

Plan Overview

A Data Management Plan created using DMPTool

Title: The role of NIK in cytosolic DNA sensing pathways

Creator: Kislay Parvatiyar

Affiliation: Tulane University (tulane.edu)

Funder: National Institutes of Health (nih.gov)

Funding opportunity number: PA20-185

Template: NIH-Default DMSP

Project abstract:

The induction of type I interferon (IFN-I) cytokines plays an essential role in the innate immune response to virus infections. Detection of viral nucleic acids in the cytosol by pattern recognition receptors (PRRs) serves as an initial first step in activating the IFN-I response. Emerging studies have linked previously unknown roles for cytosolic DNA sensing PRRs in detecting aberrant DNA species of self-origin to trigger the onset of age-related diseases, neurological disorders, and autoinflammatory conditions. Thus, there is a critical need to delineate the mechanisms by which cytosolic DNA sensing PRRs control IFN-I activation to develop the next generation of therapeutics to combat viral infections and DNA damage driven inflammatory disease states. We have recently identified a novel cross-talk phenomenon between cytosolic DNA sensing PRRs that activate IFN-I and the non-canonical NF- κ B pathway. While the non-canonical NF- κ B pathway primarily governs lymphoid organogenesis and B-cell survival and maintenance in response to extracellular ligation of select members of the TNF receptor superfamily, our data unexpectedly revealed that intracellular ligation of cytosolic DNA sensing PRRs also resulted in non-canonical NF- κ B activation. Furthermore, non-canonical NF- κ B pathway activation amplified the IFN-I response during cytosolic DNA sensing PRR stimulation. Additional analysis revealed the central regulator of the non-canonical NF- κ B pathway, NIK was critical in enhancing cytosolic DNA dependent activation of IFN-I, but surprisingly, did not require its signaling partner, IKK α , nor downstream non-canonical NF- κ B signaling. Instead, we

found NIK associated with STING, an essential signaling adaptor required for IFN-I activation downstream of cytosolic DNA sensing PRRs, and facilitated STING dependent activation of IFN-I. The mechanisms by which NIK activates STING is incompletely defined while activation of NIK/non-canonical NF- κ B signaling in the cytosolic DNA sensing pathway is unknown. Regulatory factors that control NIK signaling during cytosolic DNA sensing also remain poorly understood. The central hypothesis of this proposal is that the non-canonical NF- κ B pathway regulator, NIK, is induced via a previously unknown mechanism during cytosolic DNA sensing and upon stabilization, exerts an additional layer of control on IFN-I activation in the cytosolic DNA sensing pathway by supporting STING signaling. We will test this hypothesis proposed in our specific aims: Aim 1) Determine how NIK is induced upon cytosolic DNA sensing. Aim 2) Examine the mechanisms by which NIK sustains STING signaling. Aim 3) Characterize how NIK is regulated in the cytosolic DNA sensing pathway. We anticipate our studies will broadly impact the innate immune and inflammation biology field and will shed light into developing new therapeutic strategies in combating DNA driven inflammatory disease states.

Start date: 10-01-2023

End date: 09-30-2028

Last modified: 03-03-2023

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

The role of NIK in cytosolic DNA sensing pathways

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)

In this proposed project, data will be generated via the following methods: cell culture, confocal microscopy, real-time quantitative polymerase chain reaction (PCR), luciferase reporter assays, ELISA, viral load quantification, and immunoblot analysis. This data will be collected from a minimum of 3 independent experiments, with each independent experiment consisting of 2-3 groups, control or wild-type (+/+), and knockout (-/-) from immortalized murine embryonic fibroblasts, L929 fibroblasts, or THP-1 monocytes. The total size of the data collected is projected to be 300 GB.

We expect to generate the following data file types and formats during this project: Carl Zeiss microscopic image file images (.CZI), and (.TIFF), densitometric quantification files (.TIFF), and Excel files (.XLSX).

Raw data files will be analyzed to generate TIFF files containing images of cells and immunoblots. Quantitative raw data will be statistically analyzed via Excel or Graph pad software.

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.*

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 the interpretation and reuse of the data, a README file and data dictionary will be generated and deposited into a repository along with all shared datasets. The README file will include method description, instrument settings, RRIDs of resources such as antibodies, model organisms, cell lines, plasmids, and other tools (e.g., software, databases, services), and Protocol DOIs issued from protocols.io. The data dictionary will define and describe all variables in the dataset.

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.

The raw data generated via the confocal microscope is in the Carl Zeiss (.czi) file format while quantitative immunoblot data will be determined via Fiji ImageJ. Zeiss software or Fiji ImageJ is required to access the raw data. The raw data generated via qPCR, ELISAs, reporter assays, image quantification is in the .XLSX format. Statistical programs such as Excel and Graph pad can be used to analyze the raw data.

Fiji ImageJ is open-source software that can be downloaded freely online. Links to this or other open-source viewers will be included with the documentation for the shared dataset.

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

In accordance with FAIR Principles for data, we will use open file formats (e.g. JPEG, MP4, CSV, TXT, PDF, HTML, etc.) and persistent unique identifiers (PIDs) such as RRIDs for resources (e.g., organisms, plasmids, antibodies, cell lines, software tools, and databases) and DOIs for protocols using protocols.io. The bioimaging community has not yet agreed on a single standard data format

that is generated by all acquisition systems, but we will use OME-Files for data that will be preserved and shared.

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](#))

Imaging data will be deposited into NCI's Imaging Data Commons. All other data described above in the "data to be shared" section will be deposited into Zenodo.

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.

Zenodo provides searchable study-level metadata for dataset discovery and 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.

We will use Persistent Unique Identifiers (PIDs) to improve data findability across all dissemination outputs. PIDs used will include ORCID iDs for people, DOIs for outputs (e.g., datasets, protocols), Research Resource Identifiers (RRIDs) for resources, and Research Organization Registry (ROR) IDs and funder IDs for places, as much as possible to make data identifiable and findable. We will also use indexed metadata, such as MeSH terms with a unique URL to make scientific data easily findable. We will keep our ORCID Records up to date with DOIs for our datasets and publications, ROR, and funder IDs to increase findability.

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).

Question not answered.

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).

Kislay Parvatiyar (Lead PI) 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 as part of general Tulane University's stewardship, reporting, and compliance processes.
