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TITLE: Gut and Cartilage Microbiomes as Novel Mediators of Inflammation in and Potential Therapy for Post-Traumatic Osteoarthritis

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CONTRACTING ORGANIZATION: Oklahoma Medical Research Foundation

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

In this study we plan to evaluate both the pathogenic and potential protective effects of changes in body microbiomes along with their interactions with the immune system in PTOA, using cutting-edge microbiome and immune cell subtyping analyses. In Aim 1 (year 1), we will evaluate correlations between gut and cartilage microbiomes in PTOA-susceptible C57BL/6J (B6) and PTOA-resistant MRL/MpJ mice (MRL) before and after DMM-induced PTOA, and correlate these findings with systemic inflammatory cell responses via peripheral blood cell phenotyping. In Aim 2 (year 2), we will establish the direct contributions of the gut microbiome in both establishment of a cartilage microbiome and in PTOA pathogenesis by introducing the gut microbiota of B6 and MRL mice into germ-free (GF) mice, followed by DMM. In Aim 3 (year 2), we will examine the potential therapeutic potential of altering the gut microbiota as a preventative / therapeutic for PTOA by conducting gut microbiome transplantation of superhealer MRL into non-healer B6 mice, measuring susceptibility to OA, and quantifying associated systemic inflammatory cell changes.

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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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

In this study we plan to evaluate both the pathogenic and potential protective effects of changes in body microbiomes along with their interactions with the immune system in post-traumatic osteoarthritis (PTOA), using cutting-edge microbiome and immune cell subtyping analyses.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Osteoarthritis, post-traumatic osteoarthritis, microbiome, inflammation

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Planned milestones: Cecal and cartilage microbiome characterization (milestone completion target month 11), Systemic inflammatory cell population associations in PTOA (milestone completion target month 11), Transplantation of B6 and MRL microbiome into GF mice (milestone completion target month 19), Transplantation of MRL microbiome into B6 mice (milestone completion target month 19). **Actual completion date:** Cecal and cartilage microbiome characterization (not yet completed) Systemic inflammatory cell population associations in PTOA (not yet completed) Transplantation of B6 and MRL microbiome into GF mice (Transplant started 4/2021, sac date target 6/2021) Transplantation of MRL microbiome into B6 mice (Transplant completed 4/26, sac date 6/7)

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1. Cecal and cartilage microbiome characterization
    a. The major objectives of this activity are to characterize the cecal and cartilage microbiome pre- and post-DMM in young B6 and MRL animals.
        i. Pre-DMM B6 cecal and cartilage collection has been completed as of 4/21/21.
        ii. Pre-DMM MRL cecal and cartilage collection will be completed 5/24/21
        iii. Post-DMM B6 cecal and cartilage collection:
            1. MRL DMM planned 4/26/21, cecal cand cartilage collection 5/24/21
            2. B6 DMM cecal and cartilage collection completed as of 4/21/21
        iv. All cecal and cartilage microbiome characterization will be performed as one batch once all samples are collected, anticipate ~7/2021
2. Systemic inflammatory cell population associations in PTOA associated with gut microbiome variations
    a. The major objective of this activity is to characterize (via CyTOF) systemic inflammatory cell populations and serum cytokines (by bioplex) before and after DMM in B6 and MRL animals.
        i. Serum and inflammatory cells are collected during mouse sacrifice, following the schedule listed in major activity #1 above.
        ii. CyTOF planned for 7/2021-9/2021 following mouse sacrifice.
        iii. Serum cytokine analysis will be performed 7/2021-8/2021
3. Transplantation of B6 and MRL microbiome into germ-free mice, with subsequent analysis of gut/cecal microbiota and systemic inflammatory cell analysis pre- and post-DMM
    a. Characterize changes in the gut/cecal microbiome following transplantation of either B6 or MRL cecal microbiome into germ-free animals
        i. GF animals transplanted 3/2021 and 4/2021, planned sacrifice 4/2021 and 5/2021
        ii. 16S characterization will happen 6/2021-8/2021
    b. Characterize differences in OA outcomes in GF animals with cecal transplantation of B6 vs. MRL cecal microbiomes
        i. This has not yet occurred, planned for 7/2021-10/2021.
        ii. Systemic inflammatory analysis will occur following mouse sacrifice, planned 10/2021-12/2021

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

This project has provided a number of opportunities for training and professional development from a germ-free animal protocol perspective, as mentioned above. Both myself and the senior laboratory technician on the project, Vladislav Izda, in collaboration with the Attending Veterinarian at OMRF, Dr. Jennie Criley, have developed a number of new approaches to articular surgery, intraarticular injection, and DNA vehicle preparation for use in germ-free animals.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next reporting period, we will complete the remaining Goals as outlined above. Indeed, our germ-free animal work is proceeding ahead of schedule, owing to a more rapid germ-free mouse colony breeding protocol and additional support provided by the OMRF germ-free mouse facility. The majority of DMM work has already or will be performed within the next 2 months, with animals being held for 4-8 weeks after DMM before sacrifice; from there, microbiome characterization and data analysis will proceed rapidly.

4. **IMPACT:** D*escribe 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report during this period.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

We have developed a number of new protocols for microbiome transplantation and animal surgery (related to osteoarthritis) in germ-free animals, we will report these protocols when our topline results are available.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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:*

The only challenges we have faced are COVID19-related; specifically, our non-germ-free mouse breeding was delayed by several months while our facility was at minimum capacity; however, we are now back on track to complete our microbiome analyses. It is anticipated that we will still be able to meet the deadlines outlined in the Statement of Work; however, there is a possibility that we may have a lingering 1-2 month delay.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

None.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

None.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable.

None.

Significant changes in use of biohazards and/or select agents

Not applicable.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal;*

volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report.

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*

- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Matlock Jeffries, PI
Researcher identifier: ORCID ID: 0000-0001-9516-4312
Nearest person month worked: 2
Contribution to Project: Organized and directed the project, including project planning, collaboration with sequencing core facility and germ-free mouse facility

Vladislav Izda, Laboratory technician
Researcher identifier: ORCID ID: 0000-0002-7561-2699
Nearest person month worked: 6
Contribution to Project: Optimized cecal transplantation protocol, performed mouse surgeries, performed mouse necropsy and tissue collection

Jake Martin, Laboratory technician
Researcher identifier: n/a
Nearest person month worked: 2
Contribution to Project: Optimized cecal transplantation protocol, performed mouse necropsy and tissue collection, optimized microbiome characterization 16s protocols

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

None.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Not applicable.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: QUAD CHARTS:

9. APPENDICES: