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14. ABSTRACT Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease that affects only a subset of those who smoke tobacco. Recent studies have documented that the "susceptible" smokers are more likely to show evidence of air trapping before any other pathologies of COPD manifest themselves. However, the underlying biology that predisposes the non-obstructed smokers with air trapping to develop COPD has not been elucidated. In COPD, there is a characteristic pattern of inflammation involving both innate and adaptive immunity with increased numbers of macrophages, neutrophils, and lymphocytes in the lungs. A similar pattern of airway inflammation has also been described in smokers without airflow obstruction. However, it is unclear whether the extent and nature of airway inflammation are different in the susceptible smokers (with air trapping) compared to the smokers who are not susceptible (without air trapping). Lung macrophages are known to play a key role in orchestrating chronic airway inflammation in COPD. They are essential immune effector cells that are pivotal in both airway innate and adaptive immune responses to inhaled pollutants including tobacco smoke. However, the study of human lung macrophages has been difficult due to their wide diversity and plasticity as well as their autofluorescence making their examination using the customary fluorescent antibody approaches such as flow cytometry challenging, particularly in those with history of smoking. The main objective of this proposal is to develop methodology and preliminary data that would lay the foundation for a longitudinal cohort study to examine the underlying biological processes, in particular lung myeloid cells characteristics, that may render the smokers with air trapping their susceptibility to develop COPD. This pilot project would then position us to examine our focused hypothesis that the susceptible smokers (those with air trapping) have an altered airway innate immune system with distinct lung macrophage phenotype and function, which predisposes them to develop COPD.					
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INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a heterogeneous disease that affects only a fraction of those who smoke tobacco. The origin of this variability in susceptibility to develop COPD is unclear, but understanding its underlying biology has important implications for our ability to design suitable preventative and therapeutic strategies for its management. This DOD discovery research proposed to develop methodologies and generate preliminary data needed to lay the foundation for a large study that would investigate the underlying biological susceptibility of those who smoke tobacco to develop COPD.

KEYWORDS

COPD; smoking; innate immunity; lung macrophages; bronchoalveolar lavage; spirometry; air trapping.

ACCOMPLISHMENTS

What were the major goals of the project?

The main objective of this proposal was to develop methodology and preliminary data that would lay the foundation for a longitudinal cohort study to examine the underlying biological processes, in particular lung myeloid cells characteristics, that may render the smokers with air trapping their susceptibility to develop COPD. This pilot project would then position us to examine our focused hypothesis that the susceptible smokers (those with air trapping) have an altered airway innate immune system with distinct lung macrophage phenotype and function, which predisposes them to develop COPD. We proposed to examine the above hypothesis through the following specific aims:

Specific Aim 1- Develop and validate additional methodology for molecular and functional phenotyping of lung myeloid cells in patients with history of heavy smoking. We previously developed a 44-marker time of flight mass cytometry (CyTOF) panel and flow cytometry-based phagocytosis and efferocytosis assays for characterization of lung macrophages from smokers. Here, we propose to develop an additional CyTOF panel to allow for more comprehensive single-cell proteomics examination of the diverse subpopulations of lung myeloid cells. Furthermore, we will develop CyTOF-based phagocytosis and efferocytosis assays to allow for functional evaluation of macrophage subpopulations identified by CyTOF. We will optimize these assays for use in both luminal (alveolar) and non-luminal (tissue) lung myeloid cells.

Specific Aim 2- Generate pilot molecular and functional phenotyping data of luminal (alveolar) macrophages from a cohort of non-obstructed smokers with and without air trapping. We will recruit and perform bronchoalveolar lavage (BAL) in a pilot cohort of smokers without COPD and perform molecular and functional phenotyping of their BAL alveolar macrophages (AM) using CyTOF (single-cell proteomics), transcriptomics (single-cell RNA sequencing), protease activity measures, and phagocytosis and efferocytosis assays. We will also perform detailed clinical and physiologic characterization of subjects and will then examine whether distinct AM signatures exist that show trend association with air trapping phenotype, or other clinical characteristics, in non-obstructed smokers.

What was accomplished under these goals?

We developed and optimized our time of flight mass cytometry (CyTOF) panel for molecular phenotyping of lung myeloid cells, and evaluated the immunophenotypic landscape of airway myeloid cells in health, smoking, and COPD. Bronchoalveolar lavage cells from never-smokers ($n = 8$) and smokers with ($n = 20$) and without ($n = 4$) spirometric COPD were examined using this 44-parameter time of flight mass cytometry panel. We used unsupervised cluster analysis to identify cellular subtypes that were confirmed by manual gating, and then used the available literature to identify major populations of myeloid and lymphoid cells in BAL using CD68 surface marker and then further annotated and identified 22 distinct phenotypic clusters, of which 18 were myeloid cells.

Our research so far has provided novel evidence for the impact of smoking and COPD on airway myeloid cells and demonstrated that smoking and COPD are associated with an overall pro-inflammatory immune “tone”, and more specifically with (1) an increase in “recruited” myeloid cells into airway lumen, (2) an increase in subtypes with decreased regulation of inflammation, and (3) an overall increase in expression of proinflammatory markers. Our data also suggest a decrease in regulatory proteins (programmed death-ligand

1 and 2 [PDL1, PDL2], and AXL tyrosine-protein kinase receptor [AXL]) in myeloid cells, which may be contributing to the chronic inflammation seen in COPD.

The findings were published in the American Journal of Respiratory Cell and Molecular Biology (Vasudevan *et al. Am J Respir Cell Mol Biol. Dec 2020*).

In addition, and in a collaborative effort with another investigator at UCSF (Arun Prakash, MD, PhD), we demonstrated that the lung myeloid cells immune tone could be modulated by the metabolites produced by gut microbiomes, or so-called “gut-lung immune axis”, identifying an additional method via which smoking may affect the myeloid cells phenotype in lungs. The findings were recently accepted for publication in the American Journal of Physiology- Lung Cellular and Molecular Physiology (Liu *et al. Am J Physiol Lung Cell Mol Physiol. April 2021; in press*).

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

This study expanded the field of COPD and myeloid immunobiology by establishing a framework to evaluate airway myeloid subpopulations for future studies involving disease progression and treatment response.

How were the results disseminated to communities of interest?

The findings were published in the American Journal of Respiratory Cell and Molecular Biology (Vasudevan *et al. Am J Respir Cell Mol Biol. 2020 Dec;63(6):780-793*), now available through PubMed Central at <https://pubmed.ncbi.nlm.nih.gov/32915645/>. The findings from collaboration with Dr. Arun Prakash were recently accepted for publication in the American Journal of Physiology- Lung Cellular and Molecular Physiology (Liu *et al. Am J Physiol Lung Cell Mol Physiol. April 2021; in press*).

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

We are continuing to develop additional mass cytometry methodologies proposed in the research plan, which should allow us to perform both phenotype and functional analysis of lung myeloid cells in health and COPD.

IMPACT

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

Our findings to date generate reference baseline data that will be used as reference values for future evaluation of lung immune cells in smoking-related lung diseases including the long-term goal of this research, which is to investigate the underlying biological susceptibility to develop COPD in smokers.

What was the impact on other disciplines?

Our collaborative effort has established additional evidence for the role of gut-lung immune axis in setting the diverse immune responses in lungs.

What was the impact on technology transfer?

Not applicable.

What was the impact on society beyond science and technology?

At this point, there is no direct impact on society; however, understanding the diversity of the lung myeloid cell population and how they may contribute to the lung response to inhalation of tobacco smoke has potential direct relevance to the lung response to inhalation of other pollutants such as secondhand tobacco smoke, general air pollution, and occupational inhalational exposure including military and combat-related exposures such as exposure to burn pit smoke, and thus the findings of this study will have direct public health as well as military personnel health relevance.

CHANGES AND PROBLEMS

Changes in approach and reasons for change

None.

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

The main obstacle for this research has been the safety and regulatory restrictions that have been applied to us through the UCSF and San Francisco VA Medical Center, which is the primary location of our research laboratory, as described below:

- A. To comply with governmental policies and public health guidelines concerning COVID-19 pandemic, beginning in March 2020, both UCSF and the SF VA placed holds on all research activities and restricted the presence of research staff in both medical centers. This affected not only the research activities themselves, but also the research administration and IRB and slowed down those processes due to the many restrictions that were placed in effect.
- B. Later in June 2020, UCSF began research activities in a phased format. In July 2020, UCSF allowed the start of clinical research procedures with high-risk for the spread of COVID-19 infection (aerosol generating procedures [AGP] such as lung function testing, exercise testing, and bronchoscopy) with certain requirements including the strict use of PPE, COVID-19 symptom screening, and COVID-19 testing of subjects. However, based on a national level mandate, the SF VA continued to have a ban on all clinical research due to public health concerns about a shortage of PPE and COVID-19 testing. As a result, the current research, which includes AGP including exercise testing remained on hold. During the COVID-19 disease peaks of post-Thanksgiving and Winter holidays, UCSF research groups again ceased all AGP research procedures due to concerns for public health and safety.
- C. Anticipating normalization of research activities as COVID-19 vaccines became available, we began generating and establishing procedures for the safe performance of the research plan. Because the SF VA's current policy is to not provide PPE and COVID-19 testing for clinical research, we modified our IRB research protocol and obtained permission from UCSF to obtain PPE and COVID-19 testing through UCSF resources. This process has been extremely bureaucratic and required tremendous amounts of effort, but we have now established IRB-approved and UCSF-sanctioned procedures to obtain PPE and COVID-19 testing for the purpose of this study.
- D. In November 2020, the SF VA agreed to allow limited (12.5% capacity) clinical research activity for all the projects of our research group on the VA campus. However, in December, due to concerns about high risk AGP of this research, public health matters, and safety of our staff and subjects, and in concordance with the UCSF research community, the AGP research procedures including bronchoscopy were again restricted for the duration of the COVID-19 disease peaks of post-Thanksgiving and Winter holidays.
- E. Currently, as we have passed the COVID-19 disease peaks of post-Thanksgiving and Winter holidays, the research activities have ramped up, and we are now in preparatory steps to restart of research activities at our center.

Changes that had a significant impact on expenditures

As the study progress was hindered due to the COVID-19 pandemic, a significant amount of the budget has remained unused. We are requesting a carryforward of funds as our goal is to push forward with the performance of the study, as the pandemic allows, and request an extension at the end of the current term of the study if the planned recruitment goals are not met by that time.

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

As described above, we have had to halt all human research activities due to COVID-19 pandemic, and invent and incorporate major safety protocols to ensure subject and staff safety as we have slowly returned to in-person clinical research and subjects' recruitment.

To ensure that we can continue with the human research experiments in this study that includes bronchoscopy in the setting of COVID-19 pandemic, we spent major efforts to generate and implement safety protocols, as described below.

- A. Prepare IRB application modifications of the COVID-19 safety guidelines and protocols and obtain UCSF IRB approval.
- B. Obtain SF VA Research and Development Subcommittees and Committee approvals.
- C. Replace the staff who left our group during the pandemic and retrain the new staff.

D. Obtain the required institutional (UCSF and SFVA) research logistics including appropriately sized lab space with adequate ventilation required for (1) safe return of staff and (2) safe conduct of aerosol generating research procedures (AGP).

E. Secure ability to perform and operationalize COVID-19 testing for asymptomatic research subjects within 96 hours prior to each research visit.

F. Secure personal protective equipment (PPE) and COVID-19 vaccination for research staff to be able to perform aerosol generating procedures (AGP) involved in this study.

Significant changes in use or care of human subjects

We now have to incorporate safety protocols to ensure subjects and staff safety in the setting of COVID-19 pandemic. These include symptoms screening and COVID-19 testing prior to each subject visit, extensive cleaning and sterilization of space and devices used for human subject experimentation, and protection of staff using appropriate PPE and vaccination.

Significant changes in use or care of vertebrate animals

Not applicable.

Significant changes in use of biohazards and/or select agents

Not applicable.

PRODUCTS

Publications, conference papers, and presentations

Please see below.

Journal publications

The findings were published in the American Journal of Respiratory Cell and Molecular Biology (Vasudevan et al. Am J Respir Cell Mol Biol. 2020 Dec;63(6):780-793), now available through PubMed Central at <https://pubmed.ncbi.nlm.nih.gov/32915645/>. The findings from collaboration with Dr. Arun Prakash were recently accepted for publication in the American Journal of Physiology- Lung Cellular and Molecular Physiology (Liu et al. Am J Physiol Lung Cell Mol Physiol. April 2021; in press).

Books or other non-periodical, one-time publications

Not applicable.

Other publications, conference papers, and presentations

Not applicable.

Website(s) or other Internet site(s)

Not applicable.

Technologies or techniques

Not applicable.

Inventions, patent applications, and/or licenses

Not applicable.

Other Products

Not applicable.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Mehrdad Arjomandi, MD
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Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0002-0116-9217
Nearest person month worked:	3
Contribution to Project:	Provided consultation about the interpretation of the high dimensional analysis of generated data.
Funding Support:	Department of Veterans Affairs, BLRD

Name:	Christine Hsieh, PhD
Project Role:	Co-investigator
Researcher Identifier (e.g. ORCID ID):	0000-0003-3620-784X
Nearest person month worked:	1
Contribution to Project:	Provided consultation about the interpretation of the high dimensional analysis of generated data.
Funding Support:	Department of Veterans Affairs, BLRD

Name:	Arun Prakash, MD, PhD
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	0000-0002-9720-8179
Nearest person month worked:	1
Contribution to Project:	Collaborated on examining the diversity of myeloid cell populations in lung contributed by the gut-lung immune axis.
Funding Support:	National Institute of Health, R01

Name:	Joshua Vasquez, MD
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	0000-0002-7165-5739
Nearest person month worked:	2

Contribution to Project:	Provided consultation about the design and optimization of the CyTOF panel and interpretation of the high dimensional analysis of generated data, and with presentation and publication of findings.
Funding Support:	National Institute of Health, K08

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

No.

What other organizations were involved as partners?

None other than the original institutions of UCSF and San Francisco VA Medical Center.

SPECIAL REPORTING REQUIREMENTS

Not applicable.

APPENDICES

None.