Entero-mammary axis and its role in maternal transfer of immunity.

Background: The role of microbial and environmental factors in autoimmunity is becoming increasingly evident but the mechanisms by which they influence disease is not entirely understood. Using a mouse model, we recently showed that maternal transmission of antibodies via breastmilk, in early life, can influence intestinal immunity of their adult offspring. Antibody composition in breastmilk was determined by antibody (IgA) producing plasma cells that migrated from the intestine to the mammary gland during pregnancy and lactation, thus establishing the entero-mammary axis. The immune networks and mechanisms behind the entero-mammary axis and its role in intestinal disease susceptibility remains to be explored. This led us to investigate immune cell dynamics in the mammary gland during different stages of pregnancy and lactation, with a focus on lymphocyte populations. We performed a preliminary analysis of all immune cell populations in nulliparous (virgin), late pregnancy, early lactation, and involution (weaning) stages of the mouse mammary gland by 10X single cell RNA sequencing and validated them by flow cytometry. We identified previously unreported changes in lymphocyte populations that were unique to stages of pregnancy and lactation, specifically, proportional and transcriptional differences in $CD8^+$ T cells, $CD4^+$ T cells, and regulatory T cells (Tregs) (Figure 1, clusters 0, 3, 4). Surprisingly, the observed differences in T cell populations in the mammary gland were dependent on the microbiota. Based on our preliminary results, *we hypothesize that the migration of immune cells and other intestinal factors (microbial and non-microbial) during late pregnancy and lactation changes the immune landscape of the mammary gland. These stage specific changes affect breastmilk composition in mothers and influence intestinal immunity in their offspring.*

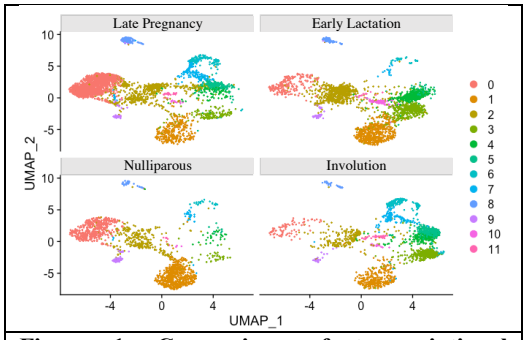

Figure 1. Comparison of transcriptional differences between T cell subsets during different stages of pregnancy/lactation. Each dot represents a cell and each number/color represents a cluster of cells that are transcriptionally similar.

Goals: Our research aims to understand the rules and lines of communication of the entero-mammary axis and their implications for intestinal disease using a mouse model. **First**, we will further characterize the immune cell changes in the mammary gland by addressing the **origin and location of T cell changes**. To test the origin of the cells, we will use photoconvertible mice to assess migration to the mammary gland (techniques previously used to establish the entero-mammary axis). We will use whole tissue imaging techniques such as iDISCO to identify the location of the different T cells. We will also identify factors/signals that drive the arrival of T cell populations to the mammary gland using RNA sequencing. **Second**, we will test the **role of microbes and microbial factors** in determining the T cell landscape of the mammary gland. We will use germ-free mice and mice treated with targeted antibiotics to identify specific microbial populations. We will also assess whether the microbial influence is intestinal in origin or from other sites near the mammary gland, such as skin. **Third**, we will study how the **immune cell landscape acquired by the mothers during lactation can protect their offspring from intestinal disease**. To determine how different T cell subsets influence breastmilk composition, we will use knockout/conditional knockout mice or depletion of T cells of interest (for example, Tregs can be depleted at specific stages of pregnancy/lactation by administering diphtheria toxin to Foxp3-DTR mice). We will expose pups of manipulated mothers to intestinal infection and inflammation (colitis) models and perform extensive immunophenotyping to test offspring immunity.

Significance: The entero-mammary axis provides a new avenue to explore host-microbe-environment interactions in both health and intestinal disease. This alliance could offer an explanation to several obscurities in intestinal disease biology, including the concept of "missing heritability", and perhaps the rapid increase in the incidence of immunological and inflammatory diseases, which is too rapid to be justified by genetic changes.

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EDUCATION

2010 – 2016 Ph.D. Immunology and Inflammation, Vilcek Institute, New York University, New York, NY
2004 – 2008 B. Sc. Cell and Molecular Biology, Winona State University, Winona, MN

RESEARCH

2023 – Assistant Professor
NOMIS Center for Immunobiology and Microbial Pathogenesis
Salk Institute of Biological Studies

2016 – 2022 Postdoctoral Fellow – Dr. Diane Mathis and Dr. Christophe Benoist
Department of Immunology, Harvard Medical School, Boston, MA
Regulatory T cells – Multigenerational Transfer of their Setpoints and Functions.

2010 – 2016 Graduate Student – Dr. Ken Cadwell
Department of Microbiology, New York University, New York, NY
Thesis title: Mechanisms of Nod2-Microbe Interactions in Intestinal Disease.

2008 – 2010 Research Associate – Dr. Edwin Chapman
Department of Physiology, University of Wisconsin, Madison, WI
Antibody Generation and Localization of Synaptotagmin isoforms.

2007 – 2008 Undergraduate Research Assistant –Dr. Emmanuel Brako
Department of Biology, Winona State University, Winona, MN
Prevalence of Equine Herpes Virus I Antibodies in Horses in Winona County.

2007 Summer Undergraduate Research Fellow – Dr. Richard Bram
Department of Immunology, Mayo Clinic, Rochester, MN
Role of CAML in the Development of γδ T cells.

2006 – 2007 Undergraduate Research Assistant –Dr. Michael Delong
Department of Biology, Winona State University, Winona, MN
Study of trophic dynamics in St. Croix River using C-14 and N-15 stable isotope ratios.

PUBLICATIONS

1. **Ramanan D**, Pratama A, Zhu Y, Venezia O, Sassone-Corsi M, Chowdhary K, Galvan-Pena S, Sefik E, Brown C, Gelineau A, Mathis D, Benoist C. (2023) Regulatory T cells in the face of the microbiota. ***Nature Reviews Immunology*** https://doi.org/10.1038/s41577-023-00890-w.
2. **Ramanan D**, Chowdhary K, Candeias SM, Sassone-Corsi M, Mathis D, Benoist C. (2023) Homeostatic, repertoire and transcriptional relationships between colon T regulatory cell subsets. *Preprint at* ***bioRxiv*** https://doi.org/10.1101/2023.05.17.541199 (in revision at PNAS).

3. Chowdhary K, Leon J, **Ramanan D**, Mathis D, Benoist C. (2023) An interwoven network of transcription factors, with divergent influences from Foxp3, underlies Treg diversity. *Preprint at* ***bioRxiv*** https://doi.org/10.1101/2023.05.18.541358 (in revision at Nature Immunology).
4. Wu M, Zheng W, Song X, Bao B, Wang Y, **Ramanan D**, Yang D, Li R, Macbeth JC, Do EA, Andrade WA, Yang T, Cho H, Gazzaniga FS, Ilves M, Coronado D, Thompson C, Hang S, Chiu IM, Moffitt JR, Hsiao A, Mekalanos JJ, Benoist C, Kasper DL. (2023) Microbiome induced complement synthesized in the gut protects against enteric infections. *Preprint at* ***bioRxiv*** https://doi.org/10.1101/2023.02.02.523770 (in revision at Cell).
5. Sassone-Corsi M, Azriel S, Simon A, **Ramanan D**, Ortiz-Lopez A, Chen F, Yissachar N, Mathis D, Benoist C. (2022) Sequestration of gut pathobionts in intraluminal casts, a mechanism to avoid dysregulated T cell activation by pathobionts. ***PNAS***. 11;119(41):e2209624119
6. Yan Y, **Ramanan D**, Rozenberg M, McGovern K, Rastelli D, Vijaykumar B, Yaghi O, Voisin T, Mosaheb M, Chiu I, Itzkovitz S, Rao M, Mathis D, Benoist C. (2021) Neuron-produced IL6 is at the center of a microbiota-neuron-Treg triangle in the gut. ***Immunity***. 54(3):499-513.
7. **Ramanan D**, Sefik E, Galván-Peña S, Wu M, Yang L, Yang Z, Kostic A, Golovkina TV, Kasper DL, Mathis D, Benoist C. (2020) An Immunologic Mode of Multigenerational Transmission Governs a Gut Treg Setpoint. ***Cell.*** S0092-8674(20)30493-1.
8. DiSpirito JR*, Zemmour D*, **Ramanan D**, Cho J, Zilionis R, Klein AM, Benoist C, Mathis D. (2018) Molecular Diversification of Regulatory T cells in Nonlymphoid Tissues. ***Science Immunology***. 3(27):eaat5861. *co-first authors.
9. Wong SY*, Coffre M*, **Ramanan D***, Hines MJ, Gomez LE, Peters LA, Schadt EE, Koralov SB, Cadwell K. (2018) B Cell Defects Observed in Nod2 Kockout Mice Are a Consequence of a Dock2 Mutation Frequently Found in Inbred Strains. ***Journal of Immunology***. 201(5):1442-1451. *co-first authors.
10. **Ramanan D***, Bowcutt R*, Lee SC, Tang MS, Kurtz ZD, Ding Y, Honda K, Gause WC, Blaser MJ, Bonneau RA, Lim YA, Loke P, Cadwell K. (2016) Helminth infection promotes colonization resistance via type 2 immunity. ***Science***. 352(6285):608-12. *co-first authors.
11. **Ramanan D**, Cadwell K. (2016) Intrinsic defense mechanisms of the intestinal epithelium. ***Cell Host and Microbe***. 19(4):434-41.
12. **Ramanan D**, Tang MS, Bowcutt R, Loke P, Cadwell K. (2014) Bacterial sensor Nod2 prevents inflammation of the small intestine by restricting the expansion of the commensal *Bacteroides vulgatus.* ***Immunity***. 41(2):311- 324.
13. Marchiando AM, **Ramanan D**, Ding Y, Gomez LE, Hubbard-Lucey VM, Maurer K, Wang C, Ziel, JW, Rooijen NV, Nunez G, Finlay BB, Mysorekar IU, Cadwell K. (2013) *Atg16L1* deficiency enhances resistance to an enteric bacterial infection. ***Cell Host and Microbe***. 14(2):216-24.
14. Zhang Z, Wu Y, Wang Z, Dunning FM, Rehfuss J, **Ramanan D**, Chapman ER, Jackson MB. (2010) Release mode of large and small dense-core vesicles specified by different synaptotagmin isoforms in PC12 cells. ***Mol Biol Cell***. 22(13):2324-36.

PRESENTATIONS

TALKS

1. Maternal factors orchestrate non-genetic transmission of immunological traits across multiple generations. June 2020, Guts and Bugs Online Seminar Series, Rutgers University, New Brunswick, NJ.

2. Maternal factors orchestrate non-genetic transmission of immunological traits across multiple generations. September 2019, Damon Runyon Fellows' Retreat, Southbridge, MA.
3. Our microbiome in health and disease. December 2015, February 2016. Know Science Talk Series. Rockefeller University, New York, NY.
4. Helminth infection promotes defensive symbiosis via type-2 immunity. Poster Blitz Talk. Skirball Retreat 2015, Lenox, MA.
5. Trans-kingdom interactions between helminths and bacteria in intestinal disease. Microbiology Retreat 2015, New York, NY.
6. Type 2 immunity protects genetically susceptible hosts from microbiota induced intestinal inflammation. The Multifaceted Roles of Type 2 Immunity. December 2014, Bruges, Belgium.
7. The role of gut microbes in inflammatory bowel disease. December 2013, New York City College of Technology, New York, NY.

POSTERS

1. Ramanan D, Sefik E, Mathis D, Benoist C. The role of microbes in non-genetic transmission of immunological traits. July 2019, International Congress of Mucosal Immunology, Brisbane, Australia.
2. Ramanan D, Mathis D, Benoist C. Identifying functions of regulatory T cell subsets in intestinal inflammation and colorectal cancer. September 2017, Damon Runyon Fellows' Retreat, Beverly, MA.
3. Ramanan D, Sefik E, Mathis D, Benoist C. Colonic rorγ+ treg phenotype is imprinted at birth. July 2017, UCB Super Meeting, London, United Kingdom.
4. Ramanan D, Cadwell K. Role of Nod2 in mucosal immunity of the small intestine. October 2011-2015, Annual Skirball Retreat, Lenox, MA.
5. Ramanan D, Cadwell K. Role of Nod2 in mucosal immunity of the small intestine. 2011-2016 Immunology and Inflammation Program, New York University, New York, NY.

HONORS

2021	Damon Runyon Dale F. Frey Breakthrough Scientist Award
2020	STAT Wunderkind Award
2017-2021	Damon Runyon Postdoctoral Fellowship – National Mah Jongg League Fellow
2017	Finalist – HHMI Hannah H. Gray Fellows Program
2016	Harold M. Weintraub Award – Fred Hutch Institute
2016	Sackler Dissertation Prize – Sackler Institute, NYU
2015	McCracken Award – Sackler Institute, NYU
2016	Best Poster Award – Immunology and Inflammation Program Retreat, NYU
2015	Best Poster Award – Skirball retreat, NYU
2014	Bio-Techne Award – The Multifaceted Roles of Type 2 Immunity, Cell Symposia
2008	Summa Cum Laude – Winona State University
2007	Best Student Poster Award – Mississippi River Research Consortium
2006-2008	Martin and Joyce Laakso Award – Winona State University
2005-2008	Cross Cultural Scholarship – Winona State University