White Paper Application

Project Title: **Role of microbiome diversity, microbial transcriptome and host gene expression in the respiratory track of humans in influenza virus infection.**

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Primary Investigator Contact:

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1. Executive Summary *(Please limit to 500 words.)*

Provide an executive summary of the proposal.

Infection susceptibility and disease severity is regulated by a complex balance between the genotype of the pathogen, the genotype of the host, and environmental factors. While many studies are being conducted to evaluate the contribution of these three factors in many infectious diseases, another variable that until recently has been overlooked is the metagenome (or microbial community including viruses, bacteria and microeukaryotes which inhabit the human body). Many viruses, including influenza virus, infect through mucosal surfaces that are colonized with normal flora. Years of co-evolution of a virus with its host and its metagenome have likely selected for viruses that are able to infect a host in the presence of the most prevalent metagenome, so the characteristics of the metagenome are likely to make an impact in infection susceptibility and disease outcome. Moreover, the host innate and adaptive immune factors elicited in response to viral infection are likely to make a big impact in the normal (basal) flora, which may influence the susceptibility to pathogenic bacteria colonization. We are specifically interested in: i) the effect of seasonality in the diversity and expression profile of the metagenome, ii) the role of the respiratory tract metagenome in promoting or inhibiting infection by influenza viruses, and iii) how change

2. Justification

Gaps in knowledge:

Our present understanding of the nature and extent of the core or at least the set of common microbial members of the upper respiratory track (URT) of humans is limited. An effort to establish these basic components is ongoing as part of the Human Microbiome Project (HMP), a NIH Roadmap initiative. Furthermore, there we have little if any understanding of the influence acute respiratory infections, such as influenza virus, have on

the URT microbiome, nor do we understand the role the URT microbiome plays in host susceptibility to infection.

Information that will be obtained:

This study will fill some of these gaps by determining the baseline status of the human URT microbiome and changes that occur during the course of an influenza viral infection. In addition, we propose to obtain the full viral genome sequence as well as the host gene expression profile. In depth analysis of the changes in the viral genome and the host gene expression profiles during an acute influenza infection of the URT will complement the results obtained through the HMP. Additionall

d) Determine the local URT gene expression levels of the host throughout the course of infection.

Our long terms goals are to:

a) Determine whether the increase or decrease of specific microorganisms correlates with the modulation of disease severity.

b) Determine whether specific metagenomes increase susceptibility to influenza virus infection and weather they participate in promoting influenza virus seasonality.

In order to do this, we will:

a) Use procedures developed for human microbiome projects to assess the transcriptome of the metagenome in the URT of humans by RNAseq.

4a. Approach to Data Production: Data Generation

Study design

We will conduct this study in two phases to optimize the type and amount of data that will be generated from the human samples analyzed. In the first phase we will determine the methodological parameters needed to obtain basal levels of the microbiome and the host gene expression of 10 age matched uninfected control individuals enrolled in the study. In the second phase, we will use the information obtained during the first phase to optimize and implement a standard procedure to examine changes in the microbiome diversity and transcription status, determine the changes in the host gene expression levels and obtain the full viral genome of samples obtained from individuals throughout the course of influenza A virus infection.

Phase 1

To optimize the overall experimental design, we will perform base line experiments to define specific parameters that will influence the analysis we are proposing. Thus, we will:

a) Conduct comparative analysis between nasal washes and nasal swabs to determine if both sampling procedures provide the same bacterial community and host gene profile information;

b) Determine if the nasal sampling disrupts the normal nasal microbial community of subjects that are being sampled longitudinally at specific intervals (e.g. days 0, 1, 2, 3, 5, 7 and 28);

c) Define the basal levels of the human URT microbiome diversity, as well as identify any changes and the host gene expression levels in uninfected individuals overtime.

d) Establish the level of human RNA present in the samples that will be processed for RNAseq to see if it will be sufficient for reliab

MEDICAL_ASTHMA; MEDICAL_CHRONIC; MEDICAL_CONGESTIVE;

that will be of great interest to clinical and basic scientists in the field.

Project sites, collaborators and roles on the project:
This study will be performed in close collaboration between Mount Sinai School of Medicine, Pontificia Universidad Católica de Chile and the J. Craig Venter Institute. The project collaborators are as follows:

Adolfo García-Sastre, Ph.D. (PI), Mount Sinai School of Medicine: Dr. García-Sastre will be the project coordinator in the USA and will lead the study and serve as liaison between the investigators at the 3 different institutions involved in the study. He will also be responsible for coordinating follow up experiments during the different stages of the pilot study and to validate the results obtained.

Rafael A. Medina, Ph.D. (Co-PI), School of Medicine, Pontificia Universidad Católica de Chile: Dr. Medina will be in charge of collecting and processing the human samples in the laboratory under the approved IRB protocol. He will maintain the patient clinical metadata

PI: Ferres, Co-PI: Medina
This grant provides funds to incorporate Dr. Medina Silva as a new investigator in the field of virology and to conduct novel collaborative and interdisciplinary research at the School of Medicine at Pontificia Universidad Catolica de Chile. U$ 104,536 (2011 – 2013)

NIH-CEIRS program HHSN266200700010C**:** Center for Research on Influenza Pathogenesis (CRIP), an NIAID funded Center for Excellence in Influenza Research and Surveillance (CEIRS).
PI: García-Sastre
This center is dedicated to determine factors affecting the pathogenicity and innate immune responses of influenza viruses. U$ 6,091,103 (this year, total costs) (2007-2014)

6. Availability & Information of Strains:

1.

We will focus this study on samples obtained from pediatric individuals with confirmed influenza virus infection, such as the H1N1 pandemic virus or the H3N2 seasonal virus (determined by qRT-PCR diagnosis). We have 16 samples during 2011 including detailed metadata that already available for this study. In addition, we have begun to recruit new individuals during the 2012 influenza season and we are currently recruiting the samples from the control individuals. Thus, we antici

7. Compliance Requirements:

7a. Review NIAID's Reagent, Data & Software Release Policy:

NIAID supports rapid data and reagent release to the scientific community for all sequencing and genotyping projects funded by NIAID GSC. It is expected that projects will adhere to the data and reagent release policy described in the following web sites.

http://www3.niaid.nih.gov/LabsAndResources/resources/mscs/data.htm

http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-013.html

Once a white paper project is approved, NIAID GSC will develop with the collaborators a detailed data and reagent release plan to be reviewed and approved by NIAID.

Accept ☒ Decline ☐

7b. Public Access to Reagents, Data, Software and Other Materials:

All sequence data generated under this project will be released to GenBank after 45 days of pre-access. The influenza virus genomes will be assembled via JCVI's assembly pipeline, annotated and deposited in GenBank, the metagenomic sequencing data will be submitted to the short-read archive (SRA) database while the attached metadata will be submitted to dbGAP. Phase 1 and phase 2 data will be released independently.

7c. Research Compliance Requirements

Upon project approval, NIAID review of relevant IRB/IACUC documentation is required prior to commencement of work. Please contact the GSC Principal Investigator(s) to ensure necessary documentation are filed for / made available for timely start of the project.

Investigator Signature: