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TITLE: Proteomics of Knee Osteoarthritis

PRINCIPAL INVESTIGATOR: Dr. Aimy Sebastian

CONTRACTING ORGANIZATION: Lawrence Livermore National Security, LLC
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14. ABSTRACT

MRL/MpJ (super healers) mice have a unique ability to repair wounds and are protected from cartilage degradation subsequent to joint trauma. The hypothesis is that in response to injury, MRL/MpJ mice synthesize proteins that (1) protect the joint from cartilage degradation and/or (2) promote cartilage regeneration. The PIs propose to generate an atlas of the injury-activated proteome in mouse models with varying susceptibility to posttraumatic osteoarthritis (PTOA): (1) C57BL/6; (2) C57BL/6 treated with streptozotocin (STZ), a model of type 1 diabetes (T1DM); (3) MRL/MpJ (super healers); and (4) STR/ort (spontaneous OA). By conducting comparative proteomics of injured and uninjured joints, the PIs will identify novel protein candidates for further exploration as potential therapies for treating injured joints. The project's specific aims are (1) application of in vivo metabolic labeling to quantify and characterize de novo protein synthesis, cellular proliferation, and mineral apposition in injured joints of mice with varying susceptibility to PTOA and (2) identification of newly synthesized RNA and proteins in the articular cartilage and immune cells of injured knees using a liquid sample interface for the AMS instrument in combination with liquid chromatography-mass spectrometry (LC-MS).

15. SUBJECT TERMS

NONE LISTED

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TABLE OF CONTENTS

	Page
1. Introduction	**4**
2. Keywords	**4**
3. Accomplishments	**4**
4. Impact	**10**
5. Changes/Problems	**11**
6. Products	**12**
7. Participants & Other Collaborating Organizations	**13**
8. Special Reporting Requirements	**15**
9. Appendices	**15**

INTRODUCTION:

MRL/MpJ (super healers) mice have a unique ability to repair wounds and are protected from cartilage degradation subsequent to joint trauma. The hypothesis is that in response to injury, MRL/MpJ mice synthesize proteins that (1) protect the joint from cartilage degradation and/or (2) promote cartilage regeneration. The PIs propose to generate an atlas of the injury-activated proteome in mouse models with varying susceptibility to posttraumatic osteoarthritis (PTOA): (1) C57BL/6; (2) C57BL/6 treated with streptozotocin (STZ), a model of type 1 diabetes (T1DM); (3) MRL/MpJ (super healers); and (4) Tlr4 KO, C3H/HeJ, Trem2 KO (susceptible to PTOA). By conducting comparative proteomics of injured and uninjured joints, the PIs will identify novel protein candidates for further exploration as potential therapies for treating injured joints. The project's specific aims are (1) application of in vivo metabolic labeling to quantify and characterize de novo protein synthesis, cellular proliferation, and mineral apposition in injured joints of mice with varying susceptibility to PTOA and (2) identification of newly synthesized RNA and proteins in the articular cartilage and immune cells of injured knees using a liquid sample interface for the AMS instrument in combination with liquid chromatography-mass spectrometry (LC-MS).

KEYWORDS:

MRL/MpJ; STR/ort; osteoarthritis, post-traumatic osteoarthritis, diabetes, streptozotocin, MetRS, superhealer, chondrocytes, knee joint, anterior cruciate ligament, ACL, de novo protein synthesis, PTOA

ACCOMPLISHMENTS

For the 4th year of this grant, our main focus has been on conducting tasks associated with Aim 1/Major Task 1 (Sub Aim 1A) of the proposal, following the original tasks and timeline we are highlighting in 'green' subtasks that have been competed, in 'yellow' subtasks that have started and are in progress, and in 'blue' subtasks that have not yet started but will initiate in the next funding period. Also in **'red'** text are some changes to the SOW that have been implemented to overcome some challenges or improve upon the original plan.

Specific Aim 1.	Timeline (months)	Status	Site 1 (LLNL)	Site 2 (UCD) Christiansen & Loots
Major Task 1. (Sub Aim 1A): Phenotype PTOA	**1-48(60)**			
Subtask 1.1. Obtain IACUC/ACURO approval; breed MRL/MpJ, C3H/HeJ, Tlr4 KO, Trem2 KO, and C57BL/6 cohorts.	1-12	completed	Breed animals	
Subtask 1.2. Induce traumatic OA in 10 week old MRL/MpJ, C3H/HeJ, Tlr4 KO, Trem2 KO mice. 576 mice will be used (4 genotypes x 24 mice per group x 6 time points).	3-48(60)	In progress	Prepare cohorts of animals, transport to UCD for injury	576 animals will be injured, ~~return animals to LLNL post injury~~ **animals remain at UCD**
Subtask 1.3. Sample collection from 576 animals from Subtask 1.2	3-48	Completed	Dissect joints, extract proteins	microCT (200+ scans)
Subtask 1.4. Induce type 1 diabetes in 6 week old C57BL/6 mice for 4 weeks.	7-9	Completed	Administer STZ	
Subtask 1.5. Induce traumatic OA in 10 week old C57BL/6 and STZ mice. 336 mice will be used (2 treatments x 24 mice per group x 7 time points).	10-18	Completed	Prepare cohorts of animals, transport to UCD for injury	336 animals will be injured, return animals to LLNL post injury
Subtask 1.6. Sample collection from 336 animals from Subtask 1.5	10-18	Completed	Dissect joints, extract proteins	microCT (168 scans)
Subtask 1.7. Histological analysis	10-~~24~~(60)	In progress	~~Embed, section,~~	**Embed, section,**

			~~visualize~~ proteins	**visualize proteins**
Milestone 1: IACUC/ACURO Approvals				
Milestone 2: Complete Sample Collection for STR, Trem2, Tlr4 KO, C3H/HeJ, and MRL strain				
Milestone 3: Complete Sample Collection for diabetic mice				
Milestone 4: Manuscript #1 describing injury-induced phenotypic and molecular changed in T1D mice Rios-Arce ND, Murugesh DK, Hum NR, Sebastian A, Jbeily EH, Christiansen BA, **Loots GG**. Preexisting Type 1 Diabetes Mellitus Blunts the Development of Posttraumatic Osteoarthritis. ***JBMR Plus***. 2022 May;6(5):e10625. doi: 10.1002/jbm4.10625. eCollection 2022 May. PubMed PMID: 35509635; PubMed Central PMCID: PMC9059474.				
Milestone 5: Manuscript #2 review article Rios-Arce ND, Hum NR, **Loots GG**. Interactions Between Diabetes Mellitus and Osteoarthritis: From Animal Studies to Clinical Data. ***JBMR Plus***. 2022 May;6(5):e10626. doi: 10.1002/jbm4.10626. eCollection 2022 May. Review. PubMed PMID: 35509632; PubMed Central PMCID: PMC9059469.				
Milestone 6: Manuscript #3 describing STR/ort mice PTOA phenotype Mendez ME, Murugesh DK, Christiansen BA, Loots GG. Antibiotic Treatment Prior to Injury Abrogates the Detrimental Effects of LPS in STR/ort Mice Susceptible to Osteoarthritis Development. ***JBMR Plus***. 2023 May 22;7(8):e10759. doi: 10.1002/jbm4.10759. eCollection 2023 Aug. PMID: 37614305 Free PMC article.				

What was accomplished under these goals?

Subtask 1.1. IACUC/ACURO approval.

Completed.

Subtask 1.2. Induce traumatic OA in 10 week old MRL/MpJ, STR/ort, C3H/HeJ, Tlr4 KO, Trem2 KO mice.

We have completed the characterization of the STR/ort, manuscript published. CH3/HeJ strain characterization is and comparison to C57BL/6 has been completed, manuscript is in preparation. Tlr4 KO strain characterization completed, manuscript in preparation. Trem2 KO characterization is in progress.

Subtask 1.3. Sample collection from 336 animals from Subtask 1.2

In progress

Subtask 1.4. Induce type 1 diabetes (T1D) 6 week old C57BL/6 mice for 4 weeks.

Completed in year 1.

Subtask 1.5/1.6. Induce traumatic OA in 10 week old C57BL/6 and STZ mice.

Completed in year 1. Manuscript (#1) and review article (#2) were published.

Subtask 1.7. Histological analysis

Histological analyses have been completed for T1DM, C57BL6, Tlr4 KO and MRL, ongoing for Trem2 and C3H/HeJ.

Major Tasks 2 work was initiated in the next fiscal year and will be completed in this fiscal year.

Major Task 2. (Sub Aim 1B) Quantify cell proliferation in injured joints	**13-60**			
Subtask 2.1. Breed necessary cohorts of MRL/MpJ, C3H/HeJ, Tlr4 KO, Trem2	24-60	In progress	Breed animals	

KO, and C57BL/6.				
Subtask 2.2 Induce traumatic OA in 10 week old MRL/MpJ, C3H/HeJ, STZ and C57BL/6 mice. Mice will receive ^{14}C-thymidine; 336 mice used (4 genotypes x 12 mice per group x 7 time points).	24-60	In progress	Prepare cohorts of animals, transport to UCD for injury	336 animals will be injured, return animals to LLNL post injury
Subtask 2.3. Sample collection from 336 animals from Subtask 2.2	24-60	In progress	Dissect joints, extract DNA	
Subtask 2.4. AMS analysis to quantify ^{14}C-thymidine levels	24-60	In progress	^{14}C measurements by AMS	
Milestone 7: Complete Sample Collection for MRL, STR, STZ and B6 mouse strain				
Milestone 8: Complete AMS analysis				
Major Task 3. (Sub Aim 1C): Quantify mineral apposition in injured joints:	**19-33**			
Subtask 3.1. Breed necessary cohorts of MRL/MpJ, C3H/HeJ, Tlr4 KO, Trem2 KO, and C57BL/6 cohorts.	19-24	completed	Breed animals	
Subtask 3.2 Induce traumatic OA in 10 week old MRL/MpJ, STR/ort mice, Tlr4 KO, Trem2 KO, STZ and C57BL/6 mice. Mice with receive ^{45}Calcium. 192 mice will be used (4 genotypes x 12 mice per group x 4 time points).	22-30	completed	Prepare cohorts of animals, transport to UCD for injury	192 animals will be injured, return animals to LLNL post injury
Subtask 3.3. Sample collection from 192 animals from Subtask 3.2.	22-30	completed	Dissect joints, extract DNA	microCT (192 scans)
Subtask 3.4. LC analysis to quantify ^{45}Calcium- levels in injured and uninjured animals	25-33	completed	Measure ^{45}Ca by liquid scintillation	
Milestone 7: Complete Sample Collection for MRL, STR, STZ, Tlr4 KO and BL6 mouse strain				
Milestone 8: Complete microCT				
Milestone 9: Manuscript #7 describing injury induced cellular proliferation and osteophyte formation in Tlr4 KO is in preparation.				

What was accomplished under these goals?

Subtask 2.1. Breed necessary cohorts of mice. This fiscal year we have added 2 additional strains of mice, Tlr4 KO, Trem2 KO, based on results we generated from single cell sequencing data generated in year 2. These mice were purchased from Jackson Labs, were bred, injured and data has been collected on PTOA phenotypes. Tlr4 manuscript is in preparation.

Ongoing

Subtask 2.2/2.3/2.4

Ongoing

Subtask 3.1/3.2/3.3/3.4. In this subtask mice were bred, injured and bones and joints were examined by microCT for structural changes. We found that ^{45}Ca data was not an improvement above microCT, therefore we proceeded to complete the analysis by microCT only.

Completed

Specific Aim 2

Specific Aim 2.	Timeline	Status	Site 1 (LLNL)	Site 2 (UCD)
Major Task 4. (Sub Aim 2A): Characterize the injury-induced transcriptome and proteome in the articular cartilage.	**10-~~48~~(60)**			
Subtask 4.1. Breed necessary cohorts of MRL/MpJ. Trem2KO and C57BL/6 cohorts	10-60	in progress	Genotype and breed animals	
Subtask 4.2 Induce traumatic OA in 10 week old. 432 mice will be used (~~2~~ 3 genotypes x 24 mice per group x ~~9~~ 6 time points).	16-60	In progress	Prepare cohorts of animals, transport to UCD for injury	432 animals will be injured, ~~return animals to LLNL post injury~~ **remain at UCD**
Subtask 4.2A. Conduct scRNA-seq on articular chondrocytes of uninjured and injured C57Bl/6.	12-24	Completed	Prepare cohorts of animals, transport to UCD for injury, isolate single cell, conduct RNA-seq and computational analyses	Injure animals, return animals to LLNL post injury
Subtask 4.2C. Conduct scRNA-seq on articular chondrocytes of uninjured and injured MRL.	24-36	Completed	Prepare cohorts of animals, transport to UCD for injury, isolate single cell, conduct RNA-seq and computational analyses	Injure animals, return animals to LLNL post injury
Subtask 4.2B. Conduct scRNA-seq on immune cells of uninjured and injured C57Bl/6.	12-24	Completed	Prepare cohorts of animals, transport to UCD for injury, isolate single cell, conduct RNA-seq and computational analyses	Injure animals, return animals to LLNL post injury
Subtask 4.2D. Conduct scRNA-seq on immune cells of uninjured and injured MRL.	24-36	Completed	Prepare cohorts of animals, transport to UCD for injury, isolate single cell,	Injure animals, return animals to LLNL post injury

			conduct RNA-seq and computational analyses	
Subtask 4.3. Sample collection from 432 animals from Subtask 4.2.	16-36	Completed	extract proteins, isolate RNA	
Subtask 4.4. Histological analysis	36-60	In progress	~~Embed, section, visualize proteins~~	**Embed, section, visualize proteins**
Subtask 4.5. LC-MS/MS analysis	36-60	In progress	~~Protein identification~~	**Protein identification**
Milestone 10: Complete Chondrocyte-Specific RNA-seq analysis for C57Bl/6 (scRNA-seq now) **Manuscript #4:** Sebastian A, McCool JL, Hum NR, Murugesh DK, Wilson SP, Christiansen BA and Loots GG. Single-Cell RNA-Seq Reveals Transcriptomic Heterogeneity and Post-Traumatic Osteoarthritis-Associated Early Molecular Changes in Mouse Articular Chondrocytes. ***Cells*** 2021 June 10(6):1462. DOI: 10.3390/cells10061462. **Manuscript #5:** McCool JL, Hum NR, Sebastian A, Loots GG. Isolation of Murine Articular Chondrocytes for Single Cell RNA or Bulk RNA Sequencing Analysis. *Methods in Molecular Biology* book entitled "Cartilage Tissue Engineering" Editors Prof. Martin Stoddart, Dr. Angela Armiento, Dr. Elena Della Bella. Jan 2023.				
Milestone 11: Complete Chondrocyte-Specific Proteomic Analysis				
Milestone 12: Manuscript #8 describing injury-mediated chondrocyte specific protein and gene expression				
Major Task 5. (Sub Aim 2B): Characterize the injury-induced transcriptome and proteome in the immune system.	**13--60**			
Subtask 5.1. Breed necessary cohorts of strains	13--60	In progress	Genotype and breed animals	
Subtask 5.2 Induce traumatic OA in 10 week old MRL/MpJ. Trem2KO and C57BL/6 cohorts mice. 432 mice (~~2~~ **3** genotypes x 24 mice per group x ~~9~~ **6** time points).	19-60	In progress	Prepare cohorts of animals, transport to UCD for injury	432 animals will be injured, ~~return animals to LLNL post injury~~ **animals will remain at UCD**
Subtask 5.3. Sample collection from 432 animals from Subtask 4.2.	19-60	In progress	~~Extract proteins, isolate RNA~~	**Extract proteins, isolate RNA**
Subtask 5.4. Breed MRL/MpJ animals.	19-60	In progress	Breeding	
Subtask 5.5. Injure MRL/MpJ animals, 104 mice (12 mice x 9 time points)	19-60	In Progress	Transport cohorts to UCD for injury	104 animals will be injured, ~~return animals to LLNL post injury~~ **animals will remain at UCD**
Subtask 5.6. Sort macrophages and T-cells, neutrophils from joints, isolate proteins	21-60	In progress	~~FACs, protein extraction~~	**FACs, protein extraction**
Subtask 5.7. LC-MS/MS analysis	36-60	In progress	~~Protein~~	**Protein identification**

			~~identification~~	
Subtask 5.8. IHC validation (based on scRNA-seq and protein analysis)	31-60	In progress	~~Visualization of C57Bl/6 and MRL/MpJ protein expression~~	**Embed, section, Visualization of C57Bl/6 and MRL/MpJ protein expression**
Milestone 13: Complete Immune-Specific RNA-seq analysis (scRNA-seq now)				
Milestone 14: Complete Immune-Specific Proteomic Analysis				
Milestone 15: Manuscript #6 Complete Immune-Specific RNA-seq analysis for C57Bl/6 (scRNA-seq now)-describe all immune cells, in a time course, post injury Sebastian A, Hum NR, McCool JL, Wilson SP, Murugesh DK, Martin KA, Rios-Arce ND, Amiri B, Christiansen BA, **Loots GG.** Single-cell RNA-Seq reveals changes in immune landscape in post-traumatic osteoarthritis. ***Front Immunol***. 2022 Jul 29;13:938075. doi: 10.3389/fimmu.2022.938075. eCollection 2022. PMID: 35967299				
Milestone 16: Manuscript #9 describing injury-mediated immune specific protein and gene expression, highlighting MRL/MpJ specific proteins that may contribute to PTOA resistance is in preparation				
Milestone 18: Manuscript #10 describing injury-mediated immune specific protein and gene expression, highlighting Trem2KO specific proteins that may contribute to PTOA vulnerabilities in this strain is in preparation				

