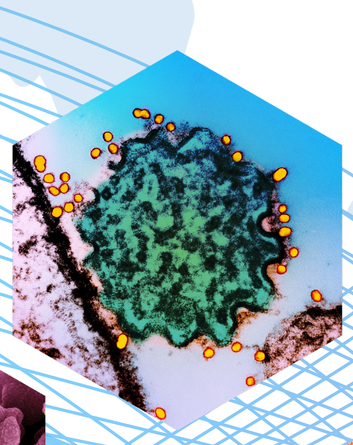
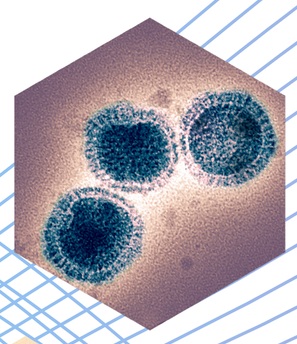
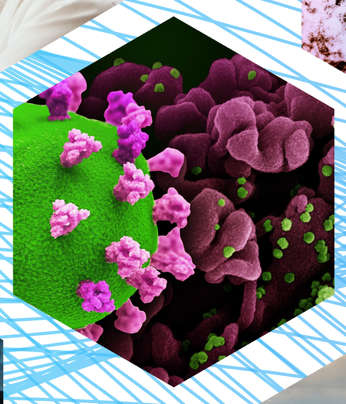
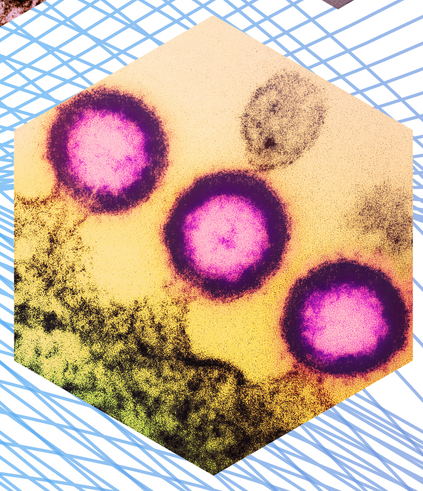
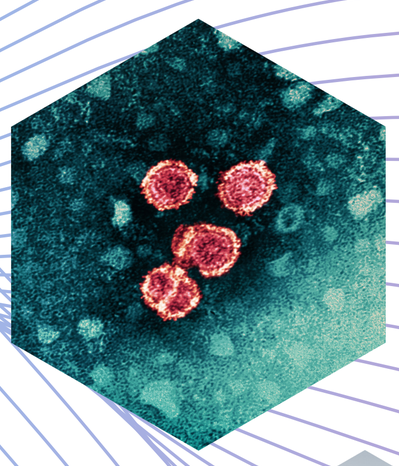
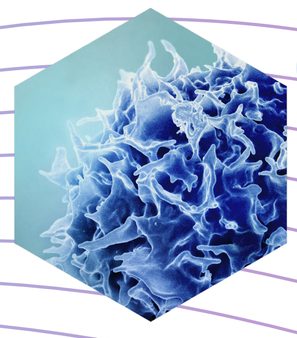
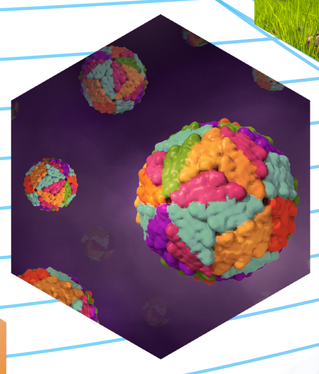
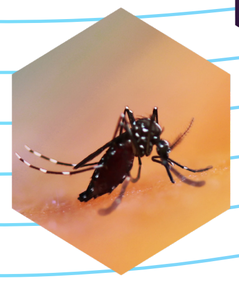
United States — Japan Cooperative Medical Sciences Program

2024 International Conference on Emerging Infectious Diseases (EID) in the Pacific Rim

And meetings of the Acute Respiratory Panel, Cancer Panel, AIDS Panel, Hepatitis Panel, Viral Diseases Panel, Immunology Board, and the 2nd International Symposium for Infectious Diseases Research Institutes Cooperation (IDRIC)


March 5-8, 2024
Hybrid and Grand Hyatt Hotel
Incheon, South Korea

Japan Agency for Medical Research and Development

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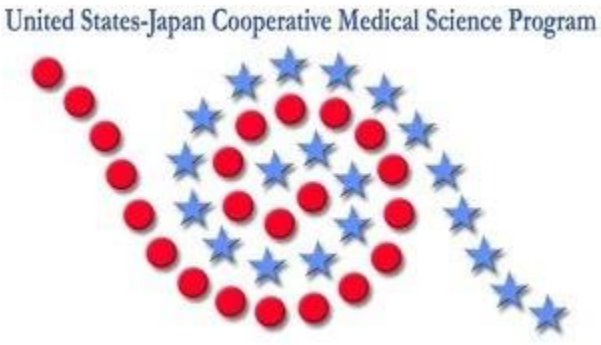


The Joint 24th International Conference on Emerging Infectious Diseases in the Pacific Rim of the U.S.-Japan Cooperative Medical Sciences Program (USJCMSP)

ABSTRACT E-BOOK

March 5-8: Poster Session

[in alphabetical order]

Dr. Jamie Arnold

Strategies to interfere with nucleotide excision by the 3’-to-5’exoribonuclease from SARS-CoV-2

Introduction: Effective antiviral therapeutics are needed to treat SARS-CoV-2 infections. While some of the most efficacious antivirals are nucleos(t)ide analogs, a coronavirus-specific complication is the virus-encoded 3’-to-5’ exoribonuclease (ExoN) which is capable of excising antiviral nucleotides. ExoN, composed of a complex of nsp14 and nsp10, is critical to viral replication as genetic inactivation of nsp14 leads to decreased replication fidelity and increased sensitivity to mutagenic nucleoside analogs. These findings underscore the need to understand ExoN activity, and the potential to antagonize ExoN proofreading to address SARS-CoV-2 infection more effectively with antiviral nucleotides.

Methods: We have expressed and purified components of ExoN: nsp14 and nsp10. We have established a robust, quantitative system to study the specificity and efficiency of ExoN activity and elucidate the kinetic and chemical mechanism of proofreading.

Results: We find that a dsRNA substrate, resembling a primed template, is preferred over ssRNA. ExoN appears to be highly processive under conditions of enzyme excess. However, consistent with the enzyme contributing to proofreading, ExoN is quite distributive, hydrolyzing only one to two nucleotides in a single binding event. The composition of the terminal basepair modulates excision. A stalled SARS-CoV-2 replicase in complex with either correctly or incorrectly terminated products prevents excision, suggesting that a mispaired end is insufficient to displace the replicase. Finally, we have discovered several modifications to the 3'-RNA terminus that interfere with or block ExoN-catalyzed excision.

Conclusion: We conclude that design of ExoN-resistant, antiviral nucleotides will be feasible.

Dr. Marc Edsel Ayes

Evaluating the Real-World Use of Cycle Threshold (Ct) Values to aid Public Health Policy in a National SARS-CoV-2 Biosurveillance Program

Introduction: Cycle threshold (Ct) values have previously been shown to predict genome coverage (Gc) for variant calling with a suggested cutoff value of Ct ≤ 27 to achieve > 90% coverage (Pillay et al., 2020). This formed the basis of sample screening guidelines used to optimize samples submitted to The Philippine Genome Center (PGC) for the Philippine Department of Health Biosurveillance Program monitoring SARS-CoV-2 Variants of Concern (VoC) in the country. A cutoff value of Ct ≤ 25 was initially used to account for possible sample degradation in transit but concerns regarding adequate sample representation across the archipelago prompted loosening of submission requirements to an average Ct ≤ 30 instead. This retrospective study evaluates the real-world use of Ct values in VoC biosurveillance and how it may be used to aid public health policy regarding healthcare and logistical resource allocation in a resource-limited setting.

Methods: Mean Historical Ct values (HCt) used to screen COVID-19 samples prior to submission to PGC for sequencing were retrieved and analyzed alongside the computed Gc (%) and SARS-CoV-2 lineage assignment (Pangolin v4.3.1) obtained after sequencing. Samples sequenced in 2022 (n = 19,223) were included in the study. Gc at different Ct values were compared using basic t-test statistic. Further sub-analysis by island group (i.e. Luzon - Northern group, Visayas - Central group, Mindanao - Southern group) was also performed using binary logistic regression to determine the log-odds of successful lineage assignment with respect to change in HCt at the island group level.

Results: A HCt cutoff value of 30 was found to be just as effective at generating samples with adequate Gc as lower cutoff values (i.e. HCt ≤ 25 and ≤ 27) with a sample HCt ≤ 30 producing a median Gc of 98.93 % (IQR 93.76 – 99.88) compared to HCt > 30 median Gc of 78.02 % (IQR 32.64 – 94.44) ($p < 0.0001$). The Luzon Island group (where PGC is located) was found to have the highest average decrease in log-odds of successful lineage assignment with each unit increase in HCt being correlated to a log-odds of -0.322 ± 0.059 ($p < 0.001$) compared to -0.149 ± 0.052 ($p = 0.004$) and -0.182 ± 0.068 ($p = 0.007$) for Visayas and Mindanao, respectively. This may indicate logistical considerations unique to each island group affecting successful lineage assignment independent of sample HCt.

Conclusion: A more lenient Ct cutoff value of Ct ≤ 30 for SARS-CoV-2 samples provides an adequate Gc for the purposes of VoC biosurveillance with minimal consequence on successful lineage assignment. Binary logistic regression can be used to identify regional differences in successful lineage assignment independent of HCt values. The discrepancy seen with the log-odds lineage assignment of samples from Luzon may indicate logistical factors and practices

that are outside the scope of this study. Such factors may need to be addressed to optimize resource allocation and utilization whilst also minimizing wastage of precious next-generation sequencing resources and may be the focus of future cost-benefit studies.

Dr. Deog-Yuong Choi

Development of in vitro - assembled multivalent VLP vaccine for norovirus infection

Introduction

Human noroviruses are the primary viral pathjirogens causing acute gastroenteritis worldwide, leading to a considerable economic burden. While the development of a norovirus vaccine has been regarded as significant, any vaccine has not been licensed yet. An effective norovirus vaccine development has been difficult due to the wide genetic and antigenic diversity of noroviruses and the co-circulation of multiple variants of various genotypes. In the context of this diversity, effective vaccine must elicit broad protective immunity.

Methods

We have developed trivalent norovirus VLP vaccine candidates based on recombinant VP1 proteins generated in E. coli system. The recombinant VP1 proteins were expressed using the InThera's proprietary technology, the fusion of the target antigen to C-terminus of RNA-interaction domain (RID). Norovirus VLP vaccine candidates were produced through in vitro assembly of the highly purified VP1 proteins. The purity, homogeneity, and impurities of the VLPs were analyzed by physiochemical methods, and the effectiveness of the vaccine candidates was tested by carrying out animal studies.

Results

We confirmed high-efficient soluble expression of the recombinant VP1 proteins in E. coli and the improvements in purity and homogeneity of the norovirus VP1-based VLPs assembled in vitro. The trivalent norovirus VLP vaccine candidates showed excellent immune responses with 2-dose administrations in the animal studies and the vaccine candidate-induced antibodies showed the ability to block the binding of norovirus VLPs and HBGA. Furthermore, GLP-based toxicology and pharmacology data sets confirmed that VLP vaccine candidates did not induce any unexpected toxicity or pharmacological outcomes associated with CNS, respiratory or cardiology aspects.

Conclusion

The results demonstrate that E. coli-derived norovirus VLPs enable mass and rapid production of norovirus vaccines. Moreover, in vitro assembly of purified VP1 proteins facilitated production of high quality VLP vaccine candidates with high homogeneity and low impurities, which might result in increased efficacy and reduced side effects. The results in preclinical studies suggest that the E. coli-derived trivalent VLP has a potential as a norovirus vaccine candidates with high efficacy and safety. Thus, this study supports that the E.coli-derived & self-

assembled norovirus VLPs are worthy to proceed into clinical study for further development as a vaccine candidate.

Dr. Yongwook Choi

Establishment of the artificial HBV cccDNA system for antiviral drug screening

Despite efficient nucleotide analog drugs, covalently closed circular DNA (cccDNA) is an important barrier to hepatitis B virus (HBV) treatment and is difficult to study because of its low copy numbers, even in primary human hepatocytes (PHH). In this study, we established a system for the expression of artificial recombinant HBV cccDNA (rcccDNA) in vitro and confirmed that this system was acceptable for seven HBV genomes, indicating the possibility of drug screening. It can overcome the barrier of low copy number in of primary human hepatocytes in HBV and is suitable for drug screening. Moreover, it is an easy detection system in vitro using direct cell lysis and qPCR. In addition, 264 antiviral compounds were tested, and six of them, including danoprevir, L-cycloserine, phenytoin sodium, amantadine, and germacrone showed a decrease between 32-35% in cccDNA. This suggests the possibility of a cccDNA target drug screening system. We will further confirm whether these chemicals are related to the decrease in cccDNA and enhance this system to a stable cell line with loxP-HBV and cre genes.

This work was supported by the intramural fund (grant numbers 2022-NG-004-02) from Korea National Institutes of Health.

Ms. Hannah Patricia Chua

The Philippine SARS-CoV-2 Genomic Biosurveillance Initiative

Introduction: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was responsible for over 772 million confirmed cases and roughly 7 million deaths globally. Since its entry, a total of 4.1 million cases and 67,000 deaths have been reported in the Philippines. The emergence of multiple variants of concern (VOC) served as an impetus for the World Health Organization (WHO) to urge countries to conduct genomic biosurveillance. The Philippines responded to this call with a streamlined biosurveillance network to effectively and efficiently monitor the virus' trajectory in the country at the height of the COVID-19 pandemic.

Methods: COVID-19 positive samples from partner hospitals and diagnostic labs were sent for whole genome sequencing. Reference-guided assembly was performed on the SARS-CoV-2 sequences to generate a consensus sequence for variant analysis and lineage classification. Phylogenetic trees were then constructed for tracking transmission and local evolution. Mutation profiling was also performed to identify possible emerging lineages and local mutations of interest.

Results: The Philippine SARS-CoV-2 Genomic Biosurveillance Network, through the Philippine Genome Center (PGC), has sequenced a total of 54,490 SARS-CoV-2 samples, among which 48,460 were successfully classified. The majority of these have been tagged as globally circulating VOC, variants of interest (VOI) and variants under monitoring (VUM). These include lineages B.1.1.7 (Alpha), B.1.351 (Beta), P.3 (Theta), B.1.617.2 and AY.x (Delta), B.1.1.529 and BA.x (Omicron), and hybrid forms of the virus. A subset of these cases (Alpha - 107, Beta - 110, Theta - 43, Delta - 254, Omicron - 485, Hybrid - 56) collected from January 2021 to February 2023 were subsequently subjected to phylodynamic analysis and mutation profiling. These analyses have provided insights into the entry and transmission of SARS-CoV-2 in the country and led to the identification of possible locally evolved lineage clusters.

Conclusions: The rapid generation time of viruses such as SARS-CoV-2 paired with their highly mutable nature necessitate the development of a rapid response and a more robust workflow in order to track their emergence and spread. Such methods, from sequencing viral genomes to generating phylogenetic trees and mutation profiles, aid users in determining causes of outbreaks, the origin of a virus, its movement between communities and the possibility of a new lineage surfacing. By having this information readily accessible and shared with the global community, the overall public health response can be better fine-tuned for understanding and mitigating epidemics as they arise.

Dr. EunJoo Chung

SARS-CoV-2 Spike Mutations In HLA-A24-Restricted Epitopes Evade CD8+ T Cell Responses In COVID-19 Vaccines

Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron variants contains more than thirty mutations within its spike protein. These genetic changes raised concerns on additional immune evasion against previous infection and vaccination. Cellular immunity plays an important role in determining the disease severity in Coronavirus disease 2019 (COVID-19) patients, therefore, vaccine-escape mutations in T cell epitopes needs to be investigated. Here, we predicted SARS-CoV-2 spike-specific T cell epitopes for major HLA-A subtypes in Koreans using web database and selected several peptides that carry mutations in variants. And then, CD8+ T cell immune responses after stimulation with original or mutated epitopes were assessed in vaccinees.

Methods & Results: First, we confirmed the mild decline of cellular immunity against peptide pools of Omicron BA.1 compared to wild-type (WT) in Ad26.COV2.S (Janssen)-vaccinated individuals. These results indicate that most of T cell epitopes in spike protein are considerably conserved in variants. However, cellular immunity may be restricted by gradual accumulation of vaccine-escape mutations. Therefore, we tracked which mutations escape the T cell immune response induced by vaccination. SARS-CoV-2 T cell epitopes in spike protein were predicted using the NetMHCpan 4.1 algorithm for the most frequent HLA-A allele in the Korean population (frequencies of ≥ 10%). A total of 12 CD8+ T cell epitopes, which recorded high HLA binding affinity (rank < 0.2) and contained mutations in variants, were finally selected. The predicted epitopes in each HLA-A alleles were validated in HLA-genotyped PBMCs from vaccinated individuals, using activation-induced marker (AIM) and intracellular staining (ICS) assay. HLA sequences of PBMCs were confirmed by sequence-specific primer (SSP)-PCR and then the samples were classified by subtype. Furthermore, we evaluated CD8+ T cell responses against original and altered epitope peptides, and found vaccine-escape mutations that led to decrease cellular immunity.

Conclusions: Our findings suggest that accumulation of vaccine-escape mutations in T cell epitopes might restrict cellular immunity and increase disease severity in vaccine breakthrough infections by SARS-CoV-2 variants.

Mr. John Michael Egana

Phil-CoVRA: A SARS-CoV-2 genomic surveillance platform for integrated data sharing and visualization

Introduction. The COVID-19 Genomic Biosurveillance project of the University of the Philippines - Philippine Genome Center (UP-PGC) is part of the Philippine COVID-19 Inter-Agency Task Force for the Management of Emerging Infectious Diseases (IATF-EID) tasked to establish a system to identify, screen, and assist Filipinos suspected or confirmed to be infected with emerging COVID-19 variants. Because of the global challenge posed by the pandemic, our research in the UP-PGC endeavors to develop a system for the surveillance of SARS-CoV-2 which includes receiving samples from different hospitals and accredited clinics across the country, sequencing the viral genome, analyzing the data through various bioinformatic analyses, storing and retrieving data, and disseminating results. Due to the highly collaborative nature of solving the pandemic, data sharing is a crucial aspect in conducting research involving various laboratories, policy-making bodies, clinics, and the general public. To greatly increase workflow efficiency, we developed the Philippine SARS-CoV-2 Research Analytics (Phil-CoVRA) web application, a local data visualization dashboard and data sharing platform tailored for the swift and comprehensive analysis of SARS-CoV-2 viral sequences.

Methods. Phil-CoVRA is implemented with a variety of open source tools to cater to different functionalities. In-house document-based databases housing the anonymized patient clinical metadata are deployed on-premise per sequencing facility across the country. To accommodate these databases housed in different facilities, we implemented Trino which connects to databases and serves as a highly parallel and distributed query engine. Superset serves as a data visualization dashboard. The larger bioinformatics analyses results files such as FASTA, FASTQ, variant call format, and phylogenetic trees are made accessible via MinIO, an S3-compatible object storage solution, which can be queried and downloaded on-demand. These services are currently deployed locally in the high performance computing cluster of the Core Facility for Bioinformatics in UP-PGC.

Results. Three main modules of Phil-CoVRA are: (1) BioViz, a dashboard to access and visualize the entirety of or a subset of currently available sequencing data as interactive graphs and charts which can be tweaked in real time, (2) BioSearch, a search function that views sample metadata information which can be filtered, and downloaded in various formats to facilitate other downstream applications, and (3) BioLinks, local instance of the Nextstrain platform which shows the phylogenetic tree of a filtered set of sequences. This interface is supported by a distributed database application that can be deployed in different collaborating laboratories, allowing for a flexible implementation and management of the data schema following certain interoperability standards for easy data aggregation.

Conclusions. We developed a platform that facilitates real-time visualization of SARS-CoV-2 genomic data, empowering researchers and healthcare professionals to discern lineage-specific patterns of transmission, monitor variants, and contribute to proactive response strategies for COVID-19. This emerges as a crucial asset for advancing the surveillance of SARS-CoV-2 in the Philippines, and in the future may cater to other emerging infectious diseases.

Dr. Wakako Furuyama

Development of a visualizing system for the Ebola virus glycoprotein

[Introduction]

Ebola virus (EBOV) causes severe EBOV disease (EVD) in humans. Limited countermeasures are currently available and EBOV must be studied in biosafety level (BSL)-4 laboratories. The EBOV glycoprotein (GP) is the single transmembrane protein responsible for the entry step, which is targeted by multiple approved drugs. However, the molecular mechanisms of the intracellular dynamics of GP in the lifecycle of EBOV are poorly understood. Thus, we attempted to develop a novel GP monitoring system under BSL-2 conditions.

[Methods]

We constructed plasmids containing the coding sequence of EBOV GP, in which the mucin-like domain (MLD) was replaced with fluorescent proteins. The effect of the substitution of fluorescent proteins on the GP-mediated entry efficacy and morphology of viral-like particles (VLPs) was assessed using a vesicular stomatitis virus-pseudotyped virus system and electron microscopy, respectively. We further examined the function of fluorescent protein-fused GP in the context of the transcription- and replication-competent VLPs (trVLPs). The morphology, replication efficacy, and intracellular dynamics of fluorescent protein-fused GP derivatives were compared with wild-type GP in a trVLP system.

[Results]

The fluorescent protein-fused GP was fully functional for virus entry and exhibited similar antibody neutralization profiles to wild-type GP, indicating that replacement of the MLD with fluorescent proteins did not affect its function. Electron microscopy revealed that the morphology of VLPs and trVLPs possessing fluorescent protein-fused GPs were as filamentous as those bearing wild-type GP. We also found that the trVLPs encoding the GP derivatives efficiently replicated for multiple generations. Furthermore, we confirmed that the system enabled the visualization of GP in real-time throughout the trVLP replication cycle.

[Conclusions]

We established a novel system to monitor the intracellular trafficking of GP using a trVLP system. This monitoring system is capable of characterizing the molecular mechanism of EBOV replication and contribute to the development of therapeutics against EBOV disease.

Dr. Rommel Gestuveo

Biosurveillance of SARS-CoV-2 across the central islands of the Philippines shows shifts in lineage dominance and local transmission

Introduction The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the pathogen causing Coronavirus Disease 19 (COVID-19) like many other viruses mutate over time. Most changes have little to no significant impact on the properties of the virus but some changes may affect its behavior – transmissibility, disease severity, vaccine efficacy, and diagnostics. Since January 2020, global strides have been made to monitor the evolution of SARS-CoV-2. Specific mutations across the viral genome led to multiple variants and lineages that are recorded in the Pango nomenclature, allowing the tracking of its transmission worldwide. With the local spread of these variants across a small group of islands in the Philippines, biosurveillance efforts in Western Visayas region has been conducted by the Philippine Genome Center (PGC) Visayas beginning in January 2022 to provide genomic information and technical expertise for better public health measures. Methods SARS-CoV-2 whole genome sequencing was performed on viral RNA extracted from nasal or pharyngeals swab specimens from confirmed COVID-19 cases. Collection and testing were performed by local testing laboratories across Western Visayas from January 2022 to December 2023. Samples were sent to subnational laboratory in Iloilo City for viral RNA extraction and quantification. Viral enrichment and sequencing were performed using the Illumina COVIDSeq Test (RUO) in either an Illumina iSeq 100 or NextSeq 1000 platform. Genome assembly was performed using an analysis pipeline developed in-house as referenced to the SARS-CoV-2 Wuhan strain (NC_045512.2). Lineage assignment was determined using PANGOLIN (v.4.1.2). Phylogenetic analysis was done using Augur (v.16.0.3) and visualized in Auspice (v.2.37. Results As of December 2023, PGC Visayas has sequenced 6,034 samples from all over Western Visayas mostly coming from Iloilo. The most common variant identified is the Omicron variant with multiple lineages. Across the region, BA.2.3 was the most dominant lineage in JanuaryApril 2022. It was surpassed by BA.5.2 in June-July 2022 but later in the same year a surge of XBB strains was circulating in the region. Minimal lineage variation across the region for a given time period was observed, indicating a conserved population of circulating strains. This could be due to increased local travel after restrictions were lifted or changes in quarantine measures. Mutation analyses of dominant lineages revealed profiles that are comparable to other Philippine isolates especially among XBB and BA.5.2 strains. These mutations were greatly observed in structural genes coding for the spike protein. A more thorough analysis could lead to the identification of mutations that are unique to the isolates from the region. Conclusion The molecular epidemiology of SARS-CoV-2 in the region has revealed the dynamics of the virus in terms of lineage dominance and diversity. Local viral spread was evident regardless of geographical borders across the island group as shown by the same changes in the dominant lineage throughout the time period.

Possible changes in virus transmission and dominant lineage may have been due to changes in public health policies, ease of lockdowns, and increased human mobility

Dr. Misao Himeno

Development of anti-HBV agents that contain alkaloidal scaffolds bearing Michael acceptors

【Introduction】

Hepatitis B virus (HBV) infection and its complications are estimated to kill 700,000 people each year all over the world. Complete elimination of HBV has not been achieved with existing treatments such as IFN therapy or nucleic acid analogs yet. Furthermore, these treatments have several problems including the requirement of long-term therapy with nucleic acid analogs, hepatitis flare caused by discontinued treatment, and the emergence of drug-resistant strains. Therefore, novel anti-HBV agent development which has different mechanisms of action is demanded.

【Methods】

The purpose of this study is to identify new anti-HBV agents and elucidate their mechanism of action. As a largely underexplored approach to generate anti-viral covalent modulators, we focused on the indole alkaloidal scaffolds with Michael acceptors as the warheads. Based on the structural motifs of covalent drugs and natural products exhibiting antiviral activity, a series of nitrogen-containing skeletons bearing Michael acceptors were designed and synthesized. Synthesized agents were provided to HBV-replicating HepAD38 cells and HBV-infected HuH-7-NTCP cells. After two weeks of inoculation, HBsAg in cell culture supernatants and HBV relaxed circular DNA (rcDNA) levels were detected. Hit agents were provided for proteomic analysis.

【Results】

Synthesized agents had a notably high hit rate and were found to exert potent in vitro anti-HBV activities, reducing viral load both in HepAD38 cells and HuH7-NTCP cells, with little cytotoxicity to hepatocytes. The proteomic analysis showed notable homologies between hit agents and HSP90 inhibitors. The binding of hit agent and HSP90 were detected as well.

【Conclusions】

We found out several potent anti-HBV agents that contain alkaloidal scaffolds bearing Michael acceptors. The mechanism of action appears the inhibition of HSP90 activity.

The discovery of anti-HBV natural product-inspired covalent modulators with significantly higher hit rates than conventional small molecule screening demonstrates the potential of this proposed design and synthetic strategy to generate novel anti-viral chemical entities.

Dr. Yun-Ho Hwang

Live attenuated smallpox vaccine candidate (KVAC103) efficiently induces protective immune responses in mice

Introduction: Smallpox, caused by the variola virus belonging to the genus Orthopoxvirus, is an acute contagious disease that killed 300 million people in the 20th century. Two strains exist: Variola major (Asian smallpox), with a mortality rate of 20%–45%, and Variola minor (alastrim), with a mortality rate of 1%–2%. Since it was declared to be eradicated and the national immunization program against it was stopped, the variola virus has become a prospective bio-weapon. Most species in the genus Orthopoxvirus are strictly host specific; however, species such as cowpox and monkeypox viruses naturally infect a variety of mammals. Therefore, humans are not safe from vaccinia, cowpox, or monkeypox viruses, and this risk is expected to increase. Monkeypox infections were associated with travel to places with circulating monkeypox (endemic areas) or with exposure to infected animals, Cases of monkeypox infection in non-endemic countries reappeared worldwide in 2022. It is necessary to develop a safe vaccine that protects people from terrorism using this biological weapon and that can be administered to immunocompromised people. Our previous study reported on the development of an attenuated smallpox vaccine (KVAC103).

Methods: This study evaluated cellular and humoral immune responses to various doses, frequencies, and routes of administration of the KVAC103 strain, compared CJ-50300 vaccine, and its protective ability against the wild-type vaccinia virus Western Reserve (VACV-WR) strain was evaluated. Additionally, we measured neutralizing antibody titers against monkeypox virus using serum inoculated with CJ-50300 or KVAC103.

Results: The binding and neutralizing-antibody titers increased in a concentration-dependent manner in the second inoculation, which increased the neutralizing-antibody titer compared to those after the single injection. In contrast, the T-cell immune response (interferon-gamma positive cells) increased after the second inoculation compared to that of CJ-50300 after the first inoculation. Neutralizing-antibody titers and antigen-specific IgG levels were comparable in all groups administered KVAC103 intramuscularly, subcutaneously, and intradermally. In a protective immunity test using the VACV-WR strain, all mice vaccinated with CJ-50300 or KVAC103 showed 100% survival. Additionally, inoculation with CJ-50300 and KVAC103 induced the production of neutralizing antibodies against monkeypox virus.

Conclusions: KVAC103 could be a potent smallpox vaccine that efficiently induces humoral and cellular immune responses to protect mice against the VACV-WR strain. Additionally, this study shows the possibility of KVAC103 being used as a vaccine against monkeypox virus.

Ms. Ma. Carmel F. Javier

A regional SARS-CoV-2 genomic surveillance program in the central islands of the Philippines reveals lineage diversity

Introduction Since January 2020, the World Health Organization and experts worldwide have been monitoring the evolution of SARS-CoV-2. The emergence of multiple lineages and variants has led to efforts to monitor its spread across a small group of islands in the central part of the Philippines. The Western Visayas region is comprised of three major islands where genomic surveillance at the start of the pandemic was limited and outsourced to national sequencing laboratories in Manila. To provide timely genomic information on local transmission, the Philippine Genome Center (PGC) Visayas has been conducting a biosurveillance effort for the region since January 2022. Collaborating with local testing laboratories and securing funding from different agencies, the capacity of PGC Visayas to conduct SARS-CoV-2 whole genome sequencing (WGS) and provide technical expertise was fast-tracked. Methods Nasal or pharyngeal swab specimens from COVID-19-infected individuals were collected from molecular testing laboratories across Western Visayas from January 2022 to December 2023 weekly. The specimens were sent to the subnational laboratory in Iloilo City for viral RNA extraction and quantification. Patient metadata was obtained from repositories of the submitting laboratories. In performing WGS, enrichment, and sequencing were performed using the Illumina COVIDSeq Test (RUO) in either an Illumina iSeq 100 or NextSeq 1000 platform. Reference-based genomic assembly was performed using an analysis pipeline developed in-house. Mapped reads to the SARS-CoV-2 Wuhan strain (NC_045512.2) were performed using minimap2 (v.2.24) with comparisons made using MUMmer (v.4.0). Lineage assignment was determined using PANGOLIN (v.4.1.2). Results Since the start of the biosurveillance project, a total of 6,034 samples have been sequenced by PGC Visayas with the majority of samples coming from Iloilo (67.6%). The confirmed cases had a median age of 50 years old (SD=29.4) with the majority of the samples from female patients (64.1%) and fully vaccinated (69.6%). In terms of disease severity, most of the cases were considered as mild (56.0%). In 2022, the number of COVID-19 cases spiked in February, July-August, and October-November, while in 2023 a rise in the number of cases was observed in May-June. As observed in other parts of the country as well as globally, 99.5% of these cases were due to the Omicron variant. Pangolin classification showed that 39.2%, 13.0%, and 7.8% of the samples were of the BA.5.2, XBB, and BA.2.3 lineages, respectively. These lineages are attributed to the observed spikes in the number of COVID-19 cases in the region. Conclusion The biosurveillance of SARS-CoV-2 in the central islands of the Philippines was a collaborative undertaking between academic, medical, and government agencies across Western Visayas that provides evidence-based genomic data on COVID-19 in the region. The objectives and findings of the project coincide with national and global efforts

in tracking the spread of SARS-CoV-2 that have implications for its epidemiology and future directions in ensuring public health. This initiative was made possible through donations and grants from both the private and public sectors. However, the continued implementation of the project will rely heavily on future funding and new collaborations

Ms. Ouek Jeong

Epidemiology and treatment status of hepatitis patients in a Korea HBV/HCV Cohort Study: a prospective multi-center cohort study

Background

A systematic longitudinal cohort study is required to generate scientific evidence for interventions, treatments and intercept strategies. We established 'the Korea HBV cohort study' and 'the Korea HCV cohort study' which is a group study of general hospitals, to investigate the antiviral treatment effects, epidemiology and clinical characteristics of chronic hepatitis B patients and anti-HCV patients in Korea (HBV cohort: 2015~, HCV cohort: 2007~). Each of the hospitals was approved by the Institutional Review Board (IRB) to participate in these cohort study. Through this cohort, we are currently collecting the electronic case report form (eCRF) and biological samples.

Methods

This cohort included basic characteristic, risk factor, socio-demographic, clinical diagnosis, endoscope, medical imaging, biopsy, and serological test for patients. Through this study, 3,028 and 3,732 patients registered, respectively. we analyzed epidemic status, clinical stage, risk factor and treatment status. We performed the descriptive analysis and Kaplan-Meier analysis All statistical analysis was conducted using SAS 9.4.

Results

In the Korea HCV cohort study, a total of 3,732 HCV-infected patients were included in the study from 2013 to 2021. Males were 50.6%, and the average age was 58 years. Most of participants were genotyped type 1 or 2, and 70% of patients had chronic hepatitis C in baseline. 68.1% of our subjects received antiviral medications for treatment of HCV infection. Tattooing 38%, piercing 33.2%, Needle stick injury 5.7% and living with HCV-infected patients are analyzed as risk factors. Overall, DAAs and pegylated interferon (PEG-IFN) treated 88% (1,469/1,469) and 12% (205/1,469), respectively. In 2013, IFN-based treatment was 96% (91/95%), but more than 97% of patients have been using direct acting antivirals (DAAs) since 2015. Recently, there were more than 99% of subjects used direct acting antivirals (DAAs) medications. In the HBV cohort study, according to our inclusion criteria, a total of 2,398 patients were included. Males were 63.7%, and the average age was 52 years. 74.7% of patients are active hepatitis and 57.8% of them are HBeAg negative. Tenofovir (44.5%) and Entecavir (29.7%) were used for the treatment of hepatitis B. Family history (28.9%), smoking (37.0%), and drinking (46.0%) were analyzed the main risk factors. Tenofovir was used to chronic hepatitis B patients in this study.

Conclusion

We reported basic characteristic, risk factor and treatment status among the hepatitis B and C patients. Further studies are needed on basic, clinical and epidemiologic studies of disease progression. We are preparing the procedures to provide the data or specimens to scientists. These activities will be enabled to us to conduct the national healthcare policy studies for chronic infectious diseases.

Soo-Kyung Jeon

Novel TLR2/3 agonists-based adjuvant can be a potent mucosal vaccine adjuvant for inducing antigen-specific mucosal immunity

Introduction: Mucosal immunity is essential to the body's defense against many different types of infectious diseases. Therefore, a great interest is in developing vaccination strategies that could induce mucosal immunity. We have developed two adjuvant systems, called L-pampo™ and Lipo-pam™, based on Toll-like receptor (TLR) 2 and 3 agonists. These adjuvant systems have been used to develop vaccines against infectious diseases and cancers. L-pampo™ is a complex of TLR2 and 3 agonists that synergistically induces both humoral and cellular immune response. Lipo-pam™ is a liposome-based formulation of L-pampo™, which serves as an antigen delivery vehicle and an adjuvant to induce a strong cell-mediated immune response. Here, we determine whether intranasal or sublingual administration of L-pampo™ and Lipo-pam™ could effectively induce mucosal immunity against Norovirus and SARS-CoV-2.

Methods: For the intranasal delivery model, we administered ovalumin, Norovirus VLP, or Influenza virus(H1N1) antigens with L-pampo™ or Lipo-pam™ after anesthetizing mice with Avertin. For the sublingual administration model, we employed sublingual delivery materials from BioLingus (Switzerland) and formulated vaccine candidates or vaccine formulation candidates with L-pampo™ and SARS-CoV-2 receptor binding domain (RBD). We applied the vaccine solutions under the tongue of the anesthetized mice using a micropipette and rolled the dorsal surface of the tongue for 30 seconds every 5 minutes to simulate the regular tongue movements in conscious animals. We placed mice in anteflexion for 10-20 minutes to prevent them from swallowing the vaccine solution.

To assess the effect of L-pampo™ and Lipo-pam™ on antigen-specific mucosal immunity, we collected samples of saliva, BALF (bronchoalveolar lavage fluid), serum, rectums, feces, spleens, and cervical lymph nodes. We analyzed each antigen-specific mucosal, humoral, and cellular immune response using ELISA, ELISPOT assays, and flow cytometry.

Results: We discovered that L-pampo™ and Lipo-pam™ can increase the levels of Noro VLP-specific histo-blood group antigen(HBGA) blocking antibodies in serum, IgA antibodies in feces, and IgG antibodies in both serum and feces and rectum samples. Additionally, these substances induced cellular immune responses. In a sublingual COVID-19 mouse model, L-pampo™ increased the level of RBD-specific IgA in both saliva and BALF, IgG in serum, and the number of IFN-γ-producing cells in splenocytes. Furthermore, L-pampo™ increased the number of tissue-resident memory T (TRM) cells critical for mucosal immunity.

Conclusions: Our study shows that L-pampo™ and Lipo-pam™ enhance mucosal immunity at the mucosal sites and humoral and cellular immune responses in mice. The results suggest that L-pampo™ and Lipo-pam™ are potent adjuvant systems that can provide protection against various infectious diseases.

Dr. Yuta Kanai

Genetic engineering strategy for generating a stable dsRNA virus vector using a virus-like codon-modified transgene

Introduction: Virus vectors are used to deliver genes of interest to target cells and organs for multiple purposes, including cell transduction, gene therapy, and vaccines. Various RNA viruses are being developed as viral vectors, but it has been reported that foreign genes inserted into the viral genome can be lost during repeated passages. We have been developing viral vectors using rotavirus (RV) and mammalian reovirus (MRV), both belonging to the family Reoviridae. This study aims to analyze the biochemical properties of foreign genes, which affect gene stabilities in RNA virus vectors. We designed artificial genes that mimic the codon usage frequency of RV and MRV genomes and their stability over long-term passages was examined.

Methods: Reporter genes similar to the codon usage frequency of the NSP1 gene of the rotavirus SA11 strain or the L1 gene of the MRV T3D were synthesized, based on commercially available luciferase genes (NLuc, Akaluc) and fluorescent protein genes (ZsGreen, AsRed). Artificially synthesized RV genome-like reporter genes (RvNLuc, RvAkaluc, RvZsG, RvAsR) were used to create recombinant RV vectors with these genes inserted into the NSP1 gene of RV. Similarly, genes with MRV genome-like reporter genes (MrvNLuc, MrvZsG) inserted into the L1 gene or S1 gene of MRV were prepared, and used to create recombinant MRV vectors. RV and MRV vectors expressing reporter genes with unmodified codons were also created, and the virus genome and reporter gene expression in cultured cells after passages were analyzed.

Results: RV vectors expressing the RvNLuc gene or the unmodified NLuc gene were created and these RV vectors were passaged through cultured cells 10 times. The NLuc gene frequently underwent deletion mutations with reduced NLuc activity, whereas the RvNLuc gene was fully maintained, with no decrease in NLuc activity observed. Similar experiments with other reporter genes revealed that reporter genes modified to resemble RV or MRV genomes were more stably maintained over a longer period in the viral vectors compared to unmodified reporter genes.

Conclusions: The stabilities of transgenes in RNA virus vectors differ between the genes of interest, but the molecular mechanisms determining genetic stability remain unknown. This study demonstrated that the stability of a transgene was affected by the nucleotide composition, and altering the codon usage of transgenes to resemble that of the viral genome significantly increased transgene stability in dsRNA virus vectors. The virus-like codon modification strategy enabled generation of stable rotavirus and mammalian orthoreovirus vectors, which could be developed as machinery for gene delivery to the intestines and/or respiratory organs. This technology has further potential to be expanded to other RNA viruses.

Dr. Sakirul Khan

Morphological and Pathological Features of Oita Virus, a Bat-Borne Rhabdovirus

Introduction: Bat-borne viruses have become a significant concern due to their potential to spill over into humans and cause severe outbreaks (ex. SARS, MERS, Nipah, etc.). Therefore, exploring bat-borne viruses is essential in assessing their pathologic and zoonotic potential. With these realities, the Oita virus (OITAV), one of the bat-borne viruses, which belongs to the Rhabdovirus family (genus: Ledantevirus) has come under scrutiny for its association with bats and possible potential implications for human health. Although some features of OITAV including its neurotropic nature and ability to infect sucking mice have been realized, many events are yet to be explored. In this study, we characterized the morphology of OITAV and assessed its pathological potential at the cellular level using transmission electron microscopy (TEM). Also, we compared the pathological features of OITAV with other Rhabdovirus, vesicular stomatitis virus (VSV) and rabies virus (RABV).

Methods: The OITAV used in this study was originally isolated from wild horseshoe bats in Oita prefecture, Japan in 1972. The primary stock of the virus used for this study was prepared from the brain homogenate of OITAV-infected mice. For the morphological characterization, the OITAVs were negatively stained and analyzed by TEM. For replication and pathological analysis, OITAV, VSV, and RABV (CVS strain) were inoculated in Neuroblastoma (NA; C1300) cells at a multiplicity of infection (MOI) of 0.01. The viral replication and cell tropism characteristics were evaluated by RT-qPCR, light microscopy, and/or electron microscopy. For ultrastructural analysis, the infected and non-infected (control) cells were fixed in 2.0% glutaraldehyde and collected at 1-, 3-, and 5-day post-infection (d.p.i.). The TEM analysis was performed using Hitachi HT 7800 electron microscope.

Results: Morphological analysis revealed that OITAV possesses a bullet-shaped structure like other rhabdoviruses (Fig. 1). The length of OITAV was found to be approximately 150–200 nm, with a diameter of around 70–100 nm (Fig. 1a). The nucleocapsid was observed to be tightly helical, measuring approximately 50–80 nm in diameter.

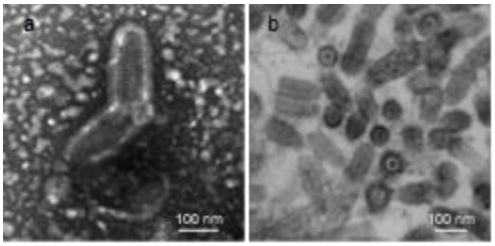


Fig. 1. Electron micrographs of Oita virus (OITAV).

Next, we evaluated the pathological features of infected cells. Results revealed that OITAV potentially infected and replicated in NA cells and was able to form larger plaques within one week of infection. TEM results also revealed that OITAV was replicated in the cytoplasm of the infected cells as observed in VSV and RABV. During the early infection (1 d.p.i.), a small number of virus particles/virions were detected in the infected cells. With increasing infection time, the number of virions was increased (Fig. 1b). Furthermore, cytopathic effects including severe cell damage of OITAV were observed by 5 d.p.i. Compared with VSV, OITAV showed moderate cytopathic potential while RABV had none or a little effect in NA cells.

Conclusions: Our findings reveal bullet-shaped morphology of OITAV which is consistent with the rhabdovirus family. Also, we observed the cellular damage potentiality of OITAV in NA cells. Although more studies are needed to explore the actual zoonotic potential of OITAV, the results presented here enhance our understanding of the replication and pathogenic dynamics of OITAV and provide a foundation for future research.

Mr. Donggun Kim

Discovery of key regulator of biological changes and therapeutic targets in COVID-19 patients using single-cell multi-omics analysis

Introduction

Despite advances in vaccines, conquering COVID-19 remains challenging due to emerging variants and persistent long-term symptoms. Epigenetic changes were highly related with gene regulation and diseases. We conducted a single-cell multi-omics analysis of PBMCs in 15 COVID-19 patients and 3 healthy controls for identifying biological changes under COVID-19 infection. The multi-omics Profiling unveils critical insights into the molecular shifts induced by COVID-19, crucial for comprehending and addressing its infection.

Results

Single-cell multi-omics profiling on the healthy and COVID-19 patients.

