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BRIEF REPORT

Society on NeuroImmune Pharmacology COVID-19 Virtual Workshop

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Abstract
This brief report collects the program and abstracts of the Society on NeuroImmune Pharmacology (SNIP) COVID-19 Virtual Workshop held on April 9, 2021. The workshop consisted of four symposia: Symposium 1: Molecular approaches to COVID-19 pathogenesis and underlying mechanisms; Symposium 2: Therapeutic and vaccine approaches to COVID-19; Symposium 3: Early Career Investigator talks; and Symposium 4: Diversity and Inclusion SNIP Committee (DISC) program: Well-being and reflections. The workshop also featured four special talks on COVID-19 and funding opportunities from the National Institute on Alcohol Abuse and Alcoholism (NIAAA); COVID-19 and funding opportunities from the National Institute on Drug Abuse (NIDA); opportunities from NIH for early career investigator (ECI) fellows; and neurologic and psychiatric complications of SARS-CoV-2 infection. Presenters included NIH officials, SNIP members, and non-member scientists whose abstracts were submitted and accepted for inclusion in the virtual event hosted by the University of Nebraska Medical Center via Zoom webinar. A special theme issue of SNIP's official journal, the Journal of Neuroimmune Pharmacology (JNIP), will collect select papers from the workshop along with other related manuscripts in a special theme issue titled "Neuroimmune Pharmacology of SARS-CoV-2."

Keywords SARS-CoV-2 · COVID-19 · Neuroimmune pharmacology · Therapeutics · Vaccines · Early career investigators · Diversity and Inclusion · Well-being

Summary

The Society on NeuroImmune Pharmacology (SNIP) organized a virtual Workshop on COVID-19 (SNIP-COVID-19 Virtual Workshop) on April 9, 2021. This special Workshop on COVID-19, in part, maintained the consistency of SNIP activity since the 26th SNIP International annual meeting has been postponed to 2022. This Workshop recognized the important contributions of SNIP members who study COVID-19, with regards to basic and clinical sciences as well as therapeutic and vaccine development.

The Workshop was opened with a warm welcome and greetings from Dr. Sulie L. Chang, the SNIP President during COVID-19 pandemic. She also briefly shared her engagement in meta-analysis studies using bioinformatics tools during the pandemic. This Workshop consisted of four special talks and four symposia. The four symposia covered a wealth of COVID-19 science, which not only comprised basic/clinical sciences and therapeutic/vaccine development, but also included the well-being of researchers. The four special talks provided by the National Institute

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of Health (NIH) officials were about the NIH priorities and funding opportunities on COVID- 19 from their respective institutions.

The first special talk was delivered by Dr. Changhai Cui on COVID-19 and funding opportunities from the National Institute of Alcohol Abuse and Alcoholism (NIAAA). She also discussed about how drinking could suppress immune response leading to increased probability of SARS-CoV-2 infection and isolation during the pandemic could increase the incidence of drinking behavior. The second special talk was provided by Dr. Yu (Woody) Lin on COVID-19 and funding opportunities from NIDA. He also mentioned about the role of drug abuse in immune suppression, which could increase the probability of SARS-CoV-2 infection and COVID-19 severity. The pandemic also increased the prevalence of drug abuse and compromised addiction cessation. The third special talk was given by Dr. John Satterlee, Special Assistant to the DNB Director, DNB, NIDA on "Opportunities from NIH for early career investigator (ECI) fellows." This was very helpful to our ECI fellows in navigating and finding an appropriate funding opportunity at NIH, which could further their career. The last, but not the least, special talk was delivered by Dr. Jeymohan Joseph, Chief, HNGTB, DAR, NIMH, Bethesda, MD, USA, delivered a special talk on "Neurologic and psychiatric complications of SARS-CoV-2 infection." Since there are reports on the role of COVID-19 on neuronal functions, COVID-19-induced neurological and psychological complications could be of important for future research.

The first symposium was on molecular approaches to COVID-19 pathogenesis and underlying mechanisms. Dr. Rosemarie Booze presented on "Post-acute SARS-CoV-2 infection (PASC) in non-human primate olfactory system and pyriform cortex." The findings from her group indicate that SARS-CoV-2 may persist in the brain and contribute to the neurovascular post-acute sequelae of SARS-CoV-2 infection (PASC). Dr. Theodore Cory presented on "Cellular senescence alters fibrogenesis in SARS-CoV-2 infected macrophage/fibroblast co-cultures." His findings suggest that cellular senescence induces fibrogenesis in SARS-CoV-2 infected macrophages/fibroblast co-culture, which needs to be investigated further to understand their molecular mechanism. Dr. Navneet Dhillon presented on "Assessment of circulating extracellular vesicles reveals pro-inflammatory and thrombosis related protein cargo in COVID-19 patients." Her findings indicated alterations in pro-inflammatory, coagulopathy, and endothelial injury protein cargo in large EVs in response to SARS-CoV-2 infection that may be a causative agent in severe illness. Dr. Howard Gendelman presented on SARS-CoV-2 induction of a pro-inflammatory human macrophage. The findings from his group suggest that SARS-CoV-2-macrophage interactions induce pro-inflammatory and IFN responses that explain the pathogenesis of virus-induced acute respiratory distress syndrome and perhaps other end organ COVID-19 disease. Dr. Supriya Mahajan presented on "Mitochondrial dynamics in SARS-CoV-2 spike protein treated human microglia: Implications for Neuro-COVID." The data obtained by her group provides important mechanistic insights into SARS-COV-2 induced mitochondrial dysfunction, which underlies neuropathology associated with Neuro-COVID-19. Dr. Servio Ramirez presented on the "SARS-CoV-2 spike protein triggers brain endothelial activation and negatively affects the function of the blood-brain barrier; implications for neuroinvasive mechanisms of emerging coronaviruses." The results presented by him may help provide an explanation to why neurological symptoms are commonly presented during COVID-19 infection and open the door to the possibility that breach to the BBB could aid in SARS-CoV-2 neuroinvasion. Overall, this symposium provided a variety of observations and different aspects of neuroimmune mechanism in SARS-CoV-2 infected cells/organs using both in vitro and in vivo models.

The second symposium presented therapeutic and vaccine approaches to COVID-19. Dr. Siddappa Byrareddy presented on "Development of targeted therapeutics for COVID-19." His study suggests that entry inhibitor and SF2523 alone or in combination with remdesivir could be a novel and efficient therapeutic strategy to block SARS-CoV-2 infection and could hence be beneficial in preventing severe COVID-19 disease evolution. Dr. Wen-Zhe Ho presented on "Epigallocatechin gallate from green tea effectively blocks infection of SARS-CoV-2 and new variants by inhibiting spike binding to ACE2 receptor." The data obtained from his group showed that green tea constituent epigallocatechin gallate (EGCG) blocks the SARS-CoV-2 infection at entry level through interfering the engagement of the receptor binding domain of the viral spike to angiotensin-converting enzyme 2 receptor of the host cells. Thus, further clinical evaluation and development of EGCG as a novel, safe, cost-effective, and natural product for prevention/treatment of SARS-CoV-2 transmission and infection is needed. Dr. Rafal Kaminski presented on "Targeting N-glycosylation and palmitoylation pathways to block coronavirus infectivity." His group showed that CRISPR-mediated specific disruption of α-glucosidase-1 (GC-1/MOGS) gene leads to a significant drop of infectivity of hCoV-OC43 progeny virions. They further performed a CRISPR-Cas9-based gene knockout screen of ten ZDHHC acyltransferases, enzymes responsible for protein palmitoylation, which reduced the viral infection. Their data provided a base for developing new, host-directed, and potentially pan-viral therapeutic strategies targeting current and future emerging coronaviruses and other glycosylation/palmitoylation-dependent enveloped viruses. Dr. Bhavesh Kevadiya presented on "Development of a layer-by-layer SARS-CoV-2 microparticle vaccine." His group has developed a

vaccine approach that deploys multiple distinct viral antigens as immunogens accomplished by slow-release layer-by-layer microparticles (LBL MPs). This approach is likely to deliver "whole" inactivated SARS-CoV-2 to elicit broad antigen exposures as well as sustained and effective antiviral immune response. Dr. Jag Khalsa, Dr. Sanjay Maggirwar, and Dr. Gregory Bunt presented on "COVID-19, cannabis/cannabidiol (CBD), and the physician." Together they discussed whether there is sufficient clinical evidence to support the use of cannabis/cannabinoids for treating COVID-19 related health effects. They also discussed the role of addiction physicians in dealing with patients with substance use disorders requesting cannabis or CBD prescription for any of the COVID-19 health effects. Dr. Kalipada Pahan presented on "AIDS for COVID-19!" His group has engineered a hexapeptide corresponding to the ACE2-Interacting Domain of SARSCoV-2 (AIDS) that inhibits the association between receptor-binding domain-containing spike S1 and ACE-2. The findings suggest that selective targeting of ACE2-to-SARS-CoV-2 interaction by wtAIDS may be beneficial for COVID-19.

The third symposium was SNIP's unique provision to the early career investigators for their presentations. Dr. Arpan Acharya presented on "HIV-1/SARS-CoV-2 coinfection and CNS pathogenesis." His preliminary data demonstrated that the glial cells in CNS only support a low level of SARS-CoV-2 replication in the presence or absence of HIV-1. A pre-doctoral student, Liana Basova, presented on "Systems biology approaches detect early neuroinflammation and calcium signaling signatures in SARS-Cov-2 infection which are not affected by H1N1 in tracheal biopsies from the ferret model." She utilized a system biology approach and facilitated the identification of gene networks affected both by SARS-Cov-2 and H1N1. Her study found significant changes in genes involved in calcium signaling and in neuroinflammation. Another pre-doctoral student, Michael Mora Stangis, presented on "Methamphetamine potentiates the S1 subunit of SARS-CoV-2 spike protein-induced downregulation of tight junction proteins of human brain endothelial cells." His preliminary data showed an increased effect on tight junction protein expression of the blood-brain barrier following exposure to both methamphetamine and the S1 subunit of the SARS-CoV-2 spike protein compared with individual treatments. His data suggests an increased blood brain barrier permeability in individuals exposing themselves to multiple harmful situations, which may in turn lead to additional comorbidities alongside the onset of the SARS-CoV-2 infection. Dr. Silvia Torices presented on "Expression of SARS-CoV-2-related receptors in naïve and HIV-1-infected cells of the neurovascular unit." She analyzed the expression pattern of the main SARS-CoV-2 receptors in naïve and HIV-1-infected cells of the neurovascular units (NVU). She showed that the receptors involved in SARS-CoV-2 infection are coexpressed in the cells of the NVU, especially in astrocytes and microglial cells.

The fourth symposium was our DISC symposium. It was to highlight SNIP's focus on diversity and inclusion within our Society to address our personal and professional well-being during COVID-19 pandemic. Dr. Thirumala-Devi Kanneganti presented on "Targeting innate immunity and inflammatory cell death, PANoptosis, for the treatment of COVID-19." The study from her group elucidated the molecular details of innate immune signaling pathways that regulate inflammation, inflammasome activation, and PANoptosis, which are relevant to not only COVID-19, but other infectious diseases. The study paved the way to mechanistically target the countless PANoptosis-dependent diseases, including COVID-19. Dr. Santosh Kumar presented on "Intervention and improved well-being of basic science researchers during the COVID-19 era: a case study." Overall, the study from his group showed that intervention strategy improved well-being (general perceived as well as COVID-19-induced stress) for basic science researchers, which was also consistent with their improved productivity during the COVID-19 era. The DISC program concluded with a panel discussion of reflection and sharing on the experience from COVID-19 in terms of the well-being and productivity of researchers, especially researchers from diverse backgrounds. In this session, a diverse group of SNIP council members shared their experiences on how they and their research groups coped with the pandemic. They also provided their opinions on how to effectively deal with such situations in the future — to stay well, as well as productive.

The COVID-19 Workshop also entailed the publication of a special issue on COVID-19 in the Journal of Neuroimmune Pharmacology (JNIP). The special theme is entitled "Neuroimmune Pharmacology of SARS-CoV-2". This JNIP special issue planned to publish selected manuscripts from the workshop talks as well as any additional COVID-19 studies.

Program

Zoom webinar hosted by the Department of Pharmacology and Experimental Neuroscience, College of Medicine, University of Nebraska Medical Center, NE 68198, USA.

Friday, April 9, 2021.

9:30 am- 9:35 am	**Welcome message** **Dr. Sulie L. Chang, PhD,** Professor of Biological Sciences and Director, Institute of Neuroimmune Pharmacology, Seton Hall University, South Orange, NJ, USA
9:35 am- 10:05 am	**Special talks, 15 min each** **Dr. Changhai Cui, PhD**, Program Official, Division of Neuroscience and Behavior, National Institute on Alcohol Abuse and Alcoholism (NIAAA), NIH, Bethesda, MD, USA **Title:** COVID-19 and funding opportunities from NIAAA **Dr. Yu (Woody) Lin, MD, PhD**, Program Official, Integrative Neuroscience Branch (INB), Division of Neuroscience and Behavior (DNB), National Institute on Drug Abuse (NIDA), NIH, Bethesda, MD, USA **Title:** COVID-19 and funding opportunities from NIDA
Symposium 1: Molecular approaches to COVID-19 pathogenesis and underlying mechanisms Co-chairs: Dr. Changhai Cui, PhD, Program Official, NIAAA, NIH, Bethesda, MA, USA Dr. Pankaj Seth, PhD, Professor and Scientist VII, National Brain Research Centre, Gurgaon, Haryana, India	
10:10 am- 10:25 am	**Speaker 1: Dr. Rosemarie Booze, PhD,** Department of Psychology, University of South Carolina, Columbia, SC, USA **Title:** Post-acute SARS-CoV-2 infection (PASC) in non-human primate olfactory system and pyriform cortex
10:25 am- 10:40 am	**Speaker 2: Dr. Theodore Cory, PharmD, PhD**, Department of Clinical Pharmacy and Translational Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN, USA **Title:** Cellular senescence alters fibrogenesis in SARS-CoV-2 infected macrophage/fibroblast co-cultures
10:40 am- 10.55 am	**Speaker 3: Dr. Navneet Dhillon, PhD**, Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Kansas Medical Center, Kansas City, KS, USA **Title:** Assessment of circulating extracellular vesicles reveals pro-inflammatory and thrombosis related protein cargo in COVID-19 patients
10:55 am- 11:10 am	**Speaker 4: Dr. Howard Gendelman, MD,** Department of Pharmacology and Experimental Neuroscience, College of Medicine, University of Nebraska Medical Center, Omaha, NE, USA **Title:** SARS-CoV-2 induction of a pro-inflammatory human macrophage
11:10 am- 11.25 am	**Speaker 5: Dr. Supriya Mahajan, PhD**, Department of Medicine, Division of Allergy, Immunology & Rheumatology Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Clinical Translational Research Center, Buffalo, NY, USA **Title:** Mitochondrial dynamics in SARS-CoV-2 spike protein treated human microglia: Implications for Neuro-COVID
11:25 am- 11.40 am	**Speaker 6: Dr. Servio Ramirez, PhD,** Department of Pathology and Laboratory Medicine, Center for Substance Abuse Research, The Lewis Katz School of Medicine at Temple University, Philadelphia, PA, USA **Title:** The SARS-CoV-2 spike protein triggers brain endothelial activation and negatively affects the function of the blood–brain barrier; implications for neuroinvasive mechanisms of emerging coronaviruses
Symposium 2: Therapeutic/vaccine approaches to COVID-19 Co-chairs: Dr. Nazira El-Hage, PhD, Associate Professor, School of Medicine, Florida International University, Miami, USA Dr. Yu (Woody) Lin, MD, PhD, Program Official, INB, DNB, NIDA, NIH, Bethesda, MD, USA	
11:45 am- 12:00 pm	**Speaker 1: Dr. Siddappa Byrareddy, PhD**, Department of Pharmacology and Experimental Neuroscience, College of Medicine, University of Nebraska Medical Center, Omaha, NE, USA **Title:** Development of targeted therapeutics for COVID-19
12:00 pm- 12:15 pm	**Speaker 2: Dr. Wen-Zhe Ho, MD, MPH,** Department of Pathology and Laboratory Medicine, Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA **Title:** Epigallocatechin gallate from green tea effectively blocks infection of SARS-CoV-2 and new variants by inhibiting spike binding to ACE2 receptor
12:15 pm- 12:30 pm	**Speaker 3: Dr. Rafal Kaminski, PhD**, Center for Neurovirology/Department of Neuroscience, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, USA **Title:** Targeting N-glycosylation and palmitoylation pathways to block coronavirus infectivity
12:30 pm- 12:45 pm	**Speaker 4: Dr. Bhavesh Kevadiya, PhD**, Department of Pharmacology and Experimental Neuroscience, College of Medicine, University of Nebraska Medical Center, Omaha, NE, USA **Title:** Development of a layer-by-layer SARS-CoV-2 microparticle vaccine

