Ninaad Lasrado

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EDUCATION

2015- 2018	BSc in Biotechnology, St. Aloysius College, Mangalore, India. **GPA:** 8.9/10
2018- Present	Graduate Research Assistant (PhD), University of Nebraska- Lincoln (UNL), Lincoln, NE. **GPA:** 3.96/4

AWARDS AND FELLOWSHIPS

2015	Best Outgoing Student – Ambika Pre-University College, Mangalore, India
2016	3rd 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
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
2021	Shear-Miles Fellowship
2021	CRWAD Travel award, SVMBS, UNL

MENTORING AND TEACHING EXPERIENCE

2015 – 2018	Mentored underprivileged kids as a part of *Make a difference,* non-governmentorganization (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)

PATENTS

"Coxsackievirus vaccines and methods of making and using such vaccines". (#63/179,980)
Inventors: Reddy J, **Lasrado N**, Gangaplara A, Massilamany C, Rasquinha M, Pattnaik A. Pending patent, filed April 26, 2021.

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) **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". *Scientific Reports*. 2021 Jun 14. DOI: https://doi.org/10.1038/s41598-021-90434-w. PMID: 34127684.

6) **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 press, *iScience*, 2022. Preprint: bioRxiv, DOI: https://doi.org/10.1101/2021.05.16.444367

7) **Lasrado N**, Arumugam R, Rasquinha M, Sur M, Steffen D and Reddy J. 2021. Mt10-CVB3 Vaccine Virus Protects against CVB4 Infection by Inducing Cross-Reactive, Antigen-Specific Immune Responses. *Microorganisms*. 2021 Nov 10. DOI: 10.3390/microorganisms9112323. PMID: 34835449.

8) Rasquinha M*, Sur M*, **Lasrado N*** and Reddy J. "IL-10 as a Th2 cytokine in mice and humans: a comparative analysis". *The Journal of Immunology*. 2021 Nov 1. DOI: 10.4049/jimmunol.2100565. PMID: 34663593. *equal contributors

9) 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

10) 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

11) 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

ABSTRACTS

1. **Lasrado N**, D'Silva P and Lasrado L. Evaluation of the phytochemical, antioxidant, anti-hemolytic and anti-cancer activities of the solvent extracts of *Imperata cylindrica*: an *in-vitro* study. February 2-3, 2018, Mangalore, India.

2. **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.

3. **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.

4. 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. 103rd Annual meeting of the American Association of Immunologists, May 9-13, 2019, San Diego, CA.

5. **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.

6. **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)

7. 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)

8. 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.

9. **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.

10. 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.

11. 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.

12. **Lasrado N**, Borcherding N, Arumugam R, Starr T, and Reddy J. Dissecting the Cellular Landscape and Transcriptome Network in Viral Myocarditis by Single-Cell RNA Sequencing. CRWAD annual meeting, Chicago, IL, December 3-7, 2021.

13. Rasquinha M, **Lasrado N**, Larman B, and Reddy J. Determination of autoantibody repertoires by PhIP-Seq analysis in the mouse model of Coxsackievirus B3 infection. CRWAD annual meeting, Chicago, IL, December 3-7, 2021.

14. Sur M, Doiphode A, **Lasrado N**, Arumugam R, and Reddy J. Generation of SERCA2a-specific TCR transgenic mice. CRWAD annual meeting, Chicago, IL, December 3-7, 2021.

15. **Lasrado N,** Rasquinha M, Sur M, Gangaplara A, Massilamany C, Arumugam M, Steffen D, and Reddy J. A live-attenuated mutant CVB3 vaccine virus protects against multiple coxsackievirus B infections. 105th Annual meeting of the American Association of Immunologists, May 5-10, 2022, Portland, OR.

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.

9. **ChIP workshop, April 2021, Diagenode.** This workshop aimed at understanding the basics of chromatin immunoprecipitation and its combination with RNAseq or qPCR. Techniques relating to the use of ChiP kits to understand chromatin modifications were taught.

10. **Secondary Effects of Antigen-Specific Vaccines, July 2021, NIAID:** This workshop focused on understanding molecular mechanisms of secondary vaccine effects, such as trained immunity. It also considered the broader public health impacts of secondary vaccine effects in vaccine schedules and future vaccine development, including regulatory issues.

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

PROFESSIONAL SERVICE ACTIVITIES
Ad Hoc Reviewer

- Vaccines
- Viruses
- Microorganisms
- Biology

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 a Coxsackievirus B (CVB) Vaccine for preventing viral myocarditis, Encephalitis and Type 1 diabetes:** We have developed a monovalent CVB vaccine that can offer cross protection against multiple serotypes of CVB such as CVB1, CBV3 and CVB4. Studies with inbred A/J and NOD mice have shown protection against these viruses with high neutralizing antibodies and antigen-specific T cell responses. Currently, we are testing the efficacy of this vaccine in outbred mice to provide a translational significance.

- **Identification of Novel transcripts in viral cardiomyopathy, which leads to autoimmune myocarditis:** We have used Single cell RNA sequencing technology to dissect the complexities and pathological mechanisms associated with post-infectious viral myocarditis caused by CVB3. Using this technology, we identified novel functions for CD4 T cells and Tregs being cytotoxic, and myeloid cells, fibroblasts and T cells having an intercellular

communication network during the fibrosis phase. We also identified novel transcriptions factors that accelerate the transition from myocarditis to cardiomyopathy phase, thus having potential to target them for therapeutic strategies.

- **Identification of self-antigens using Phip-Seq:** We are using the unique Phage-Immunoprecipitation sequencing (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. We have identified 77 potential novel autoantigens that might trigger autoimmune myocarditis. Following success in mouse studies, we are currently validating our findings using human sera and plasma from patients that have dilated cardiomyopathy or have myocarditis of viral origins such as SARS-CoV-2, CVB, Parvovirus B19 etc.

- **Induction of Treg cells for immune therapies**: We are currently using biodegradable nanoparticles (Chitosan and PLGA) encapsulated with cytokines (IL-2 and TGF-β) to promote Treg cells that can suppress autoimmune diseases.

- **Trained immunity in the prevention of viral myocarditis and pancreatitis.** We recently serendipitously noted that mice immunized with CFA provided complete protection against challenge from CVB3, thus rendering the mice free from myocarditis and pancreatitis. *In vitro* studies confirmed the elevated secretion of cytokines and metabolites involved in trained immunity. Currently, we are utilizing BCG to study the concept of trained innate protection against CVB to prevent myocarditis and pancreatitis.

- **Creation of Myhc-α and Serca2a TCR transgenic mouse models to study DCM:** We have created a TCR transgenic mouse model specific to Myhc-α-334-352 which is a target autoantigen in DCM development. We are also currently establishing a TCR transgenic mouse model specific to sarcoplasmic reticulum calcium ATPase (SERCA2a) 971-990, another 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), Myhc-α-334-352 transgenic mouse, Rag KO mouse, and CVB3-induced myocarditis mouse models for heart disease/dilated cardiomyopathy, NOD mouse model for Type-1 diabetes, and SJL mouse model in experimental autoimmune encephalitis (EAE).

Cellular Immunology and Flow cytometry: Wide experience with multi-color flow cytometric analysis of diverse immune cells; flow cytometric cell-sorting 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^s, IA^k and IE^k), and full-length viral and cardiac proteins.

Molecular biology: Standard molecular biology techniques including routine and qPCR, mutagenesis, RNA and DNA isolation, transfection, gene cloning, construction of expression vectors, creation of TCR gene constructs, expression of proteins using both prokaryotic and eukaryotic expression systems.

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

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, LLC-MK2 cells and SF9 cells) and antigen-specific-T cell hybridomas (Myhc-334-352, Serca2a 971-990, VP1 771-790 and myosin 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, 16s microbiome analysis using Mothur, and single-cell-RNA-seq analysis using SEURAT, and Cell Ranger software package by 10x genomics.

References

1. Dr. Jay Reddy, MVSc, PhD
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