The 15th Nagasaki-Singapore Medical Symposium 2024

Greetings

Welcome to the 15th Nagasaki-Singapore Medical Symposium hosted by Vaccine Research and Development Center, DEJIMA Infectious Disease Research Alliance Nagasaki University, Nagasaki University School of Medicine, and Nagasaki University Graduate School of Biomedical Sciences.

I would like to start by expressing my deepest sympathies to those who lost loved ones in the Noto Peninsula earthquake that occurred in January and the Taiwan earthquake that occurred in March of this year. I also sincerely pray for the safety and rapid recovery of everyone in the disaster-stricken areas.

In January 2020, the first case of Covid-19 was confirmed in Japan, and during the resulting pandemic, we experienced various restrictions on almost all aspects of our lives. Education, research and other activities at Nagasaki University were also affected. Having overcome many hardships, it is nice to have the opportunity to hold this symposium face-to-face for the first time in five years since May 2019.

I understand that there will be presentations on tropical infectious diseases, emerging infectious diseases, and immunology today. We hope that this event will encourage more pioneering research in the field of infectious diseases, contribute to advancements in global health, and foster researchers involved in these fields at universities in Nagasaki and Singapore.

Lastly, I sincerely hope this symposium proves to be one that is fruitful and thought-provoking for all participants. I hope you enjoy your stay here in Nagasaki.

Takeshi Nagayasu,
President, Nagasaki University

Time	Day 1: 11th July 2024 (THU)
8:30–9:00	Registration
9:00–9:30	**Opening Remarks** Takeshi NAGAYASU (President, Nagasaki University) Kevin TAN (Dean' s chair Professor, Head of Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore) **Invited Guests** Atsushi KAWAKAMI (Dean, Graduate School of Biomedical Sciences, Nagasaki University) Kazuya IKEMATSU (Dean, School of Medicine, Nagasaki University)
9:30–10:00	**Keynote lecture 1** Hiroyuki MORIUCHI (National Research Center for Control and Prevention of Infectious Diseases, Nagasaki University, Japan)
10:00–10:30	**Keynote lecture 2** Laurent Rénia (Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore)
10:30–10:50	Coffee Break
10:50–11:20	**Keynote lecture 3** Asuka NANBO (National Research Center for Control and Prevention of Infectious Diseases, Nagasaki University, Japan)
11:20–11:50	**Keynote lecture 4** Paul MacAry (Yong Loo Lin School of Medicine, National University of Singapore, Singapore)
12:00–13:00	Lunch
13:00–13:20	**Session 1** Takahiro TAKAZONO (Department of Infectious Diseases, Graduate School of Biomedical Sciences, Nagasaki University, Japan)
13:20–13:40	Kenji OTA (Department of Laboratory Medicine, Nagasaki University Hospital, Japan)
13:40–14:00	Jun Siong LOW (Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore)
14:00–14:20	Haruka ABE (Vietnam Research Station, Institute of Tropical Medicine, Nagasaki University, Japan)
14:20–14:40	Coffee Break
14:40–15:00	**Session 2** Takeshi TANAKA (Infection Control and Education Center, Nagasaki University Hospital, Japan)
15:00–15:20	Kohsuke MATSUI (School of Tropical Medicine and Global Health, Nagasaki University, Japan)
15:20–15:40	Justin Jang Hann CHU (Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore)
15:40–16:00	Vincent TK CHOW (Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore)
16:00–16:20	Coffee Break
16:20–16:40	**Session 3** Shinjiro HAMANO (Department Parasitol, Institute of Tropical Medicine, Nagasaki University, Japan)
16:40–17:00	Fumika MI-ICHI (Central Laboratory, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Japan)
17:00–17:20	Kevin S.W. TAN (Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore)
17:20–17:40	Benoît MALLERET (Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore)
18:00–18:30	Poster presentation I (Odd number) at1F Sensai Hall (with light refreshments)
18:30–19:00	Poster presentation II (Even number) at1F Sensai Hall

Time	Day 2: 12th July 2024 (FRI)
8:30–9:00	Registration
9:00–9:30	**Keynote lecture 5** Kiyoshi KITA (School of Tropical Medicine and Global Health, Nagasaki University)
9:30–10:00	**Keynote lecture 6** Xiaoyuan (Shawn) CHEN (Yong Loo Lin School of Medicine, National University of Singapore)
10:00–10:30	Coffee Break
10:30–11:00	**Keynote lecture 7** Katsunori YANAGIHARA (Graduate School of Biomedical Sciences, Nagasaki University)
11:00–11:30	**Keynote lecture 8** Nicholas RJ GASCOIGNE (Yong Loo Lin School of Medicine, National University of Singapore)
11:30–12:00	**Keynote lecture 9** Osamu KANEKO (Institute of Tropical Medicine, Nagasaki University)
12:00–13:00	Lunch
13:00–13:20	**Session 4** Toshio KODAMA (Department of Bacteriology, Institute of Tropical Medicine, Nagasaki University)
13:20–13:40	Mariko NAITO (Department of Microbiology and Oral Infection, Graduate School of Biomedical Sciences, Nagasaki University)
13:40–14:00	John CHEN (Department of Microbiology & Immunology,Yong Loo Lin School of Medicine, National University of Singapore)
14:00–14:20	Volker PATZEL (Department of Microbiology & Immunology, Healthy Longevity Research Cluster, Yong Loo Lin School of Medicine, National University of Singapore)
14:20–14:40	Coffee Break
14:40–15:00	**Session 5** Kentaro YOSHII (Department of Viral Ecology, Research Center for the Control and Prevention of Infectious Diseases, Nagasaki University)
15:00–15:20	Yuki TAKAMATSU (Department of Virology, Institute of Tropical Medicine, Nagasaki University)
15:20–15:40	ZHANG Yongliang (Department of Microbiology and Immunology, Young Loo Lin School of Medicine, National University of Singapore)
15:40–16:00	Kaiwen CHEN (Department of Microbiology and Immunology, Young Loo Lin School of Medicine, National University of Singapore)
16:00–16:20	Shusaku MIZUKAMI (Department of Immune Regulation, SHIONOGI Global Infectious Diseases Division, Institute of Tropical Medicine, Nagasaki University)
16:20–16:50	Coffee Break
16:50–17:10	**Session 6** Masato TASHIRO (Department of Infectious Diseases, Graduate School of Biomedical Sciences,Nagasaki University)
17:10–17:30	Tatsuro HIRAYAMA (Department of Pharmacotherapeutics, Graduate School of Biomedical Sciences, Nagasaki University)
17:30–17:50	Shotaro IDE (Infectious Diseases Experts Training Center, Nagasaki University Hospital)
17:50–18:00	**Closing Remarks** Koichi IZUMIKAWA (Chair of the organizing committee, Graduate School of Biomedical Sciences, Nagasaki University)

Day 1: 11th July 2024 (THU)

Bauduin Lecture Hall, Ryojun Auditorium 2F

Registration
08:30-09:00

Opening remarks
09:00-09:30 Takeshi NAGAYASU (President, Nagasaki University)
Kevin TAN (Dean's chair Professor, Head of Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore)
Atsushi KAWAKAMI (Dean, Graduate School of Biomedical Sciences, Nagasaki University)
Kazuya IKEMATSU (Dean, School of Medicine, Nagasaki University)

(Chair: Koichi IZUMIKAWA, Graduate School of Biomedical Sciences, Nagasaki University)
Keynote lecture 1
09:30-10:00 Hiroyuki MORIUCHI (National Research Center for Control and Prevention of Infectious Diseases, Nagasaki University)
"COVID-19 in Japan"

(Chair: Koichi IZUMIKAWA, Graduate School of Biomedical Sciences, Nagasaki University)
Keynote lecture 2
10:00-10:30 Laurent RENIA (A*STAR Infectious Diseases Labs, Agency for Science, Technology and Research Singapore)
"New insights in molecular mechanism of invasion of Plasmodium vivax in reticulocytes"

10:30-10:50 **Coffee Break**

(Chair: Hiroyuki MORIUCHI, National Research Center for Control and Prevention of Infectious Diseases, Nagasaki University)
Keynote lecture 3
10:50-11:20 Asuka NANBO (National Research Center for Control and Prevention of Infectious Diseases, Nagasaki University)
" Current preparation for next pandemic and BSL-4 facility in Japan"

(Asuka NANBO, National Research Center for Control and Prevention of Infectious Diseases, Nagasaki University)
Keynote lecture 4
11:20-11:50 Paul MacAry (Yong Loo Lin School of Medicine, National University of Singapore)
" Insights into serological immunity for SARS-CoV-2"

12:00-13:00 **Lunch @Pompe Hall (for guests)**

Session 1
(Chair: Akitsugu FURUMOTO, Infectious Diseases Experts Training Center, Nagasaki University Hospital)
13:00-13:20 Takahiro TAKAZONO (Graduate School of Biomedical Sciences, Nagasaki University)
"Treatment of COVID-19 in Japan"

13:20-13:40 Kenji OTA (Department of Laboratory Medicine, Nagasaki University Hospital)
"The Approach to COVID-19 through Laboratory Medicine"

13:40-14:00 Jun Siong LOW (Yong Loo Lin School of Medicine, National University of Singapore)
"Pan-coronavirus T and B cell responses"

14:00-14:20 Haruka ABE (Vietnam Research Station, Institute of Tropical Medicine, Nagasaki University)
"Characteristics of the spread of COVID-19 in Vietnam and Gabon"

14:20-14:40 **Coffee Break**

Session 2

(Chair: Koya ARIYOSHI, Institute of Tropical Medicine, Nagasaki University)

14:40-15:00 Takeshi TANAKA (Infection Control and Education Center, Nagasaki University Hospital)
"Severe Fever with Thrombocytopenia Syndrome (SFTS) virus infection: a life-threatening infection in Asia"

15:00-15:20 Kohsuke MATSUI (School of Tropical Medicine and Global Health, Nagasaki University)
" Clinical characteristics of Japanese spotted fever and risk factors for severe diseases"

15:20-15:40 Justin Jang Hann CHU (Yong Loo Lin School of Medicine, National University of Singapore)
"The Antiviral Frontier: Strategies for Emerging Viral Threats"

15:40-16:00 Vincent TK CHOW (Yong Loo Lin School of Medicine, National University Health System, National University of Singapore)
"Highly Synergistic and Potent Inhibition of Mouse Coronavirus Infection by Combination Treatment with Remdesivir and Ivermectin"

16:00-16:20 **Coffee Break**

Session 3

(Chair: Shinjiro HAMANO, Institute of Tropical Medicine, Nagasaki University)

16:20-16:40 Shinjiro HAMANO (Institute of Tropical Medicine, Nagasaki University)
" Development of a live attenuated markerless prophylactic vaccine for leishmaniasis and a leishmanin skin test"

16:40-17:00 Fumika MI-ICHI (Institute of Tropical Medicine, Nagasaki University)
" Crucial roles of very long chain dihydroceramides and cholesteryl sulfate during *Entamoeba* encystation"

17:00-17:20 Kevin S.W. TAN (Yong Loo Lin School of Medicine, National University of Singapore)
" Experimental infections with two different *Blastocystis* subtypes are associated with strikingly different microbiome features and pathobiological outcomes"

17:20-17:40 Benoît MALLERET (Yong Loo Lin School of Medicine, National University of Singapore)
" Methylene Blue Treatment Reveals Biomarkers Associated with Cerebral Malaria in Coatneyi-infected Macaque Model"

18:00-18:30 **Poster presentation I (Odd numbers) @1F Sensai Hall (with light refreshments)**

18:30-19:00 **Poster presentation II (Even numbers) @1F Sensai Hall**

Day 2: 12th July 2024 (FRI)

Bauduin Lecture Hall, Ryojun Auditorium 2F

Registration
08:30-09:00

(Chair: Osamu KANEKO, Institute of Tropical Medicine, Nagasaki University)
Keynote lecture 5
09:00-09:30 Kiyoshi KITA (School of Tropical Medicine and Global Health, Nagasaki University)
"Mitochondria as drug target: From parasites to virus"

