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TITLE: Targeting the intestinal barrier to regulate mucosal immunity in IBD and infec

PRINCIPAL INVESTIGATOR: Jerrold Turner, M.D., Ph.D.

CONTRACTING ORGANIZATION: Brigham & Women's Hospital, Inc.

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Targeting the intestinal barrier to regulate mucosal immunity in IBD and infectious enterocolitis

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6. AUTHOR(S)

Jerrold Turner, M.D., Ph.D.

E-Mail: jrturner@bwh.harvard.edu

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5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Brigham & Women's Hospital, Inc.
75 Francis Street
Boston, MA 02115-6110

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The research proposed here will explore this unanticipated interaction that has the intestinal barrier at its center. In retrospect, one might have anticipated that, as our first line of defense, the barrier would govern communication between intestinal contents and the immune system. Nevertheless, the underlying processes are almost entirely undefined. The studies described in this application will elucidate the mechanisms that regulate this communication, the means by which decreased channel activity either reduces or increases severity of IBD or infectious gastritis, respectively, and how excessive or insufficient dietary sodium impacts these processes. Further, the results will create a foundation that will help us to design a new class of therapies that modulate claudin-2 channel function. The data will also provide guidance as to whether interventions to reduce or increase channel activity are appropriate for an individual patient at a specific time, i.e. personalized medicine.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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YEAR 1 PROGRESS REPORT
Log Number: PR181271
Award Number: W81XWH-19-1-0261
Title: Targeting the Intestinal Barrier to Regulate Mucosal Immunity in IBD and Infectious Enterocolitis
Report Due Date: JULY 2020
Science Officer: Mr. Jonathan Ryder

1. **INTRODUCTION:** This application in the topic area of inflammatory bowel disease (IBD) focuses on intestinal barrier loss, a highly significant but mechanistically underexplored topic in IBD pathogenesis. As a result of limited study, opportunities for therapeutic regulation of the intestinal barrier have been neglected. It is nevertheless imperative to develop deep understanding of the causes and effects of barrier regulation and means to correct mucosal permeability defects in IBD and other disorders. The work proposed is designed to generate molecular, pathophysiological, and translational data that will enable development of barrier-directed therapies. The studies will test the central hypothesis that claudin-2-dependent regulation of the intestinal epithelial barrier negotiates communication between dietary Na^+, mucosal immunity, and microbial pathogens. Because the barrier is compromised in many intestinal and systemic diseases, the knowledge and tools generated are also expected to provide insight into other immune-mediated and infectious diseases.

2. **KEYWORDS:** diarrhea, inflammatory bowel disease, enteric infection, mucosal immunity, tight junction, sodium, claudin-2, enteropathogenic *E. coli*, *C. rodentium*, host defense, microbiome

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.*
    - **What were the major goals of the project?**
        - SPECIFIC AIMS AND MILESTONES PROPOSED (months 1-12)
            - AIM 1: To elucidate the mechanisms by which claudin-2 and dietary Na^+ interact to regulate the mucosal environment and T cell differentiation.
                - Determine the effect of claudin-2 expression on lamina propria Na^+ concentration; months 0 – 12
                - Define the impact of claudin-2 and dietary Na^+ on epithelial function; months 2 - 22
                - Define the impact of claudin-2 and dietary Na^+ on microbial populations; months 4 - 22
                - Define the impact of claudin-2 and dietary Na^+ on basal T cell differentiation; months 4 - 22
                - Define the impact of claudin-2 and dietary Na^+ on stimulated T cell differentiation; months 6 - 26
            - AIM 2: To characterize the impact of claudin-2 expression and dietary Na^+ on immune-mediated, infectious, and damage-induced colitis.
                - Define the mechanisms of by which claudin-2 and dietary Na^+ modify immune-mediated colitis; months 6 - 36

        - Define the impact of claudin-2 and dietary Na^+ modify immune responses in *C. rodentium* colitis; months 6 – 26
    - AIM 3: To define how claudin-2 limits mucosal colonization and enhances pathogen clearance during enteric infection.
        - Define effects of claudin-2-mediated Na^+ and water flux on early colonization in vitro; months 0 - 12

- **What was accomplished under these goals?**
    - All milestones have been affected by COVID-19 stay-at-home orders, which mandated cessation of all experimental activities. We were also ordered to reduce mouse colonies to the bare minimum needed to maintain each line. This changed June 1, when will entered Phase II of the research start-up progress. During this phase we have been given permission to re-initiate larger scale breeding. One round of breeding will, however, be needed to expand our population of breeders before a second round of breeding can be initiated to generate experimental animals. This will severely limit numbers of mice available for experimental use until early 2021. As expected, this has interrupted and will continue to delay progress on all in vivo aims.
    - Limited experimental work began on June 1, but only 50% of lab members are permitted to be on-site at any one time. Together with the shortage of mice resulting from the shutdown, this will require some shifting of time-lines (but no changes in overall goals). For example, while mice are being bred over the next 6 months, efforts will focus on in vitro cell culture studies.
    - Substantial progress was made in developing tools to measure lamina propria Na^+ concentrations in *Cldn2* knockout and *Cldn2* transgenic mice. Efforts had been focused on development of necessary technologies. We anticipate completing these and successfully measuring Na^+ concentrations over the next reporting period.
    - Development of bar-coded *C. rodentium* has turned out to be more difficult than anticipated. We are implementing alternative strategies to create bar-coded *C. rodentium*.
    - A manuscript entitled “Inactivation of paracellular cation-selective claudin-2 channels attenuates immune-mediated experimental colitis in mice” has been accepted and is in press at the *Journal of Clinical Investigation*.

- **What opportunities for training and professional development has the project provided?**
    - The project was not intended to provide training and professional development opportunities. Nevertheless, several postdoctoral fellows, including Preeti Raju, PhD, Nitesh Shashikanth, PhD, and Peter Steinhagen, MD (all authors on the accepted manuscript) have substantially advanced their professional skills through activities including experimental design, data analysis, data presentation, and manuscript preparation and submission.

- **How were the results disseminated to communities of interest?**

        - A manuscript entitled “Inactivation of paracellular cation-selective claudin-2 channels attenuates immune-mediated experimental colitis in mice” has been accepted and is in press at the *Journal of Clinical Investigation*.
        - Work was presented at the Annual Crohn’s and Colitis Congress in Austin, TX.
        - Presentations were scheduled at Experimental Biology and Digestive Disease Week but were canceled due to COVID19.

    - **What do you plan to do during the next reporting period to accomplish the goals?**
        - As mice become available, our efforts using in vivo models will intensify. Until that time, we will focus on studies using in vitro models.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*
    - **What was the impact on the development of the principal discipline(s) of the project?**
        - The idea that increased intestinal paracellular cation permeability can be detrimental was reported. Previous to this, paracellular cation permeability increase were only reported to be detrimental .
        - The data demonstrate benefit of a drug that reduces intestinal paracellular cation permeability and provide a pathway for development of therapeutics.

    - **What was the impact on other disciplines?**
        - Nothing to Report.

    - **What was the impact on technology transfer?**
        - Nothing to Report.

    - **What was the impact on society beyond science and technology?**
        - *Nothing to Report.*

5. **CHANGES/PROBLEMS:** *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*
    - **Changes in approach and reasons for change**
        - Nothing to Report.

    - **Actual or anticipated problems or delays and actions or plans to resolve them**
        - As described above, COVID19 has caused some delays because
            - No experimental work occurred for 12 weeks.
            - Staff continues to be limited to 50% on site.
            - Mouse colonies were dramatically cut and need to be reconstituted.
            - We have re-focused our attention on in vitro studies until mice are available.

        - An aggressive reading / journal club program for trainees has been taking place while we have been out of the lab.

    - **Changes that had a significant impact on expenditures**
        - Staff were paid while prevented from doing experimental work on site.
        - Some mouse care was required when no experimental work was permitted (see above).
        - These accommodations for COVID19 is resulted in some fund depletion with limited productivity.

    - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
        - Nothing to Report.

6. **PRODUCTS:**
    - **Publications, conference papers, and presentations**
        - **Journal publications.**
          Raju P, Shashikanth N, Tsai PY, Pongkorpsakol P, Chanez-Parades S, Steinhagen PR, Kuo WT, Singh G, Tsukita S, Turner JR (2020) Inactivation of paracellular cation-selective claudin-2 channels attenuates immune-mediated experimental colitis in mice. J Clin Invest. doi:10.1172/JCI138697; accepted; acknowledgement of federal support - yes.
          Ong M, Yeruva S, Sailer A, Nilsen SP, Turner JR (2020) Differential regulation of claudin-2 and claudin-15 expression in children and adults with malabsorptive disease. Lab Invest 100 (3):483-490. doi:10.1038/s41374-019-0324-8; published; acknowledgement of federal support - yes.

        - **Books or other non-periodical, one-time publications.**
          Nothing to Report.

        - **Other publications, conference papers, and presentations.**
          Nothing to Report.

    - **Website(s) or other Internet site(s)**
        - Nothing to Report.

    - **Technologies or techniques**
        - Nothing to Report.

    - **Inventions, patent applications, and/or licenses**
        - Nothing to Report.

    - **Other Products**
        - Nothing to Report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
    - **What individuals have worked on the project?**

Name: Jerry Turner
Project Role: PI
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1.8CM
Contribution to Project:
Funding Support:
Name: Yunuo Liu
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 12 CM
Contribution to Project:
Funding Support:
Name: Heather Rizzo
Project Role: Technical Research Assistant
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 9 CM
Contribution to Project:
Funding Support:
Name: Preeti Raju
Project Role: Research Fellow
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 6 CM
Contribution to Project:
Funding Support:
Name: Lihua Wu
Project Role: Research Lab Manager

Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 2.2 CM
Contribution to Project:
Funding Support:

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  - Nothing to Report.

- **What other organizations were involved as partners?**
  - Nothing to Report.

8. **SPECIAL REPORTING REQUIREMENTS**
   - Nothing to Report.

9. **APPENDICES:**