

## Plan Overview

---

*A Data Management Plan created using DMPTool*

**Title:** IRF3 and macrophage polarization during experimental periodontitis

**Creator:** Tom Petro

**Affiliation:** University of Nebraska Medical Center (unmc.edu)

**Principal Investigator:** Thomas M. Petro

**Data Manager:** Thomas M. Petro

**Project Administrator:** Thomas M. Petro

**Funder:** National Institutes of Health (nih.gov)

**Funding opportunity number:** PA-20-195

**Template:** NIH-Default DMSP

### **Project abstract:**

Innate and adaptive immune responses control oral mucosal infections. Macrophages of innate responses first promote inflammation that destroys microbes and then suppresses inflammation that helps to restore tissue homeostasis. Periodontitis is an oral disease where inflammatory responses to bacteria such as *Porphyromonas gingivalis* persist and restoration of mucosal homeostasis fails. The reasons for persistent periodontitis are unknown. Inflammatory macrophage responses to microbes require activation of Interferon Regulatory Factor 3 (IRF3), which is expressed constitutively, activated by phosphorylations at carboxy serine/threonines, and regulated at serine 332 in mice by ERK MAP kinase, Pin1 isomerase, and IL-33. However, because of their robust expression of inflammatory cytokines, persistent inflammatory macrophages lead to diseases, such as periodontitis. The response of macrophages to *P. gingivalis* lipopolysaccharide (PgLPS) is an in vitro model of persistent inflammation of

macrophages that can garner a more thorough understanding of macrophage transition from inflammatory to resolving phenotypes. Moreover, the repeated injection of PgLPS into the oral mucosal gingiva of mice leads to gingival inflammation and alveolar bone loss in an in vivo model of periodontitis. We found that macrophages deficient in IRF3 produce decreased IL-6 and Nitric Oxide (NO) in response to microbial stimuli. IL-6 and NO are key markers of a macrophage phenotype designated M1, considered inflammatory and found to dominate in periodontitis tissue. In contrast, IRF3 deficient macrophages express anti-inflammatory and repairing M2 phenotype markers, CD206 and Arginase1, which are depressed in periodontitis tissue. The central hypothesis of this proposal is that IRF3 activity promotes M1 macrophages and represses M2 macrophage development during periodontitis. Therefore, in aim 1 we will establish that IRF3 is critical to M1/M2 phenotypes by using primary macrophages from wild-type or IRF3KO mice stimulated with M1 (PgLPS/IFN-g) or M2 (IL-4) conditions, using IRF3KO macrophages expressing wtIRF3 or IRF3 regulatory mutants prior to M1/M2 stimulations, using primary wt macrophages treated with IRF3 modulators prior to M1/M2 stimulations. In aim 2, we will repeatedly inject PgLPS into gingiva at the first maxillary molars of wild-type and IRF3KO mice and then evaluate maxillary bone loss using microCT and conduct scRNAseq of gingival mononuclear cells. This will confirm the macrophage phenotype and RNA expression patterns during experimental periodontitis in the presence or absence of IRF3. Our ultimate goal is to treat periodontitis with IRF3 modulators. The working hypothesis here is that decreased macrophage IRF3 activity will dampen M1 phenotypes, enhance M2 markers, and effectively diminish periodontitis. The proposed work will help to explain persistent inflammatory macrophage phenotypes during periodontitis and other inflammatory diseases.

**Start date:** 10-01-2023

**End date:** 09-30-2025

**Last modified:** 02-06-2023

**Copyright information:**

The above plan creator(s) have agreed that others may use as much of the text of this plan as they would like in their own plans, and customize it as necessary. You do not need to credit the creator(s) as the source of the language used, but using any of the plan's text does not imply that the creator(s) endorse, or have any relationship to, your project or proposal

## IRF3 and macrophage polarization during experimental periodontitis

### Data Type

---

**Types and amount of scientific data expected to be generated in the project: *Summarize the types and estimated amount of scientific data expected to be generated in the project.***

Describe data in general terms that address the type and amount/size of scientific data expected to be collected and used in the project (e.g., 256-channel EEG data and fMRI images from ~50 research participants). Descriptions may indicate the data modality (e.g., imaging, genomic, mobile, survey), level of aggregation (e.g., individual, aggregated, summarized), and/or the degree of data processing that has occurred (i.e., how raw or processed the data will be)

We propose to generate scRNAseq data from experimental periodontitis tissue. We will be processing 27 tissue samples over a two-year grant period. We will use the 10x Genomics pipeline, as well as Seurat and Monocle computational tools to process DNA sequence datasets and generate mice single-cell transcriptomes. Working with a mixture of cytosolic and nuclear transcripts, gene expression will be quantified as the sum of intronic and exonic reads per gene. Ultimately, we will use and compare the best statistical methods as they are developed (e.g., unsupervised clustering and dimensionality reduction methods) to group populations of mice mononuclear cells that exhibit similar gene expression profiles while taking account of the issues of sparse per-cell information due to technical dropout effects seen using single-cell technologies. Using the computational tools mentioned above will allow us to account for statistical issues such as batch effects and help address biological “noise” such as that introduced by cells of different types being in similar states. The Single Cell Genomics Core Facility at UNL has already demonstrated that clustering methods can be used to accurately group transcriptomes of known cell types. We will also generate microCT imaging data. Palates of experimental mice will be scanned using a high-resolution ICT system (Skyscan) in the lab of Dr. Dong Wang at UNMC College of Pharmacy. Three-dimensional reconstructions will be performed using the system-reconstruction software (NRecon; Skyscan), whereby the ABC CEJ length will be evaluated. We will also generate cytokine ELISA data, qRT-PCR data, and FACS analysis data from in vitro stimulated macrophage lineage cells. These data will be stored in tabular form (.CSV). We plan to collect about 100 gigabytes of tabular data.

**Scientific data that will be preserved and shared, and the rationale for doing so: *Describe which scientific data from the project will be preserved and shared and provide the rationale for this decision.***

All data produced in the course of the project will be preserved and shared. In this proposed project,

the cleaned, item-level spreadsheet data for all variables will be shared openly, along with example quantifications and transformations from initial raw data. Final files used to generate specific analyses to answer the Specific Aims and related results will also be shared. The rationale for sharing only cleaned data is to foster ease of data reuse.

Metadata, other relevant data, and associated documentation: Briefly list the metadata, other relevant data, and any associated documentation (e.g., study protocols and data collection instruments) that will be made accessible to facilitate interpretation of the scientific data.

To facilitate interpretation of the data, *data dictionary, metadata, documentation, statistical analysis plans, bench protocols, data collection instruments* will be created, shared, and associated with the relevant datasets.

## Related Tools, Software and/or Code

---

State whether specialized tools, software, and/or code are needed to access or manipulate shared scientific data, and if so, provide the name(s) of the needed tool(s) and software and specify how they can be accessed.

Imaging data, scRNAseq, ELISA, and qRTPCR data will be made available in csv format and will not require the use of specialized tools to be accessed or manipulated.

## Standards

---

**State what common data standards will be applied to the scientific data and associated metadata to enable interoperability of datasets and resources, and provide the name(s) of the data standards that will be applied and describe how these data standards will be applied to the scientific data generated by the research proposed in this project. If applicable, indicate that no consensus standards exist**

Data will be stored in common and open formats, such as csv for our qRTPCR, ELISA, and scRNAseq data. Information needed to make use of this data [*e.g., the meaning of variable names, codes, information about missing data, other metadata, etc.*] along with references to the sources of those standardized names and metadata items will be included wherever applicable.

## Data Preservation, Access, and Associated Timelines

---

**Repository where scientific data and metadata will be archived: Provide the name of the repository(ies) where scientific data and metadata arising from the project will be archived; see [Selecting a Data Repository](#))**

All datasets that can be shared will be deposited in FACEBASE utilized by NIDCR.

**How scientific data will be findable and identifiable: Describe how the scientific data will be findable and identifiable, i.e., via a persistent unique identifier or other standard indexing tools.**

FACEBASE provides searchable study-level metadata for dataset discovery. FACEBASE assigns DOIs as persistent identifiers, and has a robust preservation plan to ensure long-term access. Data will be discoverable online through standard web search of the study-level metadata as well as the persistent pointer from the DOI to the dataset.

**When and how long the scientific data will be made available: Describe when the scientific data will be made available to other users (i.e., no later than time of an associated publication or end of the performance period, whichever comes first) and for how long data will be available.**

All scientific data generated from this project will be made available as soon as possible, and no later than the time of publication or the end of the funding period, whichever comes first. The duration of preservation and sharing of the data will be a minimum of 10 years after the funding period.

## Access, Distribution, or Reuse Considerations

---

**Factors affecting subsequent access, distribution, or reuse of scientific data: NIH expects that in drafting Plans, researchers maximize the appropriate sharing of scientific data. Describe and justify any applicable factors or data use limitations affecting subsequent access, distribution, or reuse of scientific data related to informed consent, privacy and confidentiality protections, and any other considerations that may limit the extent of data sharing. See [Frequently Asked Questions](#) for examples of justifiable reasons for limiting sharing of data.**

There are no anticipated factors or limitations that will affect the access, distribution or reuse of the

scientific data generated by the proposal.

**Whether access to scientific data will be controlled: State whether access to the scientific data will be controlled (i.e., made available by a data repository only after approval).**

Controlled access will not be used. The data that is shared will be shared by unrestricted download.

**Protections for privacy, rights, and confidentiality of human research participants: If generating scientific data derived from humans, describe how the privacy, rights, and confidentiality of human research participants will be protected (e.g., through de-identification, Certificates of Confidentiality, and other protective measures).**

### **Oversight of Data Management and Sharing**

---

**Describe how compliance with this Plan will be monitored and managed, frequency of oversight, and by whom at your institution (e.g., titles, roles).**

Thomas M. Petro, PhD, ORCID: 0000-0002-6163-4854, will be responsible for the day-to-day oversight of lab/team data management activities and data sharing. Broader issues of DMS Plan compliance oversight and reporting will be handled by the PI and Co-I team as part of general Univ. of Nebraska Med Center stewardship, reporting, and compliance.

---