(Chair: Satoshi KANEKO, Institute of Tropical Medicine, Nagasaki University)
Keynote lecture 6
09:30-10:00 Xiaoyuan (Shawn) CHEN (Yong Loo Lin School of Medicine and College of Design and Engineering, National University of Singapore)
"Cancer Theranostics"

10:00-10:30 **Coffee Break**

(Chair: Koichi IZUMIKAWA, Graduate School of Biomedical Sciences, Nagasaki University)
Keynote lecture 7
10:30-11:00 Katsunori YANAGIHARA (Graduate School of Biomedical Sciences, Nagasaki University)
"Current status and response to drug-resistant bacterial infections"

(Chair: Asuka NANBO, National Research Center for Control and Prevention of Infectious Diseases, Nagasaki University)
Keynote lecture 8
11:00-11:30 Nicholas RJ GASCOIGNE (Yong Loo Lin School of Medicine, National University of Singapore)
"Indole metabolites made by a parasite control T cell differentiation in the gut"

(Chair: Kiyoshi KITA, School of Tropical Medicine and Global Health, Nagasaki University)
Keynote lecture 9
11:30-12:00 Osamu KANEKO (Institute of Tropical Medicine, Nagasaki University)
"Cytoadhesion and rosette formation of Plasmodium knowlesi-infected red blood cells"

12:00-13:00 **Lunch Time @Pompe Hall (for guests)**

Session 4
(Chair: Katsunori YANAGIHARA, Graduate School of Biomedical Sciences, Nagasaki University)
13:00-13:20 Toshio KODAMA (Institute of Tropical Medicine, Nagasaki University)
"Host adaptation and virulence mechanisms of *Vibrio parahaemolyticus*"

13:20-13:40 Mariko NAITO (Graduate School of Biomedical Sciences, Nagasaki University)
"New findings obtained from analysis of virulence factors of periodontal pathogens."

13:40-14:00 John CHEN (Yong Loo Lin School of Medicine, National University of Singapore)
"Pathogenicity islands uncouple prophages from intra-host competition to promote their reproductive success during polylysogeny"

14:00-14:20 Volker PATZEL (Yong Loo Lin School of Medicine, National University of Singapore)
"Non-viral vectors for gene therapy, mitochondrial gene therapy and genetic vaccination"

14:20-14:40 **Coffee Break**

Session 5

(Chair: Naoki IWANAGA, Department of Respiratory Medicine, Nagasaki University Hospital)

14:40-15:00 Kentaro YOSHII (Research Center for the Control and Prevention of Infectious Diseases, Nagasaki University)
"Neuropathogenesis of tick-borne encephalitis virus infection: dendritic transport of tick-borne flavivirus RNA by neuronal granule"

15:00-15:20 Yuki TAKAMATSU (Institute of Tropical Medicine, Nagasaki University)
"Epidemiological and ecological studies of Severe Fever with Thrombocytopenia Syndrome in our laboratory"

15:20-15:40 ZHANG Yongliang (Yong Loo Lin School of Medicine, National University of Singapore)
"Deficiency of IRF3 promotes a beneficial neutrophil response to severe pulmonary bacterial infection"

15:40-16:00 Kaiwen CHEN (Yong Loo Lin School of Medicine, National University of Singapore)
"Cell death during bacterial infection"

16:00-16:20 Shusaku MIZUKAMI (Institute of Tropical Medicine, Nagasaki University)
"Induction of liver-resident memory $CD8^+$ T cells and protection against malaria at extraerythrocytic stage by mRNA-containing lipid nanoparticles"

16:20-16:50 **Coffee Break**

Session 6

(Chair: Takahiro TAKAZONO, Graduate School of Biomedical Sciences, Nagasaki University)

16:50-17:10 Masato TASHIRO (Graduate School of Biomedical Sciences, Nagasaki University)
"Analysis of chronic host-aspergilloma interactions using a novel mouse model"

17:10-17:30 Tatsuro HIRAYAMA (Graduate School of Biomedical Sciences, Nagasaki University)
"*Candida auris*: An Emerging Threat of Multidrug-Resistant Fungal Infections"

17:30-17:50 Shotaro IDE (Infectious Diseases Experts Training Center, Nagasaki University Hospital)
"Nontuberculous Mycobacterial Pulmonary Disease: An Overview and Insights from Nagasaki, Japan"

Closing remarks

17:50-18:00 Koichi IZUMIKAWA (Chair of the organizing committee ,Graduate School of Biomedical Sciences, Nagasaki University)

Abstracts

Keynote Lecture 1

COVID-19 in Japan

Hiroyuki MORIUCHI[1]

[1]Director, National Research Center for Control and Prevention of Infectious Diseases, Nagasaki University, Nagasaki 852-8523, Japan

As of May 2024, the COVID-19 pandemic in Japan resulted in 74,694 deaths, corresponding to 595 deaths among one million population. The mortality rate in Japan is among the lowest in high-income countries, despite Japan being a super-aging and densely populated society. Several factors might be involved in the relative success of pandemic control. First, the Cluster Countermeasures Group in the Ministry of Health, Labour and Welfare soon revealed the characteristics of viral transmission, warning people to avoid the “3 C’s” that contribute to clusters of infection: **C**losed spaces with poor ventilation, **C**rowded places with many people nearby; and **C**lose-contact settings such as close-range conversations. Many Japanese followed the instructions accordingly, even without implementing lockdowns. Second, most Japanese accept wearing a facemask because that is a common practice to avoid pollinosis and colds in Japan. Third, vaccination rates were high, especially in the elderly population.

The COVID-19 pandemic also revealed weak points in Japan. First, Japan is much behind in information technology and digitalisation, delaying the collection, distribution and analyses of epidemiological and clinical data. The shortage of diagnostic kits was also a big problem at the beginning of the pandemic. Second, the surge capacity of medical institutes is small in Japan, having difficulty handling the increasing number of patients. Third, socioeconomic impacts and effects on mental health were always afterthought. Fourth, Japan lost the competition for vaccine or antiviral development, having had no choice but to rely on other countries. Fifth, no command centre, like the CDC in the USA and Europe, copes with such a public health emergency of international concern in Japan.

Japanese government has adopted two strategies to overcome such weaknesses for future infectious disease emergencies. First, the Japan Agency for Medical Research and Development established “The Strategic Center of Biomedical Advanced Vaccine Research and Development for Preparedness and Response (SCARDA)”. Second, the Japan Institute for Health Security (JIHS) will be established by integrating the National Center for Global Health and Medicine (NCGM) and the National Institute of Infectious Diseases (NIID).

Speaker's Profile:

Dr. Moriuchi is a Professor of Pediatrics at the Graduate School of Biomedical Sciences and a director at the National Research Center for the Control and Prevention of Infectious Diseases, Nagasaki University, Japan. He is a President of the Japanese Society for Pediatric Infectious Diseases. He serves as a Councilor of the Japan Pediatric Society, the Japanese Society for Virology, and the Japanese Society for Vaccinology. He was the President of the 9th Asian Congress of Pediatric Infectious Diseases in 2018.

He dedicated himself to the basic research and clinical practice of herpesviruses and HIV-1 at the National Institute of Allergy and Infectious Diseases (NIAID), USA between 1990 and 1999. He received the Young Investigator Award from the American Society for Microbiology in 1996, and several awards from NIAID.

Keynote Lecture 2

New insights in molecular mechanism of invasion of *Plasmodium vivax* in reticulocytes

Laurent RENIA[1,2], Benoît MALLERET[3], François NOSTEN[4,5], Bruce Russell[6]

[1]A*STAR Infectious Diseases Labs, Agency for Science, Technology and Research (A*STAR), Singapore

[2]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

[3]Department of Microbiology and Immunology, Immunology Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, National University Health System, Singapore

[4]Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Sot, Thailand

[5]Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, UK

[6]Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand

Plasmodium vivax causes debilitating disease in human populations, particularly in tropical and subtropical regions. After exiting from the liver stage, blood stage merozoites of *P. vivax* have a strict tropism to reticulocytes. Understanding the mechanisms of its entry into reticulocytes is paramount for the development of effective *P. vivax* blood stage vaccines. While *P. vivax* Duffy-binding protein has been traditionally thought to be the sole receptor mediating *P. vivax* merozoite invasion, this parasite is now recognized to be capable of infecting Duffy-negative individuals, supporting the existence of specific pathways for human reticulocyte invasion. Two additional pathways have been discovered; the first one involves the interaction of the PvRBP2b protein with transferrin receptor (CD71), which is expressed on reticulocytes but not on mature red blood cells. The second pathway involves the PvRBP2a protein which interacts with the reticulocyte specific CD98 molecules. However, the mode of interaction between PvRBP2a and CD98 remains unknown. In addition, there are other Reticulocyte Binding Proteins (RBPs) for which no receptors have been identified. Here, we will report new information on the molecular mechanisms of infection of reticulocytes by *P. vivax* merozoites.

Speaker's Profile

Prof. Laurent RENIA

Senior Fellow and Principal Investigator

A*STAR Infectious Diseases Labs – ID Labs

Professor of Infectious Diseases
Director of the Respiratory and Infectious Diseases
Lee Kong Chian School of Medicine, Nanyang Technological University

Laurent RENIA is currently a professor of infectious diseases and the director of the respiratory and Infectious Diseases Program at the Lee Kong Chian School of Medicine, and in the School of Biological Sciences, Nanyang Technological University. He is also a senior fellow and principal investigator of the A*STAR ID Labs.

He has obtained his Ph.D. in 1991 from University Pierre et Marie Curie (now Sorbonne University) in Paris, France, and did his post-doctoral at New York University (1991-1992) under Victor Nussenzweig. He returned to Paris in 1993 where he obtained a permanent position as a research scientist at the French National Institute of Health (INSERM). Between 2001-and 2006, he became research director at INSERM, co-director, and director of the Department of Immunology at the Institut Cochin. he first joined A*STAR as a senior principal investigator in the Singapore Immunology Network (SIgN) in 2007. He became its Executive Director in 2013 from 2020. In 2020, he founded the A*STAR ID Labs (A*STAR) as its Executive director. He held an adjunct position at the French National Institute of Health (INSERM). His scientific interests cover the immunology of infectious disease, focusing on mosquito-borne and zoonotic diseases, and newly emerging viruses such as SARS-CoV-2. His research focuses on shaping new concepts based on the understanding of molecular and cellular mechanism immunity through the development of animal models and new assays and approaches. He has published more than 400 articles and book chapters.

Keynote Lecture 3

Current preparation for next pandemic and BSL-4 facility in Japan

Asuka NANBO[1]

[1]National Research Center for Control and Prevention of Infectious Diseases, Nagasaki University, Nagasaki 852-8523, Japan

In recent years, numerous emerging and reemerging infectious diseases have occurred worldwide that seriously threaten our society. As a countermeasure against high-consequence pathogens responsible for severe infectious diseases (classified as class 4 pathogens), we are preparing for the full operation of the first-suit-type biosafety level 4 (BSL-4) facility available for basic and applied research at Nagasaki University. To ensure the safe operation of these facilities, appropriate biorisk management and certification of experienced and qualified personnel with necessary knowledge and the skill are indispensable. We are currently developing an appropriate training program to ensure the safety of users involved in research in the BSL-4 laboratory by referring materials derived from multiple BSL-4 facilities in other countries. Here, we provide an overview of the BSL-4 facility at Nagasaki University and its status in the process of full operation.

Speaker's Profile

Asuka NANBO is a professor of the Department of Virus Infection Dynamics, National Research Center for Control and Prevention of Infectious Diseases, Nagasaki University. She works on elucidating the molecular mechanisms of viral infection and pathogenesis of Filovirus and Epstein-Barr virus with respect to host-virus interaction. As a divisional leader for BSL-4 Biosafety Training Unit, she is also responsible for the development of the biosafety training program for the personnel who are involved in the research and operation of the BSL-4 facility.

Keynote Lecture 4

Insights into serological immunity for SARS-CoV-2

Paul MacAry[1]

[1]Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

The scale and duration of neutralizing antibody responses targeting SARS-CoV-2 viral variants represents a critically important serological parameter that predicts protective immunity for COVID-19. In my talk, I'll describe our efforts to analyze the influence of age, co-morbidity and vaccination status on correlates of serological protection in COVID-19 in Asian volunteer cohorts.

Speaker's Profile

Dr. Paul MacAry received his BSc (Hons) in Molecular Biology from Glasgow University in 1992, his PhD in Immunology from Guy's, King's and St. Thomas' (GKT), London in 1998 followed by consecutive Wellcome Trust Postdoctoral Fellowships in Cambridge University until 2005. Since 2005 he has been an independent investigator in the Department of Microbiology and Immunology at the National University of Singapore (NUS). The multi-disciplinary research in his laboratory covers the entire spectrum of scientific endeavour, from basic research to industrial applications with an emphasis on exploiting advances in AI-based data analytics, protein modelling, automation/robotics and protein engineering to study antibody biology and immune repertoire mapping. Paul was a founding member of the Singaporean Society of Immunology (SgSI)-Singapore's first international learned society (www.sgsi.org.sg/). Paul is a Co-founder and Associate Editor of the Nature partner journal 'Vaccines' launched in 2016 (www.nature.com/npjvaccines/), the top vaccine research journal globally. Paul is a member of the Faculty of 1000 (https://f1000.com/) and his research has been featured in covering articles on the BBC, CNA and Reuters. Paul is the current director of the Life Sciences Institute (LSI), one of the most complex biomedical research institutes in Singapore comprising over forty-five research groups from five NUS faculties, >450 research staff and >SGD$160M in active research grants.

Treatment of COVID-19 in Japan

Takahiro TAKAZONO[1]

[1]Department of Infectious Diseases, Nagasaki University Graduate School of Biomedical Sciences,1-7-1 Sakamoto, Nagasaki 852-8501, Japan

The number of deaths due to COVID-19 per 100,000 population in Japan was 601, which was lower than in the United States (3392), the United Kingdom (3444), and Germany (2097). (December 2023 data) Although race may be a factor in this difference, we believe that this low number of deaths is commendable given that Japan is one of the few advanced aging countries in the world. One of the reasons for this may be the universal health insurance system and easy access to medical facilities, despite the limited number of PCR tests available in the early stages of the COVID-19 pandemic.

In Japan, the Ministry of Health, Labour and Welfare (MHLW) has established a severity classification of illness that is roughly equivalent to the NIH criteria in the United States. The available drugs are almost the same as in other countries, with the exception of Ensitrelvir, which was developed in Japan and can be used in patients with mild disease, in addition to Nirmatrelvir/Ritonavir and Molnupiravir, which are also approved in other countries. In this symposium, we would like to present data on Ensitrelvir in addition to an overview of COVID-19 treatment in Japan

Speaker's Profile

Takahiro TAKAZONO MD, PhD, is an associate professor of Department of Infectious Diseases, Nagasaki University Graduate School of Biomedical Sciences. His area of special interest is respiratory infections. He has been involved in clinical and basic research on fungal, NTM and viral respiratory infections.

Session 1-2
July 11th (THU) 13:20-13:40

The Approach to COVID-19 through Laboratory Medicine

Kenji OTA[1], Katsunori YANAGIHARA[1]

[1]Department of Laboratory Medicine, Nagasaki University Hospital, Nagasaki 852-8501, Japan

The pandemic has not only challenged the laboratory department for the establishment of capacity and serving clinical testing but provided the invaluable chances to investigate COVID-19 pathogenesis using clinical specimens. In this talk, we would like to share a part of our COVID-19 research focused on saliva.

At the beginning of the pandemic, the shortages of laboratory testing capacity as well as medical staff and sampling container limited the broad testing strategy. Nagasaki experienced a COVID-19 cluster on a cruise ship in Nagasaki port, and we reported the clinical usefulness of saliva samples as diagnostic specimen, contributed to national approval.

As high SARS-CoV-2 viral load observed in oral cavity, our next interest aimed to clarify the role of oral cavity in the development of the disease. We analyzed and compared the oral microbiome in patients with COVID-19 and healthy individuals and identified the changes in several species. In addition, time-course analysis revealed that the microbiome changes occur at a few days after the diagnosis, followed by gradual recovery.

We further moved on to the conflict between host and pathogen in the oral cavity. Though numerous studies confirmed the effectiveness of vaccination in preventing death and severe disease, the duration of infection prevention was limited. Given the character of saliva droplets containing SARS-CoV-2 virus particles, we hypothesized that the oral mucosal immunity determined by immunoglobulin in saliva could be a clue to elucidate the mechanisms of transmit or defend the infection. We collected saliva samples from the individuals before and after mRNA vaccine shot and clarified that IgA titer against whole spike protein elevated after the 1st shot, presumably reflecting its rapid and broad antigen recognition, while IgG against S1 domain showed increase after the 2nd shot, possibly indicating its more specific targeting role.

The remaining problems surrounding COVID-19, infection prevention by vaccine, assessment of infectiousness and prolonged infection (partially overlapping long COVID), must be solved by increasing research effort, which is sure to contribute to overcoming next pandemic.

Speaker's Profile

Kenji OTA is an assistant professor of Department of Laboratory Medicine, Nagasaki

University Hospital. He has contributed to scaling up the PCR testing capacity in Nagasaki University Hospital as much as 1200 tests/day and tested clinical samples around Nagasaki prefecture. His area of research encompasses the diagnostic testing as well as clarifying the pathology using clinical samples. He is going to keep on COVID-19 research, aiming to clarify the pathogenesis of long COVID.

Session 1-3
July 11th (THU) 13:40-14:00

Pan-coronavirus T and B cell responses

Jun Siong LOW [1,2,3]

[1] Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[2] Cancer Science Institute (CSI) Singapore
[3] Infectious Diseases Labs, Agency for Science, Technology and Research (A*STAR), Singapore

T and B cells are key players in host defense and in immunological memory. In the two studies presented, we used clonal approaches to probe the antigen-specific repertoire of T and B cells in SARS-CoV-2 immune individuals against coronavirus spike glycoproteins. We identified a novel class of antibodies that binds to the cryptic fusion peptide region of the spike protein. These monoclonal antibodies are pan-reactive and display unprecedented neutralizing breadth against human and animal coronaviruses across multiple genera. T cells targeting the fusion peptide are also cross-reactive and can persist long-term as memory T cells. Altogether, these findings propose the fusion peptide as an attractive candidate for consideration in the design of next generation vaccines that can elicit a robust, universal and long-lived T and B cell responses.

Speaker's Profile

Jun Siong Low received his BSc in Biomedical Science from University College London and his PhD in Immunobiology from Yale University. During his PhD study with Prof. Susan Kaech and Prof. Richard Flavell, Jun Siong identified the mechanism that determine the reactivation of tissue resident memory CD8+ T cells. He then moved to Switzerland for his postdoctoral training with Prof. Federica Sallusto and Prof. Antonio Lanzavecchia at the Institute for Research in Biomedicine and ETH Zurich, where he used clonal approaches to dissect human T and B cell responses to self and non-self antigens. In 2022, Jun Siong received the Novartis Young Investigator award and recently moved to Singapore to set up his own lab. He is a National Research Foundation Fellow, Presidential Young Professor at NUS Yong Loo Lin School of Medicine, Department of Microbiology and Immunology, and a Principal Investigator at the Cancer Science Institute and ID Labs at A*STAR. His lab focuses on understanding the antigen-specific T and B cell responses in cancers.

Characteristics of the spread of COVID-19 in Vietnam and Gabon

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COVID-19 had spread rapidly all over the world after its emergence in 2019. The causative agent SARS-CoV-2 is highly transmissible with a broad tissue tropism and has shown the increasing number of mutations relating with immune evasion. We, Institute of Tropical Medicine, Nagasaki University, set a Vietnam Research Station in 2006 in the National Institute of Hygiene and Epidemiology (NIHE) that is governed by the Ministry of Health and is a center of public health organizations in Vietnam. We have maintained close collaboration with NIHE on infectious diseases. Since the declaration of the COVID-19 pandemic, we have collaborated to support materials of diagnostic tests performed in NIHE and to analyze SARS-CoV-2 prevalent in Vietnam. We found that there had been an extraordinarily small number of COVID-19 cases/deaths until the Delta variant began to spread in Vietnam, with no wide transmission of SARS-CoV-2 original strain, D614G variant, and Alpha variant in the country, reporting only 35 deaths before May 2021. After the emergence of the Omicron variant in Vietnam in January 2022, the Omicron variant has rapidly spread in Vietnam and the largest infection peak was observed in March 2022, similar to the cases in other countries. Currently, we are analyzing the mechanism of the very small number of infection cases in the early stage of the pandemic in Vietnam. I have also collaborated with a research institute in Gabon, Central Africa, on viral diseases. I introduce the results of my COVID-19 study in Gabon, indicating a country-specific situation of COVID-19. These results may be informative to prepare for the next pandemic in each country.

Speaker's Profile

Haruka ABE is an associate professor of Vietnam Research Station, Institute of Tropical Medicine, Nagasaki University. His area of research encompasses the genetic and epidemiological analysis of viral diseases in tropical regions. Moreover, he has developed rapid diagnostic methods for viral diseases, especially for COVID-19.

Severe Fever with Thrombocytopenia Syndrome (SFTS) virus infection: a life-threatening infection in Asia

Takeshi TANAKA[1]

[1]Infection Control and Education Center, Nagasaki University Hospital, Nagasaki, 852-850, Japan

Severe Fever with Thrombocytopenia Syndrome (SFTS), is an emerging infectious disease of tick-borne viral diseases caused by Dabie bandavirus (SFTSV, formerly severe fever with thrombocytopenia syndrome (SFTS) virus), belonging to the bandavirus genus of the phenuiviridae family. This infectious disease is considered as endemic in China, Japan, Korea, and also recently reported some cases in other Asian countries such as Vietnam, Myanmar, Thailand, Taiwan and Pakistan. The typical clinical symptoms of severe SFTSV infection include hemorrhagic fever, thrombocytopenia, leukocytopenia, which can potentially lead to fatal outcomes with multi organ failure. Fatalities mainly occur in patients over 50, with reported mortality rates from 10–19%. Its emergence is threatened in Asian and surrounding countries due to the migration of ticks. Currently, there are no effective therapeutics or vaccines to combat the infection of SFTSV. Antiviral and vaccine development are urgently needed. Asian Longhorned Tick (ALHT: Haemaphysalis longicornis) is a main vector of SFTSV and is native to Asian countries. This tick was reported for the first time in the United States in 2017. It has since been found in numerous States in the US. ALHT can reproduce without a male, so a single tick can create a population in a new location. This phenomenon is a threat from a One Health perspective. In this session, updated information on the epidemiology, pathology, treatment, and prevention of this disease and the current status and future prospects for disease control will be presented.

Speaker's Profile

Takeshi TANAKA is a Senior Assistant Professor of Infection Control and Education Center, Nagasaki University Hospital. He is a senior Fellow member of the Japanese Association for Infectious Diseases (ID) and is actively engaged in ID clinical practice, research, and teaching, as well as antimicrobial stewardship and infection prevention. His current research interests are: “regulation of vascular permeability in septic shock and ARDS”, “long term epidemiology of HIV/AIDS, focusing on opportunistic infections and malignancies”, and “improvement strategy of vaccination for solid organ transplant patients in Japan”. Currently, he actively collaborates with the Japan International Cooperation Agency (JICA) as a member of the Japan Disaster Relief Team, as part of the National Infectious Diseases Response Team, and as an ID advisor for JICA project for strengthening countermeasures against ID in Latin American countries (from 2022).

Clinical characteristics of Japanese spotted fever and risk factors for severe diseases

Kohsuke MATSUI[1]

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Japanese spotted fever (JSF) is a tick-borne rickettsial disease caused by the infection of Rickettsia japonica, prevalent mainly in Western Japan. Since the first identification in 1984, the incidence has progressively risen, reaching 420 reported cases in 2020. The impact of the disease is anticipated to escalate further, as the endemic region within Japan is expanding and reports emerge from outside of Japan, including South Korea and China.

The cardinal symptoms of JSF are nonspecific, often complicating the process of timely diagnosis. Although several fatalities have been documented, scant evidence exists concerning the risk factors for severe and fatal JSF. Therefore, we have conducted retrospective multi-center risk factor analysis in Nagasaki prefecture.

Our study encompassed sixty-three cases across four study sites. One-third of cases were categorized as severe JSF, defined as having at least one abnormality in blood pressure, respiratory status, or consciousness. Multivariable analysis revealed that advanced age, male sex, and delayed treatment initiation were risk factors for the severe disease. Beyond factors associated with severity, our research showed significant aspects of the disease, such as more than half of cases being diagnosed with conditions other than rickettsiosis during the first medical consultation, and higher rate of positive blood PCR results in severe cases compared to non-severe cases.

It is important to continue enhancing awareness among clinicians to facilitate prompt diagnosis and among residents in endemic regions to prevent tick bites, particularly elderly men, who are at a high risk of severe disease.

Speaker's Profile

Kohsuke MATSUI is a clinical infectious diseases specialist with extensive experience of working at Nagasaki University Hospital as well as resource-limited settings across Asia and Africa. He currently serves as an academic coordinator at Nagasaki University School of Tropical Medicine and Global Health. His primary research focus is tick-borne infectious diseases, including Japanese spotted fever (JSF) and severe fever with thrombocytopenia syndrome (SFTS).

Session 2-3
July 11th (THU) 15:20-15:40

The Antiviral Frontier: Strategies for Emerging Viral Threats

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The continual emergence and re-emergence of viral diseases underscore the critical necessity for advancing next-generation antiviral strategies. Novel approaches, such as broad-spectrum antivirals and next generation vaccines, are imperative to combatting both known and unforeseen viral threats. In this presentation I will share how we employed an integrated system-wide approaches and technology driven platforms including genome-wide gene silencing profiling, miRNA profiling and proteomics via high-throughput assays, combined with bio-imaging and computational biology to understand the biological complexity of virus-host interactions and translating it into effective antiviral strategies against these viral pathogens. To address the urgent need for treatment options, we have evaluated an array of antiviral strategies from high throughput screening and drug repurposing for potential broad spectrum antiviral therapeutics, to the utilization of molecular intervention (CRISPR-gene editing) as well as the development of next generation live attenuated virus vaccines via self-amplifying mRNA technology. The in-vivo efficacy of these antiviral approaches are also illustrated with murine models established for these viral infections. Finally, the availability and support from high containment facilities such as BSL2-enhanced and BSL3/ABSL3 core facilities are essential to manage the pandemic preparedness research for these high risk viral pathogens. Investing in research and development for these innovative strategies is essential to enhance our preparedness and resilience against viral diseases of medical significance, safeguarding global health security for the future.

Keywords: Antiviral therapeutics; Positive Sense RNA viruses; pandemic preparedness

Speaker's Profile

Dr Justin Jang Hann Chu is currently the Assistant Dean for Academic Affairs and an Associate Professor in the Department of Microbiology and Immunology and Infectious Diseases Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore He is holding a Joint Senior Principal Investigator in the Institute of Molecular and Cell Biology (IMCB), A*STAR. He is the Director of the Biosafety Level 3 Facility NUS. Justin is actively engaged in the study of the molecular virology of positive-sense RNA viruses of medical importance including SARS CoV-2, human enteroviruses that cause HFMD as well as mosquito-borne viruses including Dengue, Zika, West Nile and Chikungunya. The outcomes from these studies are helping to pave the roadmap towards the successful development antiviral strategies including vaccines and antiviral therapeutics. Fifteen patents and numerous scientific awards have been received from his current research. Justin has published over 150 international peer-reviewed scientific publications, six book chapters and over 200 conference papers. A number of these scientific papers are published in top-notch journals including Science, Nature Immunology, Science Translational Medicine, Nature Communications, PNAS, EBioMedicine, PLoS Pathogens. A/P Chu is among the World’s Top 2% Scientists ranking published by Stanford University based on single-year data on citations received during the calendar year 2021, 2022 and 2023. He is the current elected President of the Asia-Pacific Society of Medical Virology and the elected President of the Singapore Society for Microbiology and Biotechnology.

Highly Synergistic and Potent Inhibition of Mouse Coronavirus Infection by Combination Treatment with Remdesivir and Ivermectin

Vincent TK CHOW[1], YL TAN[1], Kevin SW TAN[1], Justin JH Chu[1]

[1]Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University Health System, National University of Singapore, Kent Ridge, Singapore

The COVID-19 pandemic has highlighted the urgent need to design effective antiviral therapies against coronavirus diseases. Murine hepatitis virus (MHV) and SARS-CoV-2 both belong to the *Betacoronavirus* genus – MHV thus serves as a safe and relevant surrogate model for SARS-CoV-2 infections. Remdesivir is an approved and efficacious antiviral drug against COVID-19. Using an *in vitro* model of MHV infection of RAW264.7 macrophages, the safety and efficacy of monotherapy of remdesivir, ivermectin, doxycycline, and chloroquine were evaluated. Of the four single drugs tested, remdesivir monotherapy (at 6 μM) exerted the strongest inhibition of live virus and viral RNA replication of about 2-$\log_{10}$ and 1-$\log_{10}$, respectively. Combination drug therapy was also investigated using remdesivir (6 μM) together with ivermectin (2 μM), doxycycline (15 μM), or chloroquine (15 μM), i.e. above their IC50 values and at high macrophage cell viability of over 95%. The combination of remdesivir and ivermectin exhibited extremely potent synergism by achieving highly significant reductions of about 7-$\log_{10}$ of viable virus and 2.5-$\log_{10}$ of viral RNA in infected macrophages. This synergistic combination also resulted in the lowest cytokine levels of IL-6, TNF-α, and leukemia inhibitory factor. The next best drug combination was remdesivir with doxycycline, which decreased levels of viable virus by ~3-$\log_{10}$ and viral RNA by ~1.5-$\log_{10}$. Proteomic and transcriptomic analyses were conducted to explore the potential mechanisms of action of drug synergism. The promising potential of combination therapy for the treatment of SARS-CoV-2 and other infections will be discussed.

Speaker's Profile

Dr Vincent TK Chow is a medical virologist and molecular biologist who graduated with MD, PhD, FRCPath, MBBS, and MSc qualifications. Currently, he serves as Associate Professor of Microbiology and Principal Investigator of the Host And Pathogen Interactivity Laboratory at the Yong Loo Lin School of Medicine, National University of Singapore (NUS), and is a Principal Investigator of the NUS Infectious Diseases Translational Research Program. Since 1996, he established the Human Genome Laboratory in the Department of Microbiology

& Immunology at NUS that has isolated and characterized several novel human genes and proteins. Dr Chow previously served as President of the Asia-Pacific Society for Medical Virology as well as Chair of the Virology Section of the International Society of Chemotherapy. His laboratory has published over 290 articles in international refereed journals. He has received several awards and honors (including the Murex Virologist Award for Rapid Viral Diagnosis, the Special Commendation Award and Faculty Research Excellence Award from NUS, the Singapore Society of Pathology - Becton Dickinson Award, the Chan Yow Cheong Oration on Medical Virology, honorary Fellowship of the International Society of Antimicrobial Chemotherapy). His research interests focus on the molecular genetics and infectomics of influenza, other respiratory infections, and hand, foot and mouth disease, specifically on the cellular, molecular, and viral pathogenesis of severe influenza and enterovirus 71 infections.

Development of a live attenuated markerless prophylactic vaccine for leishmaniasis and a leishmanin skin test

Shinjiro HAMANO[1]

[1]Department of Parasitology, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan

Leishmaniasis is a neglected tropical disease caused by the protozoan parasite Leishmania transmitted by infected sandflies. Vaccination with live Leishmania major, leishmanization, has been successfully used but is no longer practiced because it results in occasional skin lesions. Thus, a critical point of leishmanization is safety.

Centrin is a calcium-binding protein and essential in the duplication of centrosomes in eukaryotes, including Leishmania. We have developed a dermotropic live attenuated centrin gene-deleted L. major (LmCen-/-) strain using CRISPR gene editing, which is antibiotic-resistant marker-free, does not have detectable off-target mutations and can not duplicate as an amastigote in mammals.

LmCen-/- continuously reduced its number after inoculated in several immune-deficient mice without any symptoms, supporting its safety as a potential live vaccine. A single dose of LmCen-/- confered mice or hamster type I immunity and resistance to not only L. major but L. donovani transmitted by sandflies, indicating its potential to confer cross-species protective immunity.

The cryopreserved LmCen-/- are stable in the liquid nitrogen and deep freezer and induced protective immunity when inoculated after thawing. The cGMP materials are produced in India. We have compiled the Chemistry, Manufacturing and Control (CMC) package for the Investigational New Drug (IND) application submitted to the US FDA.

Speaker's Profile

Shinjiro HAMANO, M.D., Ph.D. is a professor and a Vice Dean of the Institute of Tropical Medicine, Nagasaki University. Since he has been eager to control Neglected Tropical Diseases (NTDs), he specially studied "Parasitology", "Tropical Infectious Diseases", and "Immunology". He would like to develop deep insight into infectious diseases and the surrounding factors from various points of view through both field and laboratory studies. He aims to contribute to new knowledge and provide tools crucial to controlling infectious diseases.

Crucial roles of very long chain dihydroceramides and cholesteryl sulfate during *Entamoeba* encystation

Fumika MI-ICHI[1]

[1]Central Laboratory, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki 852-8523, Japan

Amoebiasis is a parasitic disease caused by *Entamoeba histolytica* infection, and is a serious public health problem worldwide. Cysts are the only form able to transmit to a new host; in other word, infection is solely established by cysts. Cysts are differentiated from trophozoites in a process termed “encystation”. During *Entamoeba* encystation, cell metabolites, components and morphology drastically change, which sequentially exert in an orchestrated manner. Lipids are plausibly among these metabolites. Here, we exploited the state-of-the-art untargeted lipidomics, and characterized 339 molecules of 17 lipid subclasses. Of these, dihydroceramide (Cer-NDS) was found to be among the most induced lipid species during encystation. Notably, in encysting cells, amounts of Cer-NDS containing very long N-acyl chains (≥26 carbon) were more than 30-fold induced as the terminal product of *de novo* metabolic pathway. We also showed that upregulation of *de novo* synthesis of dihydroceramides was induced by cholesteryl sulfate, which is synthesized by sulfation of host-derived cholesterol in *Entamoeba* encysting cells. Furthermore, these very-long-chain ceramides are indispensable for generating membrane impermeability in mature cysts. Because membrane impermeability is prerequisite for becoming dormant cyst that shows resistant to environmental assaults, we reached a conclusion that the lipid subclass of Cer-NDS and cholesteryl sulfate cooperatively play a crucial role for *Entamoeba* cell differentiation and transmission, which is a parasitic strategy.

Speaker's Profile

Fumika MI-ICHI is the Professor of Central Laboratory in the Institute of Tropical Medicine (NEKKEN) at Nagasaki University. Her research interest is the parasitic strategy of *Entamoeba histolytica* and her laboratory is focusing on the lipid metabolism from the aspects of biochemistry, molecular and cell biology, and evolution.

Experimental infections with two different *Blastocystis* subtypes are associated with strikingly different microbiome features and pathobiological outcomes

Kevin S.W. TAN[1], Lei Deng[1], Lukