Oral Inflammatory and Immune Responses During Early SARS-CoV-2 Infection

2022 Research Aid Awards (RAA)

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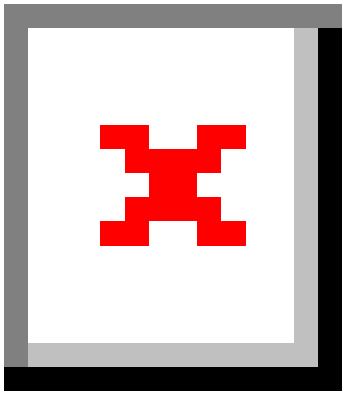
FollowUp Form

Award Information



In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

- *Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- *Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Title of Project:*

Oral Inflammatory and Immune Responses During Early SARS-CoV-2 Infection

Award Type

Research Aid Award (RAA)

Period of AAOF Support
July 1, 2022 through June 30, 2023

Institution
University of North Carolina at Chapel Hill- Adams School of Dentistry

Names of principal advisor(s) / mentor(s), co-investigator(s) and consultant(s)
1) Laura Jacox DMD, PhD, MSc, Assistant Professor, Committee Mentor, and Co-investigator 2) Shannon Wallet PhD, Full Professor, Committee Member, and Co-investigator 3) Natalie Bowmanc, MD, MPH, Assistant Professor, Committee Member, and Collaborator

Amount of Funding
$5,000.00

Abstract
(add specific directions for each type here)

Abstract and Specific Aims:

SARS-CoV-2 is transmitted primarily through aerosol and respiratory droplets, and oral mucosa and salivary glands are a site of early SARS-CoV-2 viral replication and transmission.1-3 Infected individuals can exhibit high viral titers in the oral cavity, irrespective of symptoms, and casual social encounters can generate droplets capable of transmission.4 Dental professionals, particularly orthodontists, are at increased risk for point-of-care transmission due to oral proximity, generation of aerosols, and high volumes of young unvaccinated patients.5 It is clear that children and youth, with high contact rates and poor adherence to hygiene and distancing protocols, can acquire and spread COVID-19, and resultant clusters and outbreaks can be large.6,7 Global trends point to waning vaccine immunity with a steady decline in antibody levels, and the incidence of breakthrough infections is rising.8,9 Given the high transmission potential and widespread distribution of the COVID-19 pandemic, the need to better understand the disease process remains an urgent public health need.

Local and systemic immune responses have the potential to protect against COVID-19 or, alternatively, their dysregulation can accelerate disease progression. COVID pathogenesis can take a mild course with few or no symptoms, resembling other upper respiratory diseases, with outpatient recovery in two weeks.10 In others, disease progresses to a more severe state requiring hospitalization and can lead to acute respiratory distress syndrome and even death.10 The presentation, duration, and severity of symptoms is due to the type and magnitude of the immune response to SARS-CoV-2 infection.11 A growing body of research has implicated a systemic hyper-inflammatory response as a major source of COVID-19 associated disease and death.12 Yet, there is a lack of understanding about early defense mechanisms, particularly the localized salivary immune response. The oral cavity and upper respiratory tract are the primary site of SARS-CoV-2 initial exposure, and early defenses in these areas serve as barriers, minimizing viral replication and may predict or regulate succeeding immune cascades.13,14 It remains unclear how salivary immune mechanisms relate to patient clinical outcomes and are influenced by host demographics and comorbidities.

Elucidating the profile of inflammatory biomarkers and antibody titers in early infection and understanding immune dysregulation in patients with mild versus severe COVID-19 will provide insights for prognostic and therapeutic applications. Characterizing salivary SARS-CoV-2 antibodies present in early disease (e.g. IgA, a known early defense against respiratory pathogens) could aid development and deployment of vaccines against COVID-19 and other related coronavirus diseases.15 Salivary antibody detection may also aid in assessment of individual immune response, indicating levels of vaccine protection or risk for severe infection.15,16 Illuminating site-specific cytokine signatures enables development of clinical therapies and informs treatment timing. For instance, identifying the optimal window for type 1 interferon (IFN) therapy, an immunomodulatory agent used to restrict viral replication and subsequent spread.17

Our proposed study will characterize the immunological landscape of the oral cavity during early SARS-CoV-2 infection. Salivary analysis represents a safe, feasible way to perform proteomic and genomic diagnostic assays to understand SARS-CoV-2 response in the mouth. We will use de-identified saliva, collected from 120 SARS-CoV-2+ patients within 10 days of infection. These samples had their viral load determined by quantitative PCR, and we now aim to take a targeted and unbiased approach to generate a profile of the antibody and inflammatory responses in the oral cavity during the early course of disease. Understanding the SARS-CoV-2 response in the oral cavity is a logical first step to bolster our practice management in an orthodontic environment with a largely pediatric population.

SA1: Characterize salivary inflammatory (SA1.1) and antibody (SA1.2) responses associated with early SARS-CoV-2 infection. While there are a myriad of reports on the effect of SARS-CoV-2 infection on the peripheral immune and antibody responses, there is a lack of investigations of the salivary compartment. Saliva from 120 acute SARS-CoV-2+ patients will be used for the evaluation of patient-specific immune responses including soluble mediators (cytokines, chemokines) (SA1.1) and salivary antibodies (IgA, IgM & IgG) (SA1.2). We will use a similar multiparameter technology to measure over 40 soluble mediators along with a novel assay to determine spike antibody titers (IgG, IgM & IgA in salivary samples). We will correlate our quantitative findings with patient demographics, including age, race, comorbidities, symptomatology, severity of disease progression (outpatient versus inpatient), and vaccination status. We hypothesize that in mild disease (e.g. outpatients), innate immune responses in the mouth promote a timely mucosal antibody response resulting in effective viral clearance and appropriate immune response. However, in severe disease (e.g. inpatients), the innate immune response does not efficiently control early viral replication and does not stimulate an effective antibody response, leading to uncontrolled viral replication, tissue damage and subsequent immune overreaction. Understanding innate and adaptive defense mechanisms in the oral cavity during the early course of SARS-CoV-2 infection holds the potential to identify new diagnostic, prognostic and therapeutic biomarkers.

Respond to the following questions:

Detailed results and inferences:*

If the work has been published, please attach a pdf of manuscript below by clicking "Upload a file".

OR

Use the text box below to describe in detail the results of your study. The intent is to share the knowledge you have generated with the AAOF and orthodontic community specifically and other who may benefit from your study. Table, Figures, Statistical Analysis, and interpretation of results should also be attached by clicking "Upload a file".

Babikow, Erika Final Report 2022 RAA.pdf

The oral cavity is an important site of initial SARS-CoV-2 infection and transmission, with the oral immune response having the potential to protect against or exacerbate COVID-19 disease progression. Characterizing salivary inflammatory and antibody responses associated with early SARS-CoV-2 infection has the potential to inform development of targeted diagnostic and therapeutic interventions for COVID-19. These applications

are significant to orthodontic practices experiencing increased risk of point-of care transmission due to oral proximity, generation of aerosols, and treatment of large patient pools under 12 years of age. Our proposed study aimed to characterize the immunological landscape of the oral cavity during early SARS-CoV-2 infection, investigating the inflammatory response during the early disease course.

We used de-identified saliva, collected from 175 SARS-CoV-2+ subjects within 10 days of infection. We enrolled 40 healthy controls matched for sex and age distribution. All subjects were between the ages of 18-65 and in stable physical health. Unstimulated saliva samples were loaded to a commercially available, Luminex bead-based assay kit which tested for 37 known inflammatory biomarkers. Mediator concentration was obtained based on known standards. A linear regression model was used to compare cytokine concentration between the COVID+ and control groups, as well as look at multiple host factors within the COVID+ group, including days post-symptom onset, days post-positive test, age, gender, and vaccination status.

At the start of this project, we anticipated salivary cytokines would be increased in our COVID+ subjects. Multiple groups have demonstrated a consistent trend of increased cytokines in serum samples of COVID+ subjects (IL-6, IL-10, and TNFa). Likewise, groups investigating saliva samples of COVID+ hospitalized patients also saw an increase in cytokine concentration (IL-6, IL-17 and TNFa), in their inpatient subjects who had been sick for longer periods on average. Interestingly, we found the opposite in our acutely infected, outpatient sample. Cytokines in our saliva samples appear to be largely decreased in COVID+ subjects early in the disease course.

We found 11 of 37 inflammatory biomarkers to be significantly different between COVID+ and control groups. 9 of the 11 significant mediators showed a decreased concentration in COVID+ subjects. Only 2 of the 11 significant mediators, MMP3 and CD30, were increased. This decrease in inflammatory mediators suggests that SARS-CoV-2 is capable of suppressing the early salivary innate immune response. IL-2 and IL-12 play essential roles in T-cell activation and were decreased in COVID+ subjects. In contrast, MMP3, an enzyme involved in tissue remodeling and implicated in COVID-19 associated lung inflammation, was increased.

Our findings demonstrate that SARS-CoV-2 alters the composition of the salivary immune barrier and appears to suppress the early innate immune response in the oral cavity. This immune suppression would presumably lead to enhanced viral proliferation and, ultimately, increased transmission.

Were the original, specific aims of the proposal realized?*

Objective: Our proposed study will characterize the immunological landscape of the oral cavity during early SARS-CoV-2 infection.
Aim 1: Characterize salivary inflammatory (SA1.1) and antibody (SA1.2) responses associated with early SARS-CoV-2 infection.

The original specific aims have been realized. We successfully characterized the inflammatory response (SA 1.1). Salivary antibody assays have been completed, as well, but not yet statistically analyzed (SA1.2). We are actively working on analysis of our antibody results, and are preparing a resulting manuscript from our cytokine and antibody data.

Were the results published?*

No

Have the results of this proposal been presented?*
Yes

To what extent have you used, or how do you intend to use, AAOF funding to further your career?*
I am grateful for the AAOF's support of orthodontic resident research. This project was initiated early in the COVID-19 pandemic, when it was recognized SARS-CoV-2 virus was present in saliva but very little was known about its impact on the immunological landscape. Funding from the AAOF provided meaningful support in the execution of this project. Once published, this study will provide insights to dental and medical professionals about the role SARS-CoV-2 plays in the oral cavity. Working with the AAOF has been a wonderful experience, and I hope to contribute to the AAOF as I move into my professional life as an orthodontist.

Accounting: Were there any leftover funds?
$0.00

Not Published

Are there plans to publish? If not, why not?*
The results have not yet been published. Final manuscript preparation is underway and AAOF support will be acknowledged.

Presented

Please list titles, author or co-authors of these presentation/s, year and locations:*
This work was presented by Dr. Erika Babikow for her master's thesis defense on March 9, 2023 to the University of North Carolina, Chapel Hill Division of Craniofacial and Surgical Care. Dr. Babikow also delivered an oral presentation to the entire UNC Adams School of Dentistry for UNC's Dental Research in Review Day held on March 1, 2023. AAOF support was mentioned during both of these presentations.

In addition, her research was accepted for poster presentation at the 2023 AAO Annual Meeting in both the William R. Proffit Resident Scholar Award Program and Table Clinic Session, where AAOF support was also acknowledged.
Title: Oral Inflammatory Cytokine Response During Early SARS-CoV-2 Infection
Co-Authors: Christina Graves PhD, Marcelo Freire DDS, PhD, DMedSc, Di Wu PhD, and Laura Jacox DDS, MS, PhD

Was AAOF support acknowledged?

If so, please describe:

AAOF support was acknowledged in written format as well as recognized verbally in all presentations.

Internal Review

Reviewer comments

Reviewer Status*

Approved

File Attachment Summary

Applicant File Uploads

- Babikow, Erika Final Report 2022 RAA.pdf

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AAO Foundation Final Report Form
(a/o 6/30/2023)

Please prepare a report that addresses the following:

Type of Award: Research Aid Award

Name(s) of Principal Investigator(s): Erika Babikow, DMD, MS; Laura Jacox, DMD, PhD, MS; Shannon Wallet, PhD; Natalie Bowman, MD, MPH

Institution: University of North Carolina, Chapel Hill

Title of Project: Oral Inflammatory and Immune Responses During Early SARS-CoV-2 Infection

Period of AAOF Support: 07-01-22 to 6-30-23

Amount of Funding: $5,000.00

Summary/Abstract:

SARS-CoV-2 is transmitted through respiratory droplets and orthodontists are at risk of point-of-care transmission due to oral proximity and high patient volumes. The oral cavity is a site of SARS-CoV-2 exposure, with immune defenses serving as barriers to minimize viral replication. Yet, an understanding is lacking of how oral innate defense mechanisms are impacted. We collected saliva from N=277 SARS-CoV-2+ patients and 40 uninfected controls and used Luminex xMAP technology to detect 37 known inflammatory biomarkers. Our results identified multiple cytokines whose concentration significantly differs between COVID+ and control subjects. Identifying cytokine signatures has the potential to reveal diagnostic biomarkers for COVID-19.

Results and Inferences:

The oral cavity is an important site of initial SARS-CoV-2 infection and transmission, with the oral immune response having the potential to protect against or exacerbate COVID-19 disease progression. Characterizing salivary inflammatory and antibody responses associated with early SARS-CoV-2 infection has the potential to inform development of targeted diagnostic and therapeutic interventions for COVID-19. These applications are significant to orthodontic practices experiencing increased risk of point-of care transmission due to oral proximity, generation of aerosols, and treatment of large patient pools under 12 years of age. Our proposed study aimed to characterize the immunological landscape of the oral cavity during early SARS-CoV-2 infection, investigating the inflammatory response during the early disease course.

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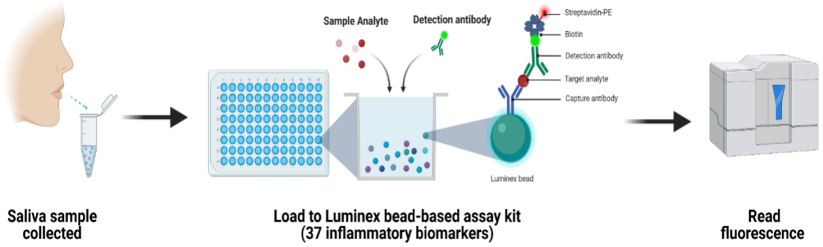

Figure 1: Study Summary. Overview of study design illustrating the sample collection and assay technique.

Table 1: Subject Demographic Information

COVID+ SUBJECTS	**N=277**
Sex	
Female	182 (66%)
Male	95 (34%)
Age	
18-29	125 (45%)
30-49	90 (32.5%)
50-65	62 (22.5%)
Days Since Positive Test	5 +/- 2.2
Days Since Symptom Onset	7 +/- 2.9
Vaccination Status	
No	75 (27%)
Yes	202 (73%)

CONTROL SUBJECTS	**N=40**
Sex	
Female	26 (65%)
Male	14 (35%)
Age	
18-29	20 (50%)
30-49	12 (30%)
50-65	8 (20%)
Days Since Positive Test	N/A
Days Since Symptom Onset	N/A
Vaccination Status	
No	0 (0%)
Yes	40 (100%)

Figure 3: Comparison of COVID+ and Control Biomarker Concentration. The graphs below display the eleven biomarkers significantly different between the COVID+ and control groups. The black dots represent individual subjects, the red line represents sample means, the gray dotted line represents the limit of detection.