To assess the transcriptome and chromatin accessibility landscape of distinct immune cell types, we performed single-cell multi-omics sequencing on three healthy samples and fifteen COVID-19 patient samples divided into four levels of severity (Mild: 2, Moderate: 3, Severe: 6, Critical: 4). After strict quality control and multimodal dimension reduction, we kept 56,383 high-quality cells (Healthy: 12,755 cells; COVID-19: 43,628 cells) and 10 major PBMCs cell types were identified. (Fig 1A) The relative ratio of cell types in the PBMC fractions of each condition reveals a higher abundance of CD14+ monocytes and a lower abundance of CD4+ T cells in COVID-19 compared with healthy. (Fig 1B) Furthermore, we discovered infection-specific changes and highlighted Monocytes as a COVID-19-sensitive cell type in PBMCs. The expression of genes associated with inflammation and immune activation was increased in Monocytes from COVID-19 patients. (Fig 1C)

Identification of COVID-19 infection-specific gene regulation network (GRN) in CD14+ monocyte.

To further investigate the heterogeneity of gene regulation in the CD14+monocyte compartment of COVID-19 patients, we performed sub-clustering the CD14+ monocytes them revealed 2 distinct condition-specific clusters. (Fig 2A) Next, we conducted transcript and epigenetic based trajectory analysis for two condition clusters to identify GRNs enriched in the COVID-19 cluster. In particular, the GRN of the transcription factor (TF) JDP2 increased in the COVID-19 cluster in both its own expression, activity, and expression of target genes. (Fig 2B)

Conclusions

Our single-cell multi-omics analysis unveils the impact of COVID-19 PBMCs, emphasizing the

significance of CD14+ monocytes as sensitive cell types of infection. The observed elevation in inflammation-related gene expression and the identification of a COVID-19-specific gene regulation network, notably featuring the transcription factor JDP2, provide valuable insights into the molecular responses underlying the disease. These findings contribute to the understanding of immune functional changes and long-term sequelae in COVID-19 patients.

Methods

Isolation of single nuclei from human blood samples was performed according to the protocol developed by 10x Genomics (Chromium Single Cell Multiome ATAC + Gene Expression Reagent Kits v1). To analyze single-nuclei-level multi-omics profiling, the raw data were processed using the 10x Genomics Cell Ranger arc v2.0 pipeline. The downstream analysis of single cell multi-omics data was performed using Signac in R.

Figure 2. A. Sub-clustering and trajectory in CD14+ Monocytes **B**. COVID-19 cluster enriched JDP GRN.

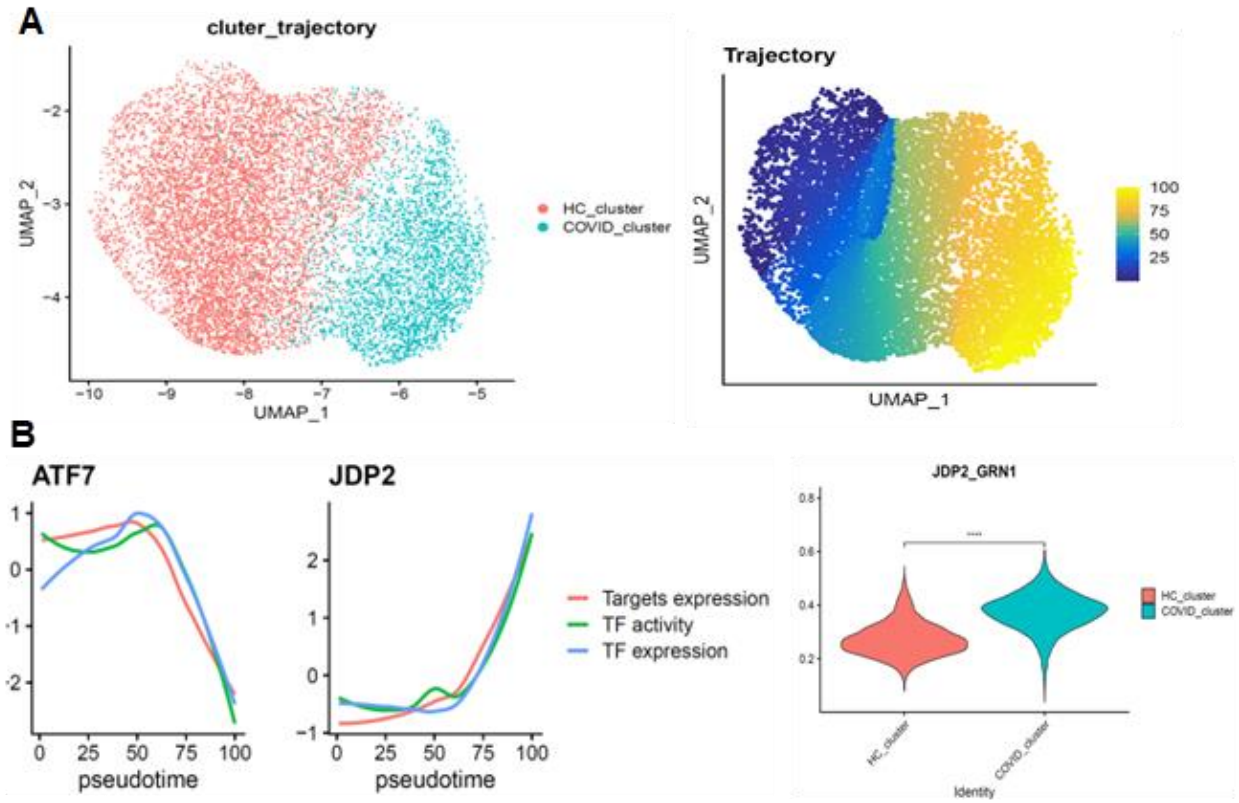
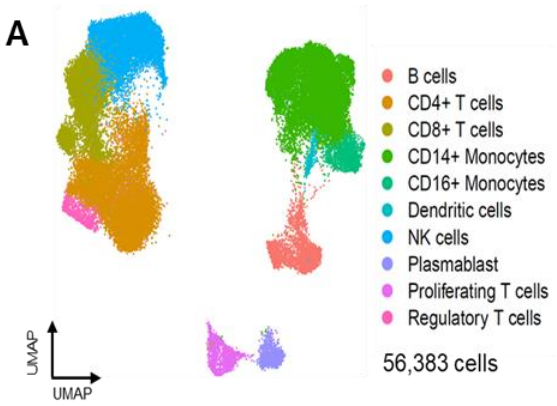
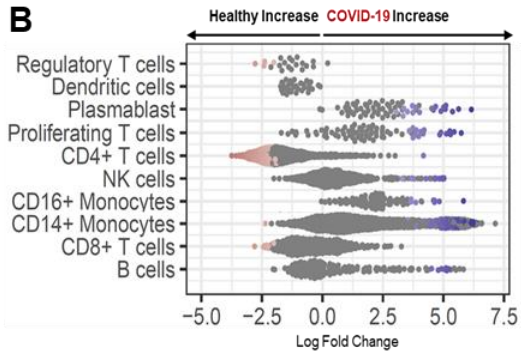
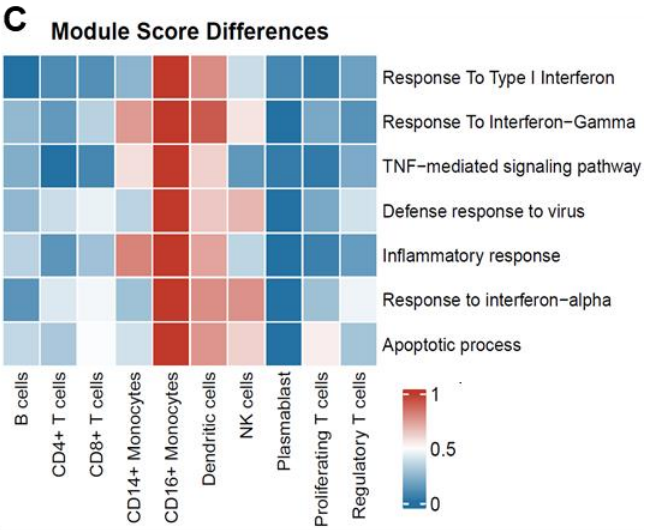


Figure 1. A. The UMAP plot displays 10 major cell clusters **B**. Cell type population plot. **C**. Heatmap of COVID-19 related gene module expression


A
B cells
CD4+ T cells
CD8+ T cells
CD14+ Monocytes
CD16+ Monocytes
Dendritic cells
NK cells
Plasmablast
Proliferating T cells
Regulatory T cells
56,383 cells
UMAP
UMAP


B
Healthy Increase
COVID-19 Increase
Regulatory T cells
Dendritic cells
Plasmablast
Proliferating T cells
CD4+ T cells
NK cells
CD16+ Monocytes
CD14+ Monocytes
CD8+ T cells
B cells
−5.0
−2.5
0.0
2.5
5.0
7.5
Log Fold Change


C
Module Score Differences
Response To Type I Interferon
Response To Interferon−Gamma
TNF−mediated signaling pathway
Defense response to virus
Inflammatory response
Response to interferon−alpha
Apoptotic process
B cells
CD4+ T cells
CD8+ T cells
CD14+ Monocytes
CD16+ Monocytes
Dendritic cells
NK cells
Plasmablast
Proliferating T cells
Regulatory T cells
1
0.5
0

Dr. Tomohiro Kotaki

Dissection of the mechanism of rotavirus inclusion body formation with a focus on viral NSP2 protein

Introduction: Rotavirus, a causative agent of severe diarrhea in infants, is an 11-segmented double-stranded RNA virus. To our knowledge, currently, there are no antiviral drugs against rotavirus infection. Understanding the mechanism of viral replication is vital for the development of antiviral agents. During rotavirus infection, membrane-less inclusion bodies, called “viroplasms,” are formed in infected cells. These structures are essential for viral particle formation and viral RNA replication. Several viral non-structural proteins (NSPs) and host factors are thought to be involved in viroplasm formation. However, the detailed mechanism underlying viroplasm formation remains unclear. In this study, we aimed to elucidate the mechanism of viroplasm formation by focusing on viral NSP2.

Methods: NSP2-mutant rotaviruses were generated using a reverse genetics system. Mutations were introduced into the 23 highly conserved residues of NSP2. Replication of the recombinant viruses was investigated in MA104 cells. Viral RNA kinetics and protein expression were analyzed using qRT-PCR and western blotting, respectively. Viroplasm formation was examined by confocal and immunoelectron microscopy. Fluorescence recovery after photobleaching (FRAP) assay was performed to measure the liquid–liquid phase separation (LLPS) in viroplasms. Furthermore, co-immunoprecipitation and proximity labeling were performed to identify host factors involved in viroplasm formation.

Results: Eighteen recombinant viruses were successfully rescued, of which three exhibited more than a 100-fold reduction in viral replication. These recombinant viruses exhibited decreased protein expression and RNA replication in infected cells. Additionally, they exhibited aberrant viroplasm formation in terms of size, number, and degree of LLPS. These data indicate that the mutated residues in the recombinant viruses are involved in viroplasm formation. To further dissect the mechanism, host proteins interacting with NSP2 and the residues involved were explored through co-immunoprecipitation and proximity labeling. Several host proteins were found to be incorporated into the viroplasms.

Conclusions: This study identified critical residues of NSP2 for viroplasm formation. Additionally, host proteins involved in viroplasm formation were identified. The relationship between the critical residues and the identified host factors is currently under investigation. Further examination of viroplasm formation is important for the development of antiviral drugs against rotavirus as well as other viruses that form membrane-less inclusion bodies.

Dr. Dong Sook Lee

Development of Plant-based Vaccine Candidates for SARS-CoV-2 and Influenza Virus Using Inactivated Lactococcus

Introduction

Since December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused the COVID-19 pandemic. The virus has resulted approximately 680 million cases of human infection with mild to severe clinical symptoms, such as fever, cough, pharyngolaryngitis, and pneumonia. During the pandemic period, seasonal influenza viruses started to recirculated in the winter of 2022, which might increase public health burdens with the twindemic of SARS-CoV-2 and influenza.

Up to date, researchers have explored promising vaccine platforms utilizing diverse approaches, including reverse genetics vaccines, viral vector vaccines, bacterial vector vaccines, DNA vaccines, subunit vaccines, and VLP vaccines, although these are currently only in the stage of laboratory research. In this study, we investigated the efficacy of the vaccine candidates developed using a bacterium-like particle (BLP) method to address the potential threat of the twindemic, we utilized plant-based BLP expressing two proteins simultaneously: SARS-CoV-2 spike protein (SP) and influenza A (H1N1) hemagglutinin (HA), and evaluated vaccine efficacy using a mouse model.

Method

The ectodomain of the SARS-CoV-2 spike protein (SP) and influenza A (H1N1) hemagglutinin (HA) were expressed from plant leaves (Nicotina benthamiana) as a trimer, respectively, and extracted for binding to inactivated Lactococcus lactis (iLact). The iLact-tSP and iLact-tHA were then mixed and monitored for immune responses in a mouse model by immunizing three times at two-week intervals.

Results

Humoral immune responses (IgG, neutralizing antibodies) against SP and HA were detected in the mouse blood sera collected at two, four, and six weeks after immunization, respectively. The titers of both antibodies increased in portion to the antigen concentration, and also rose with the number of immunizations.

Conclusion

In this study, we demonstrated the feasibility of plant-based BLP vaccine development for COVID-19 and influenza. Furthermore, our results also suggest that the BLP can be utilized for vaccine development against viral disease.

Mr. Renato Jacinto Mantaring

A Method for Selecting Compact Markers for Viral Identification

Introduction: Updated tools and algorithms for genomic biosurveillance have paved the way for a deeper understanding of the dynamics of circulating viruses to inform policy as evidenced by evolving classification systems. The establishment of a universal virus nomenclature system for SARS-CoV-2 has inspired researchers to adapt similar conventions for other viruses under surveillance. These systems, however, generally rely on whole genome or whole gene sequencing of large datasets for phylogenetic evaluation which can be expensive and computationally demanding. Furthermore, unlike SARS-CoV-2, data availability for other viruses under surveillance may be sparse and whole genomes may be incomplete or unavailable. Here, we present a method for the selection of smaller marker regions that can create phylogenetic trees of comparable resolution around which single primer pairs can be designed. The method leverages the calculation of conservation and acceleration scores to identify a marker region composed of an accelerated region flanked by conserved regions. We demonstrate this method using a rabies virus dataset which has its own proposed universal nomenclature system, MADDOG (Campbell et al., 2022).

Methods: Whole genome sequences of locally sampled rabies viruses (RABV) were integrated into a pre-existing dataset of global samples curated by Campbell et al. (2022) previously used for the proposed MAD-DOG method of RABV classification. Multiple sequence alignment was performed using MAFFT (Katoh et al., 2002) and regions of conserved and/or accelerated evolution were determined using the PHAST package tool phyloP (Hubisz et al., 2011). Phylogenetic trees of WGS and selected marker regions were generated using IQTREE 2 (Minh et al., 2020) and the Robinson-Foulds (RF) and quartet distance (QD) metrics against the WGS tree were calculated using IQTREE and tqDist to estimate similarity between tree topologies. (Sand et al., 2014).

Results: Using a plot of wig scores generated by phyloP, a selected region of 700 bases long was qualitatively selected between the M1 and M2 genes of the rabies genome. Phylogenetic trees generated were compared using normalized RF (nRF) and QD (nQD) metrics with the phylogenetic tree of whole genome sequences referred to as the reference tree. The tree generated using whole nucleoprotein (N) gene sequences (the current standard marker for rabies virus identification) has an nRF of 0.47 and a normalized QD of 0.044 when compared to the tree generated from whole genome sequences. In comparison, the tree generated from sequences of the abovementioned test region has a normalized RF score of 0.50 and an nQD of 0.050.

Conclusions: Overall, the presented method provides a simple method to identify shorter regions of interest which can generate comparable phylogenetic trees relative to current

standard markers. This approach can easily be adapted to other genomes or viruses under surveillance. The prospect of generating targets that can be captured within a single primer pair as opposed to resorting to multiple primer sequences to capture entire genes may lead to more cost-effective protocols without sacrificing phylogenetic resolution when it comes to identifying viral strains or lineages.

Dr. Meng Ling Moi

The internalization of virus-immune complex by Fc receptor-bearing cells

The internalization of virus-immune complex by Fc receptor-bearing cells plays a pivotal role in antibody-dependent enhancement (ADE), thus amplifying viral replication and potentially worsening the severity of infection. This phenomenon has been observed in flavivirus infections, including Dengue virus (DENV), Zika virus (ZIKV) and Japanese Encephalitis virus (JEV) during secondary infection with a heterologous serotype or different flavivirus. The interaction between IgG Fc receptors and virus-bound antibodies in the development of flavivirus infections serves as a significant mechanism in which ADE operates. To gain a deeper understanding of the mechanism of antibody-dependent mechanism among flaviviruses in vitro, we developed two stable cell lines which express the wild-type (WT) FcγRIIA and, an FcγRIIA that do not possess the cytoplasmic region (CT). By using conventional plaque assay, we determined the ADE activity of these cell lines, using monoclonal antibody and human serum. Initial results demonstrated differential ADE patterns across flaviviruses, in the presence and absence of FcγRIIA cytoplasmic region. The results demonstrate the diversity of ADE activity between flaviviruses in the presence of non-neutralizing antibodies. Further studies are being conducted to determine the role of cytoplasmic region in regulating ADE during flavivirus infection.

Ms. Augustine Natasha

Southern Hantaan Virus Genotype Is Distinct and Responsible for Hemorrhagic Fever with Renal Syndrome, Republic of Korea

Introduction

Hantaviruses, known for causing life-threatening diseases such as hemorrhagic fever with renal syndrome (HFRS), continue to be a significant health concern, particularly in the southern provinces of the Republic of Korea (ROK). Our previous studies identified a novel southern genotype of Hantaan orthohantavirus (HTNV) harbored by Apodemus agrarius chejuensis (A. chejuensis) on Jeju Island. This research aims to investigate deeper into the potential of the southern HTNV genotype as an etiological agent of HFRS, emphasizing its prevalence in the southern ROK.

Methods

Samples of 22 patients with HFRS and 193 small mammal tissues were collected in the southern ROK. The clinical characteristics of patients infected with the southern HTNV genotype were analyzed. Molecular screening using reverse transcription polymerase chain reaction (RT-PCR) was done on small mammal tissues. To obtain whole-genome sequences, we employed multiplex PCR-based MinION sequencing on both patient and rodent samples, facilitating subsequent analyses involving phylogenetics and genetic reassortment.

Results

Nearly complete genome sequences of southern HTNV were successfully recovered from six patients with HFRS and seven A. agrarius. Phylogenetic analysis of the southern HTNV genomes displayed a genetic clustering with genotype harbored by A. chejuensis on Jeju Island. Intriguingly, a genetic reassortment event between the M and S genome segments was identified in a single clinical sample. These results indicate the potential pathogenicity of the southern HTNV genotype in causing HFRS in humans.

Conclusions

The study provides evidence supporting the hypothesis that the southern HTNV genotype is a causative agent of HFRS in the southern ROK. The observed genetic reassortment underlines the dynamic characteristics of southern HTNV tripartite genomes. This emphasizes the necessity for active surveillance and awareness among physicians regarding the emergence of diverse orthohantavirus genotypes capable of inducing HFRS in the ROK.

Dr. Takuto Nogimori

Specific T-cell responses by saRNA vaccine expressing membrane-anchored RBD against SARS-CoV-2

Introduction

The COVID-19 pandemic has driven mRNA vaccine research, offering rapid development and high effectiveness. Although there are many reports regarding the induction and maintenance of antibody responses by LNP-mRNA vaccination, reports on cellular immune responses induced by mRNA vaccines are limited.

In our previous study, we observed that the LNP-mRNA vaccinees with high titers of antibody responses have less spike-specific CD8 T-cell responses compared to CD4 T-cell responses. To overcome this weak induction of CD8 T-cell responses, we explore the potential of a self-amplifying RNA (saRNA) vaccine platform, which theoretically enables longer-lasting and high antigen expression, resulting in less vaccine dose required compared with traditional mRNA vaccines for eliciting T-cell responses.

Methods

We constructed saRNA encoding receptor binding domain (RBD) in spike protein with extracellular transport and transmembrane sequences (saRNA RBD-TM). We measured the antibody titers, antigen-specific CD4 and CD8 T-cell responses in the vaccinated mice and non-human primates (NHPs).

Results

saRNA RBD-TM and mRNA S2P vaccination in mice yielded robust antibody and T-cell responses. Notably, CD4 and CD8 T cells induced by saRNA RBD-TM expressed multiple cytokines compared to mRNA S2P. In NHPs, saRNA RBD-TM induced CD8 T-cell responses in lymph nodes and peripheral blood. Besides saRNA RBD-TM induced potent Th1-biased CD4 T-cell responses in lymph nodes, correlating positively with RBD-specific B cells.

Dr. Philip Ian Padilla

Water-based Epidemiologic (WBE) surveillance reveals presence of SARS-CoV-2 RNA signals in wastewater and river water in Iloilo City, Philippines

Introduction

Global research efforts have revealed the persistence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in feces. This discovery unlocked opportunities for the application of wastewater-based epidemiology (WBE) on COVID-19 monitoring. More than 60 countries, majority with centralized wastewater facilities, have adopted WBE as a surveillance tool. In the present study, wastewater samples from wastewater treatment facilities in Metro Manila were collected, as well as environmental samples along the Iloilo River systems in Iloilo City, Philippines, to detect the presence of SARS-CoV-2 mRNA signals. In addition, a cross sectional study was conducted in the local community along the sampling sites to assess their sanitary practices and knowledge, attitudes, and perceptions towards COVID-19.

Methods

Sampling was performed four times during two periods: October to November 2022 (wet season) and March to April 2023 (dry season). Viral RNA from grab water samples were concentrated using the 0.45 µm membrane filters, and gene copy numbers were estimated using quantitative reverse transcription polymerase chain reaction (RT-qPCR). The amplification targeted the N1 and N2 regions in the viral genome. E. coli count, pH and temperature profiles of sampling sites were also obtained. Additionally, a 47-item questionnaire was administered together with key informant interviews among households along the Iloilo River.

Results

In a survey of 17 sites in Iloilo, we detected SARS-CoV-2 RNA signals in several sites during both dry and rainy seasons with the lowest detection limit of up to 1.3 x 10^5 copies/L (Ct value: >35). Five communities along the Iloilo River consistently displayed the presence of the viral RNA fragments. E. coli count was notably high (1.0 - 91.5 x 10^2 CFU/100 ml) during the wet season and this was observed across 4 to 5 sampling sites. In contrast, during the dry season, E.coli counts were low (1.5 x 10^2 CFU/100 ml) and were detected in a single site. Meanwhile, pH and temperature profiles ranged from 4.0 - 7.5 and 25.0 - 34.0 °C, respectively. Community survey revealed that the majority of the households (80.6%) have a shared single toilet facility with an exclusive septic tank (75.2%) for an average of 4 members. Moreover, the survey indicates that most households utilize cleaning agents before and after defecation and other toilet activities.

Conclusion

The current study elucidated the potential application of WBE as a surveillance tool for SARS-CoV-2 in areas with decentralized wastewater systems in the Philippines. Enforcement of sanitation standards will strengthen prevention and surveillance efforts against emerging viral diseases.

Mr. Carl Justin Palpal-latoc

VARGRAM - Visual ARrays for GRaphical Analysis of Mutations

Introduction: Identifying the genetic variations among a group or groups of viral samples provides information on the functional capabilities of and characteristic differences among infectious diseases. At the Philippine Genome Center, we developed an internal data visualization tool written in Python that shows present mutations among batches of samples that may be compared against a reference lineage. Although there are current resources that visualize mutation counts such as Nextclade, our tool offers more granular flexibility and customization in terms of design and faceting, meant to create static figures for reports or publications. We are actively expanding this tool into a public user-friendly package to offer more biosurveillance visualization features in the future.

Methods: VARGRAM is meant to visualize mutations in a batch or batches of samples. Its data processing module takes in the analysis output file(s) of the batch(es) from Nextclade, an open-source tool that performs mutation calling among other functions, to obtain the amino acid changes per sample. Alternatively, VARGRAM can take in FASTA files directly and call Nextclade. Once the Nextclade output is obtained, the number of occurrences of mutations is counted which is finally visualized (see Figure 1). Depending on the user preference, VARGRAM can additionally show key mutations of reference lineages for comparison. This reference may be provided directly by the user. The generated figure can be saved with the usual file formats (PNG, JPEG, PDF) and a custom resolution set by the user.

Results: VARGRAM provides intuitive visual maps for analysis of mutations. While there are existing tools, ours is focused on generating visually appealing summary figures that allow for quick comparison against reference lineages and among batches of samples. Leveraging the generality of Nextclade, our tool works for any pathogen, provided that at least a reference FASTA (and gene annotation for uncovered pathogens in Nextclade) is provided. By giving the user more customization power and focusing on generating static figures, VARGRAM is a quick and easy-to-use tool for effective communication of insights.

Conclusion: One of the key needs of any biosurveillance program is a summary or analysis of data by way of visualization. VARGRAM addresses this need by providing a visualization tool that shows how a pathogen may be changing through mutation profiling. We are currently developing VARGRAM further to have more capabilities and bolster its relevance in the community. Additional features such as but not limited to transmission mapping (by way of phylogenetic analysis of samples) are underway VARGRAM - Visual ARrays for GRaphical Analysis of Mutations

Ms. Jieun Park

Amplicon-based Sequencing, Genomic Characterization, and Zoonotic Potential Prediction of Chikungunya Virus from a Korean Traveler in Thailand

Introduction

The Chikungunya virus (CHIKV), which is transmitted by arthropods, is an emerging virus that is currently presenting a significant global health threat. The movement of individuals across international borders has played a pivotal role in facilitating the spread of this virus to regions that were previously unaffected. Consequently, it is of utmost importance to conduct genomic analysis of CHIKV strains originating from diverse geographical locations in order to acquire a comprehensive understanding of its evolutionary patterns and transmission dynamics.

Methods

A patient who displayed symptoms of CHIKV infection subsequent to a trip to Thailand was subjected to a collection of clinical and laboratory information. The viral RNA was extracted from the patient's specimen and subsequently subjected to nanopore sequencing. A comprehensive bioinformatics analysis was conducted to genotype the genome sequence of CHIKV. Further analysis was conducted using zoonotic prediction program by machine-learning model.

Results

The CHIKV strain's near-complete genome sequence was successfully determined, with this particular sequence representing the initial documented instance of whole-genome sequencing of CHIKV in South Korea. Through the process of genetic analysis, significant observations have been made regarding the viral genome, providing insights into its origin and potential modifications.

Conclusions

The discoveries made in this study underscore the significance of genomic surveillance in monitoring the emergence and spread of infectious diseases such as CHIKV. The identification of CHIKV in South Korea emphasizes the necessity for robust monitoring systems and preparedness strategies. This study serves as a demonstration of how next-generation sequencing can be utilized to elucidate the molecular epidemiology of pathogens that are disseminated globally.

Ms. Julia Theresa Regalado

Whole genome sequencing and analysis of SARS-CoV-2 genome in samples collected from the Lung Center of the Philippines

Introduction COVID19, a disease caused by the SARS-CoV-2 virus, has rapidly spread causing a global pandemic beginning in 2020. The widespread activity of SARS-CoV-2 has brought about the importance of studying its molecular characteristics which can be done through whole genome sequencing. Mutations have caused an increase in pathogenicity such as by affecting ACE2 association, replication, protein folding, and fusion. Studying these will enable proper biosurveillance of the virus to monitor lineages, viral activity, and another possible surge. Methods SARS-CoV-2 patient samples from the Lung Center of the Philippines underwent whole genome sequencing for lineage assignment, mutation analysis, and the construction of a phylogenetic tree. Results The samples collected from March 2020 to December 2020, showed a variation of lineages which were: B.1, B.1.1, B.1.1.263, B.1.1.28, B.1.1.63, B.1.177, B.1.617.2, and B.6. The phylogeny of each lineage indicated major clustering for B.1.1.263 and between B.1.177 and B.1.617.2. Mutation analysis noted similar mutations among amino acids D614G, P314L, S84L, and R203K/G204R. Lineage B.1.617.2 showed mutations for L452R, T478K, and P681R. Conclusion Through whole genome sequencing, proper biosurveillance practice allows for the detection of the current dominant lineage during time of collection. Lineage diversity indicates that the virus is extremely capable of mutational adaptation in order to combat the host immune system. Further understanding of the diversity, relationships, and mutations of the virus enables for proper mitigation practices including vaccine design

Dr. Cynthia Saloma

Long-Term COVID-19 Sequelae by Variant: Insights from a Philippine Cohort

Introduction: Vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has significantly reduced the severity and mortality of symptomatic cases of COVID-19 worldwide. Although the majority of vaccinated individuals contracting the disease go on to make a full recovery, there are many reports of patients experiencing chronic debilitating sequelae post-infection commonly referred to as Long COVID or post COVID-19 condition. A highly heterogeneous, multi-system illness, symptoms associated with Long COVID may include dyspnea, tinnitus, muscle pains, and cognitive dysfunction lasting from weeks to months, to even years. At present, there is a limited but growing body of knowledge regarding the pathophysiology of this condition, and clinical definitions on the diagnosis and management of Long COVID varies between clinical reporting units. Thus, this study aims to document, profile, and compare the long-term sequelae of individuals previously infected with confirmed SARS-CoV-2 Variants of Concern in a Philippine cohort. Characterization of variant-specific long-term sequelae post-infection will help enhance post-COVID patient care by providing additional clinical insight for the effective identification and management of Long COVID.

Methods: SARS-CoV-2 cases confirmed via Next Generation Sequencing as part of a national genomics biosurveillance effort were recruited into the study through a retrospective cohort design with the consent of all patients and approved by the ethics committees of their respective hospitals. Cases of Alpha (n=57), Beta (n=56), Delta (n=55), Omicron (n=55), and Theta (n=45) were identified from selected regions of the Philippines and recruited for interview. Interview questions focused on identifying and qualifying key symptoms and long-term sequelae related to Long COVID based on CDC definitions. Patients were then followed up for a total of three (3) visits at a minimum three-month interval to document changes in symptoms throughout the interim.

Results: Long COVID19 symptoms were reported in 88% of participants (n=235) with more than 80% reporting symptoms per variant. General symptoms such as headache, fatigue, and post-exertional malaise were some of the most frequently reported symptoms across all variants. Neuropsychiatric symptoms such as brain fog, sleep problems, and mood changes were the second most frequently reported symptoms across all variants. Nearly half of all the patients (48%) reported having difficulty thinking or concentrating (brain fog), making this the most frequently reported symptom overall. Upon stratification into variants, brain fog was the most frequently reported symptom in Alpha, Beta, and Delta patients. Fatigue and headache were the second and third most commonly reported symptoms accounting for 45% and 37% of all participants, respectively. Across all variants, fatigue was the most frequently reported symptom in Omicron and Alpha participants alongside brain fog, and the second most

frequently reported symptom in Beta and Delta participants. Changes in smell or taste was reported in 21% of all participants, but over one third of respondents were from the Theta group (38%).

Conclusion:

A large proportion of cases presenting with brain fog as a neurologic sequelae is consistent with other studies on post COVID-19 condition. Further investigations involving functional impact are being considered in a follow up study to determine interventions to help address issues expressed by participants in the study.

Dr. Hye Jin Shin

RSV induces activation of intracellular EGFR on the mitochondrial membrane for virus Abstract

Respiratory syncytial virus (RSV) infects people of all ages, but is one of the most common causative agents of lower respiratory tract infections, such as pneumonia, especially in infants under one year of age. However, no direct treatment has been developed for RSV infections. Epidermal growth factor receptor (EGFR) acts as a homeostatic regulator, orchestrating essential cellular processes, including proliferation, apoptosis inhibition, cell migration and differentiation, mucus production, activation of inflammatory response, and overall maintenance of cell survival. Positioned prominently on the plasma membrane, EGFR exhibits ubiquitous expression across diverse intracellular organelles, including endosomes, mitochondria, nucleus, and lysosomes. EGFR is not only important for understanding diseases related to cell survival and proliferation but also plays a crucial role in understanding viral infections. EGFR trafficking plays a pivotal role in virus-host interactions by translocating EGFR into an array of intracellular organelles, including endosomes, lysosomes, mitochondria, and even the nucleus. Maintenance of mitochondrial homeostasis and EGFR activity is important for human cell growth. This study reported that RSV infection maintained the total cellular ATP levels and promoted the intracellular activity of EGFR to replicate RSV. RSV activates the intracellular EGFR-mediated cell survival signaling cascade and maintains mitochondrial EGFR expression for viral production during early events after infection. The approved EGFR inhibitor, vandetanib, markedly reduces RSV propagation, suggesting that EGFR is an attractive host target for RSV therapeutics. Our results suggest that RSV infection maintains cellular ATP levels and promotes the activation of intracellular EGFR in the mitochondrial membrane, significantly contributing to robust RSV propagation.

Dr. Nikunj Shukla

Non-canonical Adjuvants and Co-adjuvants That Enhance the Immune Response of Vaccines

Introduction: In the face of emerging infectious diseases, there remains an unmet need for vaccine development where adjuvants that safely induce, enhance or sustain immune responses to pathogenic antigens are highly desired. Most of the current vaccines provide only short-term protection and have limited efficacy in immune-compromised individuals such as elderly. Thus, adjuvants play a critical role in boosting the efficacy of such vaccines. While traditional approved adjuvants include aluminum salts, Toll-like receptor (TLR) agonists, emulsion systems, etc. we embarked on the identification of adjuvants that boost the immune responses especially of an approved TLR4 agonistic adjuvant monophosphoryl Lipid A (MPLA). Here we present 3 such adjuvants that boost the antigen-specific immune responses. Methods and Results: 1. Substituted sulfamoyl benzamidothiazole 2D216: Compound 2D216 in this series demonstrated sustained activation of NF-κB after a primary stimulus with a TLR-4 agonist and also enhanced release of immunostimulatory cytokines in human and murine antigen-presenting cells. In vivo murine vaccination studies demonstrated that compound 2D216 acted as a potent co-adjuvant in combination with MPLA that enhanced antigen-specific antibody equivalent to that of AS01B (an approved co-adjuvant system). The combination adjuvant MPLA/2D216 produced Th1 dominant immune responses and importantly protected mice from lethal influenza virus challenge. This combination adjuvant 2D216/MPLA also demonstrated minimal local reactogenicity and no systemic inflammatory response. The Structure-activity relationship studies led to identification of more potent analog 2E151. The mechanism of action (MOA) of these compounds was linked to intracellular Ca2+ elevation via Ca2+ channel(s) at the plasma membrane and nuclear translocation of the nuclear factor of activated T-cells (NFAT). 2. Benzothiadiazole-sulfonamide-thiophene 2G272: Compound 2G272 is also Ca2+ influx inducer that was identified from high throughput screening as enhancer of release of extracellular vesicles (EVs). It enhanced EV release in murine bone marrow-derived dendritic cells (mBMDCs) and increased costimulatory molecule expression on the surface of EVs and the parent cells. EVs isolated from 2G272-treated mBMDCs induced antigen specific T cell proliferation. 2G272 was also found to be a good Th2 adjuvant in vaccination studies enhancing antigen-specific antibody titers. Thus, compound 2G272 enhances the release of EVs with immunostimulatory potency that is a novel tool for EV-based immune studies and vaccine development. 3. Bis-aryl sulfonamide 2F52: Compound 2F52 demonstrated sustained NF-κB and ISRE activation after a primary stimulus. Vaccination with inactivated influenza virus adjuvanted with 2F52 demonstrated protective effects in a murine lethal virus challenge study. The adjuvant activities were mediated by induction of mitochondrial stress and MAVS aggregation. Conclusions: Thus, we have identified several non-canonical novel adjuvants that are safe to use, potent as

adjuvant and which can serve to enhance the immunogenicity of known vaccines as a toolbox for novel vaccine adjuvants.

Dr. Satoshi Taniguchi

Substitution of E1497K in L protein changes plaque phenotype and growth kinetics of Guanarito virus S-26764 strain

[Introduction] Guanarito virus (GTOV), a bi-segmented ambisense RNA virus, is the causative agent of Venezuelan hemorrhagic fever. GTOV belongs to genus Mammarenavirus, family Arenaviridae and has been classified as a Category A bioterrorism agent by the US Centers for Disease Control and Prevention. Despite being a high priority agent, vaccines and drugs against Venezuelan hemorrhagic fever remain to be developed. GTOV S-26764 strain, isolated from a non-fatal human case, shows unclear cytopathic effect (CPE) in Vero cells, posing a significant obstacle to research and development efforts. In this report, we aimed to generate Vero cell-adapted GTOV S-26764 and analyze its characteristics.

[Methods] GTOV strain S-26764 was passaged in Vero cells 17 times with 4-to 7-day intervals. The whole genome sequence of adapted GTOV (wtGTOV-VP17) was determined and compared with that of original strain S-26764 (wtGTOV). In vitro viral growth and plaque morphology of wtGTOV-VP17 were compared with those of wtGTOV. Recombinant GTOVs were generated by the reverse genetics system to characterize wtGTOV-VP17.

[Results] wtGTOV-VP17 showed clear CPE and high yield in Vero cells, compared to wtGTOV. Four amino acid changes [R346K and V484I in nucleoprotein (NP), and E1497K and I2057V in L protein (L)] were found in wtGTOV-VP17 genome. Recombinant GTOV possessing E1497K amino acid change in L showed clear CPE and high yield in Vero cells. Minigenome assay developed in this study revealed that E1497K amino acid change in L was involved in enhancing viral gene replication and transcription efficiency.

[Conclusions] Vero cell-adapted GTOV S-26764 capable of generating CPE will allow us to perform neutralization assays and anti-drug screening against GTOV. Moreover, the developed reverse genetics system will accelerate vaccine and antiviral drug development.

Ms. Akeno Tokunaga

Induce Severe Inflammatory Cytokine Response and Elevated the Number of Infiltrating White Blood Cells in Mice.

Introduction: In our previous reports, epidemic influenza A/H1N1, A/H3N2, and B viruses showed a wide range of growth capability in vitro. The study demonstrated growth-dependent cell death in a growth kinetics assay. However, the relationship between the viral growth capabilities and the severity of inflammation in vivo remains unknown. In this study, we inoculated a wide variety of epidemic influenza virus strains into the mouse nasal cavity to assess the bronchoalveolar inflammation.

Methods: Nasopharyngeal swabs were collected from influenza virus antigen-positive patients living in Tottori Prefecture, Japan in each 3 month-epidemic seasons from 2009 to 2020 (n=4,926). Influenza virus isolation was performed in MDCK cells (n=4,680). The growth capabilities of all types/subtypes (A/H1N1, A/H3N2, B) were analyzed (n=156). Nine strains including high or low growth capability strains were selected for inoculation into the mouse nasal cavity (2x10^9 copies/ml, 30 µl each per nostril), and mouse weights were measured every day. On the third day, mouse bronchoalveolar lavage fluid (BALF) and mouse lungs were collected. Inflammatory cytokine levels (IL-6, TNF-α, IL-1β) in BALF were measured by ELISA. The number of infiltrating white blood cells in BALF was counted with trypan blue staining. The right-side lungs were stained with hematoxylin and eosin (HE) for microscopic observation.

Results: Mice infected with high growth capability strains showed higher concentrations of IL-6 ($r = 0.84$, $P < 0.01$), TNF-α ($r = 0.79$, $P < 0.01$) and IL-1β ($r = 0.83$, $P < 0.01$) in BALF. Additionally, Mice infected with high growth capability strains exhibited an elevation in the number of infiltrating white blood cells in intrabronchial and intra-alveolar space ($r = 0.70$, $P < 0.05$). Lungs of mice infected with high growth capability strains revealed the infiltration of inflammatory cells into the intra-alveolar space and the thickening of the alveolar walls. Mice infected with high growth capability strains showed severe weight loss rate on the third day post-virus inoculation ($r = 0.76$, $P < 0.05$).

Conclusions: Mice infected with high growth capability strains exhibited high levels of inflammatory cytokines and infiltrating white blood cells in BALF, along with pronounced lung tissue damage and severe weight loss. These results suggested that epidemic influenza virus with high growth capability induced severe inflammation in the mouse bronchoalveolar space. Some reports indicated that severe influenza cases were caused by a robust inflammatory response. Therefore, growth capability might be considered as a key factor in influenza prognosis.

Mrs. Ana Vidal

Invasion of bronchioles and alveoli by Streptococcus pneumoniae is driven by a mechanism that results in downregulation of hydrogen peroxide production

Background: Streptococcus pneumoniae (Spn) invades the lung causing pneumonia, a main cause of morbidity and mortality worldwide. In vivo, pneumococci rapidly invade bronchioles and alveoli, translocating to the bloodstream, yet details of this mechanism are scarce. We therefore investigated the mechanistic basis of invasion of the lung. Methods and Results: We first stablished that human bronchiole Calu-3 cells became polarized in a Transwell system device, as evidenced by a stable transepithelial resistance (TEER), 10 days post-seeding. Spn strain TIGR4, or EF3030, infecting polarized Calu-3 cells translocated to the bottom of the Transwell system within 1 h post-inoculation. Isogenic mutant derivatives, Dcps, DlytA, or DglnL, lacking production of capsule, autolysin, or an ABC transporter, respectively, attached and translocated through Calu-3 cells at a similar rate as that of the wt strain. Notably, the translocation of a hydrogen peroxide-deficient mutant either TIGR4DspxBDlctO (lacking capsule) or EF3030DspxBDlctO (encapsulated), was significantly increased after 1 or 2 h post-inoculation, compared to its respective wt strain. A similar increased on translocation of DspxBDlctO, compared to the wt strain, was observed when human alveolar A549 cells were assessed. Regardless of the strain, the TEER of infected bronchial cells remained unchanged after 2 h of infection. XY, XZ and YZ confocal optical middle sections of infected cells revealed abundant wt pneumococci and DspxBDlctO bacteria. Unlike wt pneumococci that were randomly located in the cell cytoplasm, DspxBDlctO pneumococci were observed intracellularly in structures resembling membrane-containing vesicles. Balb/c mice were then infected intranasally with TIGR4, and lungs were harvested, sectioned and the capsule, membranes, and DNA were stained with fluorescence. Confocal studies revealed Intracellular pneumococci in membrane-containing vesicles. Conclusions: This mechanistic study provides evidence that pneumococcal invasion is not a consequence of cytotoxicity but a dynamic mechanism that, as a consequence, results in downregulation of hydrogen peroxide production.

Dr. Mis Su Yim

Nipah Virus

Introduction:

Nipah virus (NiV) was named Nipah after it first appeared in the village of Kampung Sungai Nipah in Malaysia in 1998. NiV is a single-stranded negative RNA virus belonging to the Henipavirus genus of the Paramyxoviridae family. NiV infections have been reported in various parts of Bangladesh almost every year from 2001 to present. NiV is a very deadly virus that causes sporadic infections in humans, resulting in fatal outcomes such as severe respiratory disease, severe nervous system damage, encephalitis, and death, with a mortality rate of 40-75%. Based on phylogenetic analysis, NiV is divided into two lineages: Malaysian (NiVM) and Bangladeshi (NiVB), which are composed of multiple proteomes. Among these, the surface glycoproteins, the glycoprotein (G) protein and the fusion (F) protein, are exposed on the outer surface of the virus envelope. The G protein binds the virus particle to the host cell and promotes the bond between NiV and the host cell. In contrast, the F protein undergoes a conformational change to mediate NiV entry into host cells. The similarity between the G and F proteins of NiVM and NiVB is 95.7% and 98.5%, respectively, indicating very high similarity. Until recently, there was no approved human or animal NiV vaccine or treatment, therefore development is urgently needed. In this study, we aimed to develop and then evaluate a recombinant protein vaccine using G and F recombinant proteins involved in NiV-host cell binding.

Methods:

Total eight sequences retrieved from F or G protein of NiV were selected based on their consensus or ancestor sequences. The selected sequences of F or G protein of Nipah virus for mammalian expression were synthesized from Genscrip and cloned into pcDAN 3.4 by usion restriction enzymes Ecor Ⅰ and Hind Ⅲ at 5’ end and 3’ end, respectively. The G proteins of NiVM and NiVB were synthesized as monomers and tetramers, and the F proteins were synthesized as monomers and trimers. In addition, chimeric proteins containing two forms were also expressed and through various combinations, 17 NiV recombinant protein vaccine candidates were ultimately developed.

Results:

The 17 vaccine candidates developed were evaluated for vaccine antigen immunogenicity after priming and boosting inoculation into BALB/c mice. As a result, humoral immune responses

were induced in almost all vaccine candidates, and in particular, the tetraG2 group (NiVM+NiVB) group induced the highest immune response.

Conclusions:

We confirmed that the NiV recombinant protein vaccine developed through this research can be used as a NiV vaccine candidate. In the future, we plan to proceed with experiments using additional adjuvants and cell-mediated immune response experiments.