12:45 pm-1:00 pm	**Speaker 5: Dr. Jag Khalsa, PhD**, Formerly, Chief, Medical Consequences of Drug Abuse and Infections Branch, National Institute on Drug Abuse, NIH; Currently a Special Volunteer NIDA/NIH, Bethesda, MA, USA **Dr. Sanjay Maggirwar, PhD,** Department of Microbiology, Immunology, and Tropical Medicine, The George Washington School of Medicine and Health Sciences, Washington, DC, USA **Dr. Gregory Bunt, MD,** NYU and Samaritan Day Top Village Addiction Treatment Center, New York, NY, USA **Title:** COVID-19, cannabis/CBD, and the physician
1:00 pm-1.15 pm	**Speaker 6: Dr. Kalipada Pahan, PhD**, Department of Neurological Sciences, Rush University Medical Center, Chicago, IL, USA **Title:** AIDS for COVID-19!

15-min break

Symposium 3: Early career investigators (pre- and post-doctorate fellows)

Co-chairs: Dr. Gurudutt Pendyala, PhD, Associate Professor, College of Medicine, University of Nebraska Medical Center, Omaha, NE, USA

Dr. John Satterlee, PhD, Special Assistant to the DNB Director, Division of Neuroscience and Behavior (DNB), National Institute on Drug Abuse (NIDA), NIH, Bethesda, MD, USA

1:30 pm-1:45 pm	**Special talk: Dr. John Satterlee, PhD,** Special Assistant to the DNB Director, DNB, NIDA, NIH, Bethesda, MD, USA **Title:** Opportunities from NIH for ECI fellows
1:45 pm-1:53 pm	**Speaker 1: Dr. Arpan Acharya**, **PhD**, College of Medicine, University of Nebraska Medical Center, Omaha, NE, USA **Title:** HIV-1/SARS-CoV-2 coinfection and CNS pathogenesis
1:53 pm-2:01 pm	**Speaker 2: Liana Basova, BA,** San Diego Biomedical Research Institute, La Jolla, CA, USA **Title:** Systems biology approaches detect early neuroinflammation and calcium signaling signatures in SARS-Cov-2 infection which are not affected by H1N1 in tracheal biopsies from the ferret model
2:01 pm-2:09 pm	**Speaker 3: Michael Mora Stangis, BA,** Miller School of Medicine, University of Miami, FL, USA **Title:** Methamphetamine potentiates the S1 subunit of SARS-CoV-2 spike protein-induced downregulation of tight junction proteins of human brain endothelial cells
2:09 pm-2:17 pm	**Speaker 4: Dr. Silvia Torices, PhD,** Miller School of Medicine, University of Miami, FL, USA **Title:** Expression of SARS-CoV-2-related receptors in naïve and HIV-1-infected cells of the neurovascular unit
2:17 pm-2:25 pm	**Panel discussion: Dr. Yisel Cantres-Rosario, PhD,** Department of Microbiology and Medical Zoology, University of Puerto Rico Medical Sciences Campus, San Juan, PR, USA

Symposium 4: Diversity and Inclusion SNIP Committee: Well-being and reflections

Co-chairs: Dr. Sowmya Yelamanchili, PhD, Assistant Professor, Department of Anesthesiology, College of Medicine, University of Nebraska Medical Center, Omaha, NE, USA

Dr. Jeymohan Joseph, PhD, Chief, HIV Neuropathogenesis, Genetics and Therapeutics Branch (HNGTB), Division of AIDS Research (DAR), National Institute of Mental Health (NIMH), Bethesda, MD, USA

2:30 pm-2:45 pm	**Special talk: Dr. Jeymohan Joseph, PhD,** Chief, HNGTB, DAR, NIMH, Bethesda, MD, USA **Title:** Neurologic and psychiatric complications of SARS-CoV-2 infection
2:45 pm-3:00 pm	**Speaker 1: Dr. Thirumala-Devi Kanneganti, PhD,** Immunology Department, St. Jude Children's Research Hospital, Memphis, TN, USA **Title:** Targeting innate immunity and inflammatory cell death, PANoptosis, for the treatment of COVID-19
3:00 pm-3:15 pm	**Speaker 2: Dr. Santosh Kumar, PhD,** Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN, USA **Title:** Intervention and improved well-being of basic science researchers during the COVID 19 era: a case study
3:15 pm-3:55 pm	**Panel discussion: Reflection and sharing** **Panelists:** Drs. Jean Bidlack, Sylvia Fitting, Santhi Gorantla, Cecilia Marcondes, Loyda Melendez, Ilker Sariyer
3:55 pm	**Conclusion**

Abstracts

SNIP COVID-19 Virtual Workshop abstracts are not included for the welcome message, special talks by NIH officials, panels, and by presenter opt-out (with manuscript under consideration for publication elsewhere).

Symposium 1: Molecular Approaches to COVID-19 Pathogenesis and Underlying Mechanisms

Post-acute SARS-CoV-2 Infection (PASC) in Non-Human Primate Olfactory System and Pyriform Cortex

Rosemarie M. Booze[1], Kristen A. McLaurin[1], Hailong Li[1], Jay Rappaport[2], Prasun K. Datta[2], and Charles F. Mactutus[1].
[1]*Department of Psychology, University of South Carolina, Columbia, South Carolina, USA.*
[2]*Tulane National Primate Research Center, Covington, Louisiana, USA.*

Loss of smell, or anosmia, is a commonly reported long-term consequence of SARS-CoV-2. However, the extent of viral invasion and persistence in the olfactory system and brain has not been determined post-infection. In these studies, we examined SARS-CoV-2 infected non-human primate brains using RNAscope in situ hybridization. The viral distribution of SARS-CoV-2, angiotensin-converting enzyme 2, and transmembrane serine protease 2 were identified in the olfactory epithelium and pyriform cortical region. Despite full recovery from viral inoculation, viral infection and a viral reservoir were observed in both the olfactory epithelium and pyriform cortex, evidenced by the presence of SARS-CoV-2 mRNA and DNA, respectively. Furthermore, dual-labeling of platelet-derived growth factor receptor β, a marker for pericytes, and SARS-CoV- 2 indicated that pericytes were the predominant cell type to harbor the virus in the pyriform cortex. Critically, there was no relationship between mucosal swab viral load, clinical assessment or lung histopathologic score and SARS-CoV-2 level in the pyriform cortex. These findings indicate that SARS-CoV-2 may persist in the brain and contribute to the neurovascular post-acute sequelae of SARS-CoV-2 infection (PASC).

Cellular Senescence Alters Fibrogenesis in SARS-CoV-2 Infected Macrophage/Fibroblast Co-Cultures

Theodore Cory[1] and Brandt Pence[2].
[1]*University of Tennessee Health Science Center College of Pharmacy, Memphis, TN, USA.*
[2]*University of Memphis College of Health Sciences, Memphis, TN, USA.*

COVID-19 outcomes are worsened in elderly individuals as compared to younger individuals infected with SARS-CoV-2. While the reasons for this are unknown, and are likely multifaceted, one potential factor is alterations in cellular function due to increased rates of cellular senescence in aging individuals. Cellular senescence is a state of irreversible cell cycle arrest and apoptosis resistance, and is associated with pro-inflammatory cytokine, chemokine, and growth factor production, referred to as inflammaging. We hypothesized that senescent cells would contribute to increased pro-fibrotic responses during SARS-CoV-2 infection.

Senescence was induced in monocyte derived macrophages through treatment with 250 nM doxorubicin or 10 GY X-ray irradiation. MDM were co-cultured for 24 h with IMR-90 fibroblasts, media replaced, and infected with SARS-CoV-2 isolate USA-WA1/2020 at an MOI of 0.5. Cells were incubated for 48 h, and supernatants collected. Soluble collagen production was assessed using the Sircol soluble collagen assay, and total TGF-β1 and fibronectin concentrations were assessed via ELISA.

In non-senescent cells, there was no increase in supernatant collagen concentrations. Collagen concentrations in infected co-cultures were non-significantly increased in doxorubicin induced senescence cells, and significantly increased in radiation induced senescence cells. Surprisingly, TGF-β concentrations were unaffected by infection with SARS-CoV-2. Soluble fibronectin concentrations were decreased in infected conditions, significantly in non-senescent and radiation induced senescence conditions. Future work will determine the mechanisms by which senescence induces fibrogenesis, and investigate strategies to reverse senescence-induced fibrogenesis.

Funds for this research were provided by a UTHSC/Uof M SARS-CoV-2/COVID-19 Research CORNET award.

SARS-CoV-2 Induction of a Pro-Inflammatory Human Macrophage

Mai M. Abdelmoaty[1], Jatin Machhi[2], Pravin Yeapuri[2], Farah Shahjin[2], Kabita Pandey[2], Arpan Acharya[2], Siddappa Byrareddy[2], R. Lee Mosley[2], and Howard E. Gendelman[2].
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[2]*Department of Pharmacology and Experimental Neuroscience, College of Medicine, University of Nebraska Medical Center, NE 68,198, USA.*

Background: Life threatening coronavirus disease 2019 (COVID-19) is typified by the acute respiratory distress syndrome (ARDS). This is heralded by systemic hyper-inflammation associated with the macrophage activation syndrome (MAS), a cytokine storm. However, it is now known that macrophages are not productively infected by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Thus, their contribution to COVID-19 pathology remains unclear. The mechanisms that lead to pulmonary macrophage activation and secretion of tissue injurious factors were examined in the present study.

Methods: Human monocytes were isolated by leukapheresis from HIV-1,2 and hepatitis B seronegative donors. Peripheral blood mononuclear cells purified by centrifugal elutriation and cultivated in tissue culture media containing macrophage colony stimulating factor to facilitate differentiation into macrophage-like cells. During monocyte-macrophage differentiation, expression of angiotensin-converting enzyme 2 (ACE2) receptor, a SARS- CoV-2 entry receptor, was monitored by flow cytometry. Five days following cell culture, differentiated macrophages were subjected to SARS-CoV-2 at a multiplicity of infection of 0.01. On days 1, 3 and 5 after viral exposure, culture fluid was collected to measure viral RNA expression and monocyte-derived macrophages (MDMs) were collected for transcriptomic and proteomic assay. Data sets were compared against mock challenged MDMs. SARS-CoV-2 challenged and uninfected MDMs were prepared for transmission electron microscopy (TEM).

Results: ACE2 receptor expression on the MDMs surface peaked at day 5 post-differentiation. Viral RNA levels were at the limit of detection and could not be differentiated from the infecting inoculate. Levels decreased during the 5 days of analysis following viral exposure. Transcriptomic analyses of infected MDMs showed induction of inflammatory mRNAs that included interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and interleukin-1 alpha (L-1α). Notably, expression of interferon (IFN)-pathway linked genes including IFN-α1, IFN- β1, IFN-γ and IRF7 were increased. Most cytokines remained upregulated up to 3 days post-viral exposure but were decreased after that. Proteomic profiles of infected MDMs were reflective of inflammation, immunoregulation and antiviral responses that included TNF-α, proinflammatory ILs, and IFNs upregulation. TEM images

revealed presence of viral particles and inclusion bodies in the macrophage cytoplasm and within the mitochondria after one week of viral exposure.

Conclusions: We demonstrated that exposure of SARS-CoV-2 to MDMs yields an abortive infection without evidence of progeny infectious virus production. SARS-CoV-2 triggers macrophages to express pro- inflammatory cytokines (IL-6, TNF-α, and IL-1α) which can be viewed as a critical driver of the COVID-19- associated cytokine storm. Most importantly, the virus-exposed macrophages elicited a sustained high-level type I IFN response supporting a pathway for viral restriction and disease pathogenesis. Together, these data suggest that SARS-CoV-2-macrophage interactions induce pro-inflammatory and IFN responses explaining the pathogenesis of virus-induced ARDS and perhaps other end organ COVID-19 disease.

Keywords: macrophages, SARS-CoV-2, cytokine storm, Type I IFN responses.

Mitochondrial Dynamics in SARS-CoV-2 Spike Protein Treated Human Microglia: Implications for Neuro-COVID.

Erin Clough[1], Khoo Ting Chean[2], Joseph Inigo[3], Kate E. Tubbesing[2], Dhyan Chandra[3], Marissa Baird[1], Jessica L Reynolds[1], Stanley A Schwartz[1], Alexander Khmaladze[3], and Supriya D. Mahajan[1].
[1]*Department of Medicine, Division of Allergy, Immunology & Rheumatology Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Clinical Translational Research Center, Buffalo, NY 14,203, USA.*
[2]*Department of Physics, University at Albany SUNY, 1400 Washington Avenue, Albany, NY 12,222, USA.*
[2]*Department of Pharmacology & Therapeutics Roswell Park Comprehensive Cancer Center, Buffalo, NY 14,263, USA.*

Background & Rationale: Emerging clinical data from the current COVID-19 pandemic suggests that ~ 40% of the patients with COVID-19 developed neurological symptoms attributed to viral encephalitis and in the COVID long haulers, the resulting chronic neuro-inflammation, neuronal damage results in a syndrome is described as Neuro-COVID. We recently showed that SARS- CoV-2 spike proteins interact with receptors for angiotensin-converting enzyme 2 (ACE2) on human brain microvascular endothelial cells (hBMVEC) contributing to Blood Brain Barrier dysfunction and potentially facilitates viral entry into the brain resulting in neuroinflammation and associated neuropathology. SARS-CoV-2 spike protein and ACE2 interaction results in viral entry and modulation of levels of ACE2 which cleaves Angiotensin II into Angiotensin 1–7 which regulates mitochondrial function. A recent report suggests that once the SAR-COV-2 virus enters the host cell, open-reading frames (ORFs) such as ORF-9b directly manipulate mitochondrial function to evade host cell immunity and facilitate virus replication and COVID-19 disease progression. A combination of events, such as oxidative stress, neuroinflammatory responses, and mitochondrial dysfunction, may possibly converge to mediate SAR-COV-2 induced microglial dysfunction and neuronal death contributing to COVID 19 neuropathology.

Hypothesis: We hypothesize that SAR-COV-2 induces mitochondrial dysfunction and activation of the mitochondrial-dependent intrinsic apoptotic pathway, resulting in microglial and neuronal apoptosis.

Methods: We treated human microglia (HMC3 -ATCC) with SARS-CoV-2 spike protein (Cat # NR – 52,307 BEI Resources Inc). The goal of our study is to monitor cell apoptosis in human microglia non-invasively in real time using Raman spectroscopy, which provide unique spatio- temporal information on mitochondrial function in live cells and can be used to study the release of cytochrome C from mitochondria in neuronal cells undergoing apoptosis. Additionally, we did a Seahorse flux analysis (Agilent Seahorse XF96) to determine the effect of SARS-COV-2 on Mitochondrial biogenesis.

Results: Seahorse assay showing increased Oxygen consumption rate (OCR) in microglial cells treated with SARS- COV-2 spike protein. Increases in OCR can be indicative of increased reactive oxygen species (ROS) production suggesting that SARS- COV-2 induced cell death and increases in OCR are associated with oxidative stress. Raman spectroscopy yielded information on, lipid composition, stress response, nuclear division, respiratory activity and cell death. We observed significant differences in Phospholipids such as Phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylcholine (PC), which account for ~ 80% of mitochondrial membrane lipids between SARS-COV-2 treated and untreated microglial cells. Further, we are using Raman spectroscopy to identify the spectral signatures of cytochromes found in mitochondrial oxidative phosphorylation (OXPHOS) complexes between SARS-COV-2 treated microglia and untreated controls.

Conclusion: These data provide important mechanistic insights into SARS-COV 2 induced mitochondrial dysfunction which underlies neuropathology associated with Neuro-COVID.

The SARS-CoV-2 Spike Protein Triggers Brain Endothelial Activation and Negatively Affects the Function of the Blood–Brain Barrier; Implications for Neuroinvasive Mechanisms of Emerging Coronaviruses

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The SARS-CoV-2 coronavirus is responsible for causing the respiratory illness known as COVID-19. Since the onset of the pandemic, it has become increasingly clear that the negative consequences of SARS-CoV-2 infection is not only restricted to the respiratory system, but it can also a have dire impact on the cardiovascular system and the central nervous system (CNS). Regarding the CNS, COVID-19 patients have presented neurological symptoms that includes headaches, nausea, dizziness, forgetfulness along with ischemia due to microclot formation and in rare cases, encephalitis. How this devastating pathogen may induce neurological deficits remains largely unknown. To this end we sought to characterize how the spike protein (the receptor engaging surface protein of SARS-CoV-2) affects brain endothelial cell biology and the function of the blood–brain barrier. First, using postmortem brain tissue, our results show that the angiotensin converting enzyme 2 or ACE2 (a known binding target for the SARS-CoV-2 spike protein), is ubiquitously expressed throughout various vessel calibers in the frontal cortex. Moreover, ACE2 expression was upregulated in cases of hypertension and dementia. ACE2 was also detectable in primary human brain microvascular endothelial cells (from multiple donors) under cell culture conditions. Analysis of cell viability revealed that neither the S1, S2 or a truncated form of the S1 containing only the RBD had minimal effects on hBMVEC viability within a 48 h exposure window. However, our results showed that S1 promotes loss of barrier integrity in an advanced 3D microfluidic model of the human BBB, a platform that more closely resembles the physiological conditions at this CNS interface. In addition to the above permeability studies, our analyses were also extended to the evaluation of changes to key transporter proteins essential to the BBB. Lastly, experimental evidence is provided that suggests that the SARS-CoV- 2 spike protein trigger a proinflammatory response on brain endothelial

cells that may contribute to an altered state of BBB function. Together, these results may help provide an explanation to why neurological symptoms are commonly presented during COVID-19 infection and open the door to the possibility that breach to the BBB could aid in SARS-CoV-2 neuroinvasion.

Keywords: SARS-CoV-2, BBB, cerebrovasculature, neuroinflammation, hypertension, neuro- COVID-19.

Symposium 2: Therapeutic/Vaccine Approaches to COVID-19

Development of Targeted Therapeutics for COVID-19

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Since the discovery of SARS-CoV-2, more than 120 million people have been infected to date and 2.6 million people have succumbed to death worldwide. Large efforts were made to develop an emergency vaccine and make it available to all adults. However, children, pregnant women, and immunosuppressed people are still at risk of acquiring and suffering from diseases. Therefore, alternative strategies are needed to block/inhibit viral entry to host cells. Herein, we identified two lead compounds, one blocks the attachment of spike with ACE2, while the other is a PI3K-α-mTOR/(BRD2/BRD4) inhibitor that blocks two orthogonal pathways necessary for SARS-CoV-2 pathogenesis in human cells. Using a computer-aided drug design approach, we first performed a virtual screening of a library of drug like compounds that had potential to bind at the ACE2/Spike interface to identify the SARS-CoV-2 entry inhibitors. We identified several lead compounds and tested them in vitro for antiviral and biophysical activities. Recently, a proteomic analysis study identified several host cellular targets essential for SARS-CoV-2 pathogenesis. A small molecule SF2523 was identified which inhibits two of the critical targets, the bromodomain and extra-terminal domain proteins (BETs: BRD2/BRD4) and mTOR: at nanomolar potency. The inhibitory activities of these small molecules were tested in Vero- STAT1 knockout cells and a human bronchial epithelial cell line (UNCN1T). We identified the top compound based on Spike interface with binding energy and showed very potent antiviral activity with high selectivity index (SI). Furthermore, the cellular target, SF2523 effectively blocks SARS-CoV-2 replication in lung bronchial epithelial cells in vitro, showing a low IC50 value, which is comparable to IC50 value of remdesivir. Finally, we demonstrated that the combination of doses of SF2523 and remdesivir is highly synergistic and allows for the reduction of doses of SF2523 and remdesivir by several folds to achieve the same potency observed for a single inhibitor. Currently, the synergetic effect of both viral and cellular inhibitors is ongoing in lung epithelial cells. In summary, we report the identification of two lead compounds, the first of which blocks the cellular entry of SARS-CoV-2 acting through Spike/ACE-2 interface, and the second inhibits host proteins essential for viral pathogenesis in submicromolar concentration in biologically relevant human bronchial epithelial cells. Furthermore, our data suggest that entry inhibitor and SF2523 alone or in combination with remdesivir could be a novel and efficient therapeutic strategy to block SARS-CoV-2 infection and could hence be beneficial in preventing severe COVID-19 disease evolution. Future studies involving targeting both viral and cellular proteins as a synergistic effect including new mutant viruses (B.1.1.7, B.1.351, P.1 and CAL.20C) are planned to develop potential therapies to overcome resistance to all the SARS- CoV-2 variants and to reduce doses required for a better outcome to treat COVID-19.

Epigallocatechin Gallate from Green Tea Effectively Blocks Infection of SARS-CoV-2 and New Variants by Inhibiting Spike Binding to ACE2 Receptor

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As the global COVID-19 pandemic rages on, the new SARS-CoV-2 variants have been identified in the different regions of the World. These variants are spreading quickly and may resist to vaccine-induced immunity and the existing therapeutics. Therefore, there is still urgent need of safe, effective, and affordable agents for prevention/treatment of SARS-CoV-2 infection. Here, we demonstrate in a panel of in vitro studies that green tea and its major ingredients are highly effective (> 90%) in inhibiting infection of the pseudovirus expressing the spikes of SARSCoV-2 and the newly emerged variants (UK-B.1.1.7, SA-B.1.351, Brazil-P.1 and US-B.1.429). Among the 4 active green tea catechins at noncytotoxic doses, epigallocatechin gallate (EGCG) is the most potent in the action. More importantly, EGCG could block live SARS-CoV-2 and coronavirus infection of the host cells. The highest inhibitory activity was observed when the viruses were pre- incubated with EGCG prior to the infection. Mechanistic studies showed that EGCG blocked the infection at entry level through interfering the engagement of the receptor binding domain (RBD) of the viral spike to angiotensin-converting enzyme 2 (ACE2) receptor of the host cells. These data support further clinical evaluation and development of EGCG as a novel, safe, cost-effective, and natural product for prevention/treatment of SARS-CoV-2 transmission and infection.

Targeting N-glycosylation and Palmitoylation Pathways to Block Coronavirus Infectivity

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The emergence of new SARS-CoV-2 variants partially resistant to vaccine-induced immunity and disparities in vaccine distribution underscores an urgent need for new broad-spectrum treatment options for COVID-19. The spike protein S of coronaviruses, including current SARS-CoV-2, undergoes several heavy post-translational modifications as it moves through the endoplasmic reticulum (ER) and Golgi along host cellular secretory pathways. The ectodomain of protein S can be N-linked glycosylated on a total of 22 Asn-X-Ser/Thr motifs. Its cytoplasmic tail can be palmitoylated on up to 10 cysteine residues. Both modifications were shown to regulate viral protein stability, conformation, intracellular trafficking, receptor interaction, antigenicity, and host cell invasion, thus impacting viral replication and infectivity of many enveloped viruses (HIV-1, influenza virus, Ebola, Zika, and coronaviruses, including MERS and SARS). Using a model Betacoronavirus

hCoV-OC43, we demonstrate a critical role of N-glycosylation and palmitoylation pathways in maintaining the infectivity of coronavirus in epithelial cells. Post-infection treatment with Celgosivir, a widely used inhibitor of α-glucosidases necessary for N-glycosylation, suppressed both virus release to supernatants and the number of plaques generated from infectious supernatants. Moreover, CRISPR-mediated specific disruption of α-glucosidase-1 (GC-1/MOGS) gene leads to a significant drop of infectivity of hCoV-OC43 progeny virions. Additionally, we performed a CRISPR-Cas9-based gene knockout screen of the first ten (out of a total of 23) ZDHHC acyltransferases, enzymes responsible for protein palmitoylation. The viral infections were reduced, as measured by intracellular and extracellular levels of viral RNA, in ZDHHC3, 5, 7, 8, and 9 knockout cells compared to controls. These data provide a base for developing new, host-directed, and potentially pan-viral therapeutic strategies targeting current and future emerging coronaviruses and other glycosylation/palmitoylation-dependent enveloped viruses.

Development of a Layer-by-Layer SARS-CoV-2 Microparticle Vaccine

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Even after about one year from the declaration of the COVID-19 pandemic, there is still no antiviral agent capable of ensuring the proper treatment or full protection against SARS-CoV-2 virus. The COVID-19 pandemic has required rapid action and the vaccine development in an unprecedented timeframe. Currently, three SARS-CoV-2 vaccines are immediately available for public distribution while others are in varying phases of preclinical or clinical trial development. The humoral and cellular immune response induction after vaccination is required for protection against SARS-CoV-2 infection. Despite the recorded efficacy of the existing vaccines, there remain a number of limitations. *First*, the vaccines are given under emergency use authorization with as yet undefined duration of action. *Second*, each of the deployed vaccines deploys the spike protein as the immunogen. *Third*, there is a putative lack of long-term memory responses well known to prevent reinfection. Such responses remain difficult to assess as cross-reactive CD4+ memory T cells and herd immunity both impact transmission. *Fourth*, there are no long- term toxicity profiles. *Fifth*, there are known SARS-CoV-2 genomic variabilities. These linked host immune response could become limitations in long-term efficacy. *Sixth*, the lead vaccines require repeated doses. Thus, there is an immediate need to develop alternative safe and effective vaccines that take into account viral genomic variability, toxicities and induction of antiviral CD4+ T cell memory cells. We have developed a vaccine approach that deploys multiple distinct viral antigens as immunogens accomplished by slow-release layer-by-layer microparticles (LBL MPs). This approach would deliver "whole" inactivated SARS-CoV-2 in order to elicit broad antigen exposures. Our intended strategy will allow pulsatile release of viral antigens and a sustained memory and humoral antiviral immune response to viral variants. Therefore, *a pulsatile release of SARS-CoV-2 antigens from polymeric layer MPs was formed using a means for controlled degradation. This vaccine format is capable of eliciting a sustained and effective antiviral immune response*. Extensive physicochemical characterization of the particles was completed and will be presented using electron microscopy and Raman spectral mapping methods. In process are studies of particle toxicity, biodistribution (by PET and SEPCT CT) and biodegradation. T and B cell immune response will be evaluated after a single dose administration. We posit that pulsatile release multi-layered particles are a versatile platform for efficient induction of anti-SARS-CoV- 2 immune responses.

COVID-19, Cannabis/CBD, and the Physician

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The world is facing one of the most devastating viral pandemics of our time where almost 120 million people have been infected with a novel corona virus- known as severe acute respiratory syndrome cornonavirus-2 (SARS-CoV-2), and more than two million people have died from the corona virus induced disease (COVID-19).[1] The COVID-19 pandemic is responsible for unprecedented loss of life, economic, social, and health consequences including severe acute respiratory syndrome (SARS), cardiovascular, mental health including anxiety, depression, and neurological complications like tremors, seizures and impaired consciousness.

Extensive efforts are underway to develop preventive vaccines and therapeutics such as remdesivir, dexamethasone, convalescent plasma and others to treat COVID-19 but many 'long- haulers' patients report residual mental health and other problems after recovery.[3,4] Cannabis and its products such as cannabidiol (CBD) are being advertised for the treatment of COVID-19 associated mental health problems, anxiety, depression, PTSD and substance use disorders. In this workshop, Dr. Maggirwar will discuss the health effects of COVID-19, Dr. Khalsa will present whether there is sufficient clinical evidence to support the use of cannabis/cannabinoids for treating COVID-19 related health effects,[5] and Dr. Bunt will discuss the role of addiction physicians in dealing with patients with SUDs requesting cannabis or CBD prescription for any of the COVID-19 health effects.

AIDS for COVID-19!

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COVID-19 is an infectious respiratory illness caused by the virus strain severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Many COVID-19 patients in intensive care units suffer from cytokine storm. Although anti-inflammatory therapies are available to treat the problem, very often these treatments cause immunosuppression. Since SARS-CoV-2 binds to angiotensinconverting enzyme 2 (ACE2) for entering into host cells, to target COVID-19 from therapeutic angle, we engineered a hexapeptide corresponding to the ACE2-Interacting Domain of SARSCoV-2 (AIDS) that inhibits the association between receptor-binding domain-containing spike S1 and ACE-2. Accordingly, wild type (wt), but not mutated (m), AIDS peptide inhibited SARSCoV-2 spike S1-induced activation of NF-κB and expression of IL-6 in human lungs cells. However, wtAIDS remained unable to reduce activation of NF-κB and expression of proinflammatory molecules in lungs cells induced by bacterial flagellin, HIV-1 Tat, and viral double-stranded RNA mimic poly IC, indicating the specificity of the effect. The wtAIDS, but not mAIDS, also hindered the association between ACE2 and spike S1 of SARS-CoV-2 and inhibited the entry of pseudotyped SARS-CoV-2, but not VSV, into human ACE2-expressing HEK293 cells. Interestingly, intranasal intoxication of C57/BL6 mice with recombinant SARS-CoV-2 spike S1 led to fever, increase in IL-6 in lungs, infiltration of neutrophils into the lungs, arrhythmias, and impairment

in locomotor activities, mimicking some of the important symptoms of COVID19. However, intranasal treatment with wtAIDS, but not mAIDS, peptide reduced fever, protected lungs, improved heart function, and enhanced locomotor activities in SARS-CoV-2 spike S1intoxicated mice. Therefore, selective targeting of ACE2-to-SARS-CoV-2 interaction by wtAIDS may be beneficial for COVID-19. This study was supported by grants (AG050431, AT010980, and NS108025) from NIH.

Symposium 3: Early Career Investigators (Pre- and Post-Doctorate Fellows)

HIV-1/SARS-CoV-2 Coinfection and CNS Pathogenesis

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Background: Being an enveloped RNA virus, SARS-CoV-2 has several common features with HIV-1 in disease pathogenesis. Using a systemic analysis of literature, we documented that people living with HIV-1 (PLWH) taking combinational antiretroviral therapy (cART) and completely suppressed plasma viremia did not have a higher risk of SARS-CoV-2 infection or COVID-19 severity (1, 2). Meanwhile, PLWH with common comorbid conditions with COVID-19 had a poor disease prognosis. Nonetheless, the current data is limited to small geographical coverage and information on the long-term sequelae of COVID-19 among coinfected patients is lacking. Recent clinical data from cohort studies suggest that COVID-19 patients may experience short and long- term neurologic symptoms with the compartmentalization of innate/adaptive immune responses. Furthermore, HIV-1/SARS-CoV-2 coinfected individuals' long-term neurological sequelae remain elusive. Therefore, we designed in vitro studies to understand the pathophysiology of HIV- 1/SARS-CoV-2 coinfection in primary cells from central nervous system (CNS).

Methods: Human primary astrocytes and pericytes were purified from healthy donors and grown. The cells were divided into four groups. Group-1 was used as uninfected controls, group-2 was infected with SARS-CoV-2, group-3 was infected with HIV-1, and group-4 received HIV/SARS- CoV-2 coinfection. Initially, cells (Gr. 3 &4) were infected with HIV-1 ADA (0.1 MOI), and after establishing productive infection, the cells (Gr. 2 & 4) were infected with SARS-CoV-2 (1.0 MOI). We measured the HIV-1/SARS-CoV-2 replication kinetics, expression of SARS-CoV-2 entry receptors and cytokines expression longitudinally up to 72 hrs post SARS-CoV-2 infection. Finally, cells from all groups were lysed, and cell-associated RNA and protein were subjected to proteomics and transcriptomics analysis to understand the host-virus interaction in the context of coinfection.

Results: For astrocytes, in SARS-CoV-2 mono infected cells, we detect up to a 5-fold increase in viral replication compared to co-infected cells, where there is a 2/3-fold increase from 0 to 72 hrs post-infection. Whereas in pericytes, in SARS-CoV-2 mono infected cells, we detect up to a 2/3-fold increase in viral replication while in coinfected cells, we observed a 4/5-fold increase. While we await the transcriptomics and proteomics data, the preliminary result from the gene expression analysis indicates downregulation of all of the reported SARS-CoV- 2 entry factors including angiotensin I converting enzyme 2 (ACE-2), transmembrane serine protease 2 (TMPRSS-2), neuropilin 1 (NRP-1), and tripartite motif-containing 28 (TRIM28) longitudinally in both mono and coinfected astrocytes/pericytes (3, 4). On the other hand, we observed overexpression of IL-6 and TNF-a, which are more profound in SARS-CoV-2 mono infected cells compared to HIV- 1/SARS-CoV-2 coinfected cells.

Conclusion: Our study's preliminary data demonstrate that the glial cells in CNS only support a low level of SARS-CoV-2 replication in the presence or absence of HIV-1. However, post- exposure to SARS-CoV-2, most of the host factors (ACE-2, TMPRSS-2, NRP-1, and TRIM28) that facilitate viral entry get down-regulated. On the other hand, overexpression of proinflammatory cytokines observed in SARS-CoV-2 mono-infected cells is decreased during coinfection. The upcoming transcriptomics and proteomics data will help us understand the host-virus interaction and detect molecular targets for therapeutic interventions to control post-COVID- 19 neurological consequences in SARS-CoV-2 mono or HIV/SARS-CoV-2 coinfected patients.

Systems Biology Approaches Detect Early Neuroinflammation and Calcium Signaling Signatures in SARS-Cov-2 Infection Which are Not Affected by H1N1 in Tracheal Biopsies From the Ferret Model.

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Ferrets have been described as a model of severe acute respiratory syndrome virus-2 (SARS-CoV- 2) infection and transmission of the new (2019) coronavirus disease (COVID-19), recapitulating aspects of human disease. SARS-CoV-2, along with SARS-CoV, Middle East respiratory syndrome (MERS-CoV), and influenza A viruses such as the avian H1N1 strain, primarily target the respiratory system (nasal, trachea, lungs), as a site of early exposure. In the case of SARS-Cov- 2, the angiotensin converting enzyme 2 (ACE2) is a key viral entry receptor expressed by lung alveolar cells, and several other cells including enterocytes, endothelium, as well as neurons and glia, causing cellular and biological process targets to be very diverse. For instance, clinical outcomes are largely respiratory, but enteric, vascular and neurological symptoms are prevalent. In this study, we have performed a systems analysis on transcriptome profiles from ferrets' trachea biopsies, metadata provided in DOI: https://doi.org/10.1016/j.cell.2020.04.026 and GSE147507, for comparing SARS-Cov-2 (n=6)/Control (n=4) and H1N1 (n=6)/Control (n=4) transcriptome profiles in that tissue 3 days after virus (10^5pfu) or saline intranasal inoculation. Our goal was to identify early molecular signatures with the potential to inform the diversity of outcomes resulting from infection. The log2 ratio of gene counts between each virus and respective controls, and p values, were calculated. Interactome databases, and Z-scores were used to estimate overrepresented and perturbed pathways in Ingenuity Pathway Analysis (IPA). Visualization in Cytoscape (http://www.cytoscape.org/) via GeneMania (http://www.genemania.org/) facilitated the identification of gene networks affected both by SARS-Cov-2 and H1N1, in different directions or intensities. These included leukocyte extravasation, cardiac, cancer and Th1 response signaling pathways. SARS-Cov-2, however, disrupted a few gene networks that were not affected by H1N1. These exclusive signatures included significant changes in genes involved in calcium signaling and in neuroinflammation, characterized by a suppression. The implications of these findings are discussed in the light of findings in the literature, and the value of ferrets as an animal model for mild COVID-19.

Methamphetamine Potentiates the S1 Subunit of SARS-CoV-2 Spike Protein-Induced Downregulation of Tight Junction Proteins of Human Brain Endothelial Cells

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Among the many symptoms and issues caused by SARS-CoV-2, the neurological impacts of the disease may not be known for years to

come. Exposure of human primary endothelial cells to the S1 subunit of the SARS-CoV-2 spike protein resulted in decreased levels of tight junction proteins, the alteration that is consistent with the blood brain barrier disruption, which may allow for the virus to pass and infect the brain parenchyma. This was also shown to be the case when these cells were also exposed to the SARS-CoV-2 virus. In addition, we are now examining how co-exposure to methamphetamine may further influence tight junction protein expression. Human primary endothelial cells were exposed to 100uM of methamphetamine for 1 hour prior to treatment with 15nM of the S1 subunit of the SARS-CoV-2 spike protein for timeframes ranging from 1 hour to 12 hours. Protein expression levels of ZO-1 and Claudin-5 were measured by immunoblotting. Preliminary data show that there was an increased effect on tight junction protein expression following exposure to both methamphetamine and the S1 subunit compared with individual treatments. This data could point to increased blood brain barrier permeability in individuals exposing themselves to multiple harmful situations, which may in turn lead to additional comorbidities alongside the onset of the SARS-CoV-2 infection.

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Expression of SARS-CoV-2-Related Receptors in Naïve and HIV-1-Infected Cells of the Neurovascular Unit

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Neurological complications are common in patients affected by COVID-19 due to the ability of SARS-CoV-2 to infect brains. While the mechanisms of this process are not fully understood, it has been proposed that SARS-CoV-2 can infect the cells of the neurovascular units (NVU), which form the blood-brain barrier (BBB). The aim of the current study was to analyze the expression pattern of the main SARS-CoV-2 receptors in naïve and HIV-1-infected cells of the NVU to elucidate a possible pathway of the virus entry into the brain and a potential modulatory impact of HIV-1 in this process. The gene and protein expression profile of ACE2, TMPRSS2, ADAM17, BSG, DPP4, AGTR2, ANPEP, cathepsin B and cathepsin L was assessed by qPCR and immunoblotting, respectively. In addition, we investigated if brain endothelial cells can be affected by the exposure to the S1 subunit of the S protein, the domain responsible for the direct binding of SARS-CoV-2 to the ACE2 receptors. The receptors involved in SARS-CoV-2 infection are coexpressed in the cells of the NVU, especially in astrocytes and microglial cells. These receptors are functionally active as exposure of endothelial cells to the SARS CoV-2 S1 protein subunit altered the expression pattern of tight junction proteins, such as claudin-5 and ZO-1. Additionally, HIV-1 infection upregulated ACE2 and TMPRSS2 expression in brain astrocytes and microglia cells. These findings provide key insight into SARS-CoV-2 recognition by cells of the NVU.

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Symposium 4: Diversity and Inclusion SNIP Committee: Well-being and reflections

Targeting Innate Immunity and Inflammatory Cell Death, PANoptosis, for the Treatment of COVID-19

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The innate immune system is the critical first line of defense against pathogenic infections. In the context of viral infections, activation of the innate immune response is key to controlling viral replication and eliminating the infection. However, overactivation of this response can lead to systemic hyperinflammation and significant morbidity and mortality. Recently, a novel coronavirus, SARS-CoV-2, has emerged, leading to the disease COVID-19 and a global pandemic. Targeted therapeutic strategies are critically lacking, and understanding the role of the innate immune responses in this disease is essential to inform treatment. Clinical data show that patients with COVID-19 experience a cytokine storm and significant tissue damage, both of which contribute to disease severity and mortality. Recent work from our group showed that increased TNF-α and IFN-γ levels following SARS-CoV-2 infection lead to inflammatory cell death with molecular characteristics of pyroptosis, apoptosis, and necroptosis, termed PANoptosis. PANoptosis is defined as a unique, physiologically relevant, inflammatory programmed cell death pathway activated by specific triggers and regulated by the PANoptosome complex. The PANoptosome provides a molecular scaffold for contemporaneous engagement of key molecules from pyroptosis, apoptosis, and necroptosis. PANoptosis has been implicated in infectious and autoinflammatory diseases, cancer, and beyond, and targeting this pathway is likely to have therapeutic benefits. For example, we found that neutralizing TNF-α and IFN-γ reduced SARS-CoV-2–induced mortality in mice. We have also previously elucidated the molecular details of innate immune signaling pathways that regulate inflammation, inflammasome activation, and PANoptosis, identifying upstream sensors and important molecules in these pathways, including ZBP1, caspase-8, RIPK1, and many others that can serve as therapeutic targets. These findings are applicable to not only COVID-19, but also other infectious diseases and conditions associated with a hyperactive innate immune response, cytokine release, and severe inflammation; this work paves the way to mechanistically target the countless PANoptosis-dependent diseases.

Intervention and improved well-being of basic research researchers during the COVID 19 era: a case study

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The coronavirus disease-19 (COVID-19) pandemic has affected individuals of all categories, irrespective of their geographical locations, professions, gender, or race. As a result of full or partial lockdown and stay-at-home orders, the well-being and productivity of individuals were severely affected. Since basic science research requires laboratory experiments, the work-from-home strategy hurt their productivity. In addition, the combination of decreased productivity and staying at home is likely to compromise their well-being by causing stress and anxiety. In this case study, a strategy was developed to engage researchers through listening and learning, motivation, and empowerment, using regular virtual sessions. Through these virtual sessions, research work was prioritized and coordinated, from idea conception to writing research papers and grant proposals. Perceived stress scores (PSS) and COVID-19-related stress (COVID-SS) scores were measured to evaluate general and COVID-19-induced stress, respectively, every month from March to July 2020 during the COVID-19 era. The result showed a significant improvement in both the PSS and the COVID-SS scores of the intervention group compared to the control group. In addition, while there was no/minimal change in PSS and COVID-SS scores from March to subsequent months until July for the control group, the intervention groups showed significant and consistent improvement in both scores in the intervention group. Overall, the intervention strategy showed improved well-being for basic science researchers, which was also consistent with their improved productivity during the COVID-19 era.

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Declarations

Conflict of Interest The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.