detect the presence of viral antigens in the cells from virus-infected animals.
- Identification of Novel transcripts in viral cardiomyopathy, which leads to autoimmune myocarditis: We are currently using next generation Single cell RNA sequencing technology to discover novel cellular subpopulations that may go beyond our traditional understanding of immune and non-immune cells, and also to identify the transcriptome signatures in the development of DCM.
- **Identification of self-antigens using Phip-Seq:** We are using the unique Phip-seq technology to detect autoantibodies generated against self-antigens from the serum of virus infected animals to check for potential autoantigen candidates that might cause autoimmune myocarditis.
- Induction of Treg cells for immune therapies: We are currently using biodegradable nanoparticles encapsulated with cytokines to promote Treg cells that can suppress autoimmune diseases.
- Identification of small molecules as viral inhibitors: We identified one viral protein fragment that blocked the viral replication in Coxsackievirus. Using this fragment of the viral protein, we will be screening small molecules that could potentially block virus replication.
- Creation of Serca2a TCR transgenic mouse model to study DCM: We are currently establishing TCR transgenic mouse model specific to sarcoplasmic reticulum calcium ATPase (SERCA2a) 971-990, a target autoantigen in the development of DCM, to evaluate the role of antigen-specific T cells in the induction of DCM and atrial myocarditis.

b) Technical skills and expertise

Mouse models: Successfully established coxsackievirus B4-induced pancreatitis mouse model for human autoimmune diseases. I also have vast experience on working with other mouse models such as experimental autoimmune myocarditis (EAM) and coxsackievirus B3-induced myocarditis models for heart disease/dilated cardiomyopathy.

Cellular Immunology and Flow cytometry: Wide experience with multi-color flow cytometric analysis of diverse immune cells; flow cytometric cell-sorting (basics) and magnetic-activated cell-sorting.

Baculovirus expression system: Hands-on experience in baculovirus expression systems. Expressed several major histocompatibility complex (MHC) class II molecules that are covalently linked with various antigenic T cell peptide epitopes (alleles IA^k and IE^k), and full-length cardiac proteins.

Molecular biology: Standard molecular biology techniques including PCR, RNA and DNA isolation, transfection, gene cloning, expression of proteins using both prokaryotic and eukaryotic expression systems.

Protein biochemistry: Purification of proteins based on antibody affinity chromatography; SDS-PAGE, ELISA.

Immunological assays: MHC tetramer/dextramer staining (*ex vivo* and *in vitro*), *in situ* staining, thymidine-incorporation assay, intracellular cytokine staining, cytokine and serum ELISA, cytometric bead array, CTLL-2 assay, virus neutralization assay.

Cell culture: In vitro culturing of a wide range of primary cell types (T cells, macrophages, dendritic cells), cell lines (HeLa cells, Vero cells, CTLL-2 cells, $58\alpha^{-}/\beta^{-}$ cells and SF9 cells) and antigen-specific-T cell hybridomas (Myhc-334-352, Serca2a 971-990, VP771-790 and cross-reactive CRX)

Virological techniques: Virus production and titration, immunofluorescence test and infection into animals *in vivo* and recovery/isolation of virus from infected animals.

Handling of microorganisms: Culturing of a wide range of microorganisms including viruses (Coxsackievirus B1, B3, B4, and Baculovirus) and bacteria (*Mycobacterium spp.*).

Histology: Tissue cutting, embedding and H & E staining and immunohistochemistry. Interpretations of histological analysis of various mouse organs such as heart, brain, skeletal muscle, and liver.

Microscopy: Confocal microscopy, fluorescence microscopy and light microscopy.

Computer skills: Microsoft office, flow cytometric data analysis by Flow Jo, Graph Pad Prism and bioinformatics analysis of gene sequences and protein sequences and T-cell and B-cell epitope prediction tools of IEDB.

Bioinformatics: Basic Linux command language, revision control software GitHub, R programming, microbiome transcriptome analysis using Mothur and single-cell-RNA-sequence analysis using SEURAT, MONOCLE R package and Cell Ranger software package by 10x genomics.