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

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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.

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IRF3 and macrophage polarization during experimental periodontitis

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 lCT 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.

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.

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

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.

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.

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

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.

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.

There are no anticipated factors or limitations that will affect the access, distribution or reuse of the scientific data generated by the proposal.

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

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.