What was accomplished under these goals?

Subtask 4.1/2. Breed necessary cohorts is ongoing at LLNL.

In progress.

Subtask 4.2A-D. Conduct scRNA-seq on articular chondrocytes of uninjured and injured C57Bl/6 and MRL.

Completed, this work resulted in 1 published manuscript (#4) and 1 published methodology book chapter (#5)

Subtask 4.3/7. Animals are injured as planned, joints are digested, chondrocytes are purified, proteins are isolated, identified and quantified by LC-MS/MS. Samples are being stockpiled and will be examined in the upcoming year.

In progress.

Subtask 5.1/2. Continuing to breed necessary cohorts at LLNL, injure joints, and utilize FACS to sort out individual immune subpopulations from different strains (B6, MRL and Trem2 KO) based on cell surface markers identified by scRNA-sequencing. In particular we have focused on two types of immune cells, macrophages and neutrophils.

In progress.

Subtask 5.3. Conduct scRNA-seq on immune cells of uninjured and injured C57Bl/6 and MRL.

Completed, this work resulted in one published manuscript (#6) and one manuscript in preparation.

Subtask 5.4-5.11. Samples are being stockpiled and will be examined in the upcoming year. Special focus will be on macrophages and neutrophils, since those are the subpopulations that seem to differ the most, by RNA-seq between C57Bl/6 and MRL/MpJ.

In progress.

What opportunities for training and professional development has the project provided?

At UCD, three postdocs, Alireza Nasoori, Benjamin Osipov and Cesar Morfin are trained on this project. They are developing technical and managerial skills. Dr. Osipov is currently interviewing for faculty positions and his OMICs training will be valuable for his future research. We also had a graduate student from UC Merced, Ms. Jillian McCool who has been participating on this project, she has learned animal husbandry, preparing mice for injuries, carryout dissections, preparing tissues for histology and conducting immunohistochemistry on sectioned joints. Ms. McCool graduated and is now a postdoc at LLNL. All postdocs and graduate students are encouraged to attend the two premier Bone and Cartilage national meetings, ORS and ASBMR. Dr. Melanie Mendez, a postdoc who was trained on this grant at LLNL, presented a poster 'Tlr4 Deficiency Accelerates Post-Traumatic Osteoarthritis Development in Mice' at ASBMR 2022 in Austin, TX. She is involved in early investigator subcommittee for ASBMR. Dr Alireza Nasoori, submitted an abstract to ORS 2024 describing the osteoarthritis and bone phenotype of Trem2 KO mice.

How were the results disseminated to communities of interest?

Results were communicated through publications or presentations at conferences.

Publications

Mendez ME, Murugesh DK, Christiansen BA, Loots GG. Antibiotic Treatment Prior to Injury Abrogates the Detrimental Effects of LPS in STR/ort Mice Susceptible to Osteoarthritis Development. JBMR Plus. 2023 May 22;7(8):e10759. doi: 10.1002/jbm4.10759. eCollection 2023 Aug. PMID: 37614305

McCool JL, Hum NR, Sebastian A, Loots GG. Isolation of Murine Articular Chondrocytes for Single-Cell RNA or Bulk RNA Sequencing Analysis. Methods Mol Biol. 2023;2598:187-196. doi: 10.1007/978-1-0716-2839-3_14. PMID: 36355293

Conference presentations

2023 ORS Musculoskeletal Biology Workshop, Zermatt, Utah; 22-27 July 2023

Session: ***New insights into osteoarthritis pathogenesis from single-cell-omic technologies***. Chair: Gabriela Loots (University of California-Davis)
Invited talk: ***Single cell analysis identifies strain specific differences in immune populations associated with resistance to PTOA*** (presenter: Gabriela Loots)

McCool, JL, Sebastian, A, Hum, NR, Murugesh, DK, Wilson, SP, Amiri, B, Christiansen, BA, Loots, GG (2021). ***Characterizing Immune Cell Infiltration in the Murine Joint Microenvironment after Traumatic Knee Injury***. Oral Poster Presentation. American Society for Bone and Mineral Research, Sept 11-15th 2022 (presenter: Jillian McCool)

- ASBMR 2022 Annual Meeting Young Investigator Award Recipient: $1,000 honorarium
- University of California Merced GSA Travel Award

Mendez, M, Wilson, SP, Murugesh, DK, Hum, NR, Jbeily, EH, Sebastian, A, Christiansen, BA, Loots, GG (2021). ***Tlr4 Deficiency Accelerates Post-Traumatic Osteoarthritis Development in Mice.*** Poster Presentation. American Society for Bone and Mineral Research, Sept 11-15th 2022 (Presenter Melanie Mendez)

What do you plan to do during the next reporting period to accomplish the goals?

Main focus for the next period will be to complete **all remaining tasks, publish 3-4 manuscripts and seek follow up funding.**

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Our Sebastian et al. manuscript describing injury induced changes in chondrocytes has 40 citations in ~2.5 years.

Sebastian A, McCool JL, Hum N, Murugesh DK, Wilson SP, Christiansen BA and Loots GG. Single-Cell RNA-Seq Reveals Transcriptomic Heterogeneity and Post-Traumatic Osteoarthritis-Associated Early Molecular Changes in Mouse Articular Chondrocytes. ***Cells*** 2021 June 10(6):1462. DOI: 10.3390/cells10061462.

Sebastian et al. manuscript describing injury induced changes in immune cells has 13 citations in ~1.5 years.

And our Rios-Arce et al manuscript already has 8 citations.

What was the impact on other disciplines?

We anticipate that the single cell RNA-seq chondrocyte methods book chapter we published in *Methods in Molecular Biology* book entitled "Cartilage Tissue Engineering' will become a standard protocol for purifying chondrocytes from articular cartilage in many laboratories that plan to employ single cell RNA-seq for molecular profiling. We are already getting many inquiries about sharing our data and our protocols for cell isolation.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

The original PI of this grant, Dr. Loots moved institutions on 1/18/2023, as the funds had to remain at LLNL, a subcontract had to be established between LLNL and UCD to allow Dr. Loots to complete the tasks. This move significantly delayed productivity on this grant, and most tasks that are still pending will require additional time to complete.

Changes that had a significant impact on expenditures

Due to Dr. Loots terminating her employment at LLNL and moving to UCD, where she is now a faculty member, many individuals working on this project at LLNL moved onto different projects and Dr. Loots hired new staff at UCD who are now working on this project. This transition caused a 6+ month delay in productivity and slowed expenditure. The subcontract was established on 05/31/2023, and now the project has commenced at UCD.

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

None

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals.

None

Significant changes in use of biohazards and/or select agents

None

PRODUCTS:

Publications, conference papers, and presentations

Journal publications from this work:

Published Manuscripts acknowledging federal support by Grants PR180268/PR180268P1

Mendez ME, Murugesh DK, Christiansen BA, Loots GG. Antibiotic Treatment Prior to Injury Abrogates the Detrimental Effects of LPS in STR/ort Mice Susceptible to Osteoarthritis Development. JBMR Plus. 2023 May 22;7(8):e10759. doi: 10.1002/jbm4.10759. eCollection 2023 Aug. PMID: 37614305

Books or other non-periodical, one-time publications.

McCool JL, Hum NR, Sebastian A, Loots GG. Isolation of Murine Articular Chondrocytes for Single-Cell RNA or Bulk RNA Sequencing Analysis. Methods Mol Biol. 2023;2598:187-196. doi: 10.1007/978-1-0716-2839-3_14. PMID: 36355293

Other publications, conference papers, and presentations

2023 ORS Musculoskeletal Biology Workshop, Zermatt, Utah; 22-27 July 2023

Session: ***New insights into osteoarthritis pathogenesis from single-cell-omic technologies***. Chair: Gabriela Loots (University of California-Davis)
Invited talk: ***Single cell analysis identifies strain specific differences in immune populations associated with resistance to PTOA*** (presenter: Gabriela Loots)

McCool, JL, Sebastian, A, Hum, NR, Murugesh, DK, Wilson, SP, Amiri, B, Christiansen, BA, Loots, GG. ***Characterizing Immune Cell Infiltration in the Murine Joint Microenvironment after Traumatic Knee Injury***. Oral Poster Presentation. American Society for Bone and Mineral Research, Sept 11-15th 2022 – (presenter: Jillian McCool)

- ASBMR 2022 Annual Meeting Young Investigator Award Recipient: $1,000 honorarium
- University of California Merced GSA Travel Award

Mendez, M, Wilson, SP, Murugesh, DK, Hum, NR, Jbeily, EH, Sebastian, A, Christiansen, BA, Loots, GG. ***Tlr4 Deficiency Accelerates Post-Traumatic Osteoarthritis Development in Mice.*** Poster Presentation. American Society for Bone and Mineral Research, Sept 11-15th 2022 – (Presenter Melanie Mendez)

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

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Aimy Sebastian*
Project Role:	*Research Scientist*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-7822-7040*
Nearest person month worked:	*3*
Contribution to Project:	*Dr. Sebastian has been conducting statistical analyses, analyzing both bulk and single cell RNA-seq data. She leads all aspects of the project at LLNL, meeting with the UCD Sub PI weekly, and with all team members monthly.*
Funding Support:	*This grant*

Name:	*Amiri, Beheshta*
Project Role:	*Research Scientist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6*
Contribution to Project:	*Ms. Amiri has been working on histology generating IHC and histological joint data. She also help prepare samples for microCT and for RNA-sequencing, images joint sections and prepares images for figures and reports*
Funding Support:	*This grant*

Name:	*Wilson, Stephen*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-0125-1149*
Nearest person month worked:	*2.4*
Contribution to Project:	*Dr. Wilson has been conducting statistical analyses, analyzing both bulk and single cell RNAseq data with emphasis on immune cells, particularly neutrophils*
Funding Support:	*This grant*

Name:	*Deepa Murugesh*
Project Role:	*Animal Technician*

Researcher Identifier (e.g. ORCID ID):	*0000-0002-4232-8480*
Nearest person month worked:	*0.8*
Contribution to Project:	*Ms. Murugesh oversees the animal colony, she sets up breedings, genotypes the animals, prepares cohorts for experiments. Drives the animals to Partner PI laboratory for injury, oversees the health of the animals post injury, assists with harvesting tissue, histology, etc.*
Funding Support:	*This grant*

UCD Subcontract Personnel

Name:	*Gabriela G. Loots*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-9546-5561*
Nearest person month worked:	1
Contribution to Project:	*Dr. Loots oversees the project at UCD, she meets weekly with LLNL PI, Dr. Sebastian and team members. She also meets weekly with UCD postdocs to go over experimental designs, data analysis, and set out future directions. She drafts reports and dissipates data to the scientific community.*
Funding Support:	*This grant*

Name:	*Alireza Nasoori*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-8753-730X*
Nearest person month worked:	*4*
Contribution to Project:	*Dr. Nasoori has been focused on characterization of the Trem2 Kos, to characterize the PTOA phenotype and determine molecular mechanisms contributing to the enhanced PTOA development phenotype in these mice, relative to C57Bl/6 strain. He has been conducting histology.*
Funding Support:	*This grant*

Name:	*Cesar Morfin*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*0009-0005-6929-6975*
Nearest person month worked:	*4*
Contribution to Project:	*Dr. Morfin has been working on generating single cell RNAseq data and IHC validation.*

Funding Support:	*This grant and LLNL*

Name:	*Benjamin Osipov*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-9456-3311*
Nearest person month worked:	*1.75*
Contribution to Project:	*Dr. Osipov has been working on generating proteomics data, conducting microCT analysis and analyzing the data..*
Funding Support:	*This grant*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes, Dr. Loots is now a UCD faculty and has separated from her employment at LLNL. She transferred PI of this grant to Dr. Sebastian at LLNL, and she is now funded on this project as a subcontractor. She will devote 5% effort on this project for the remainder of this no cost extension.

What other organizations were involved as partners?

Dr. Loots is now at UC Davis, a subcontract has been created, and her effort is now conducted at UC Davis.

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Dr. Blaine Christiansen, the Partner PI will be submitting a copy of this report in parallel, his contribution to the partner PI project remains unchanged now that Dr. Loots has moved to UC Davis.

QUAD CHARTS:

N/A

APPENDICES:

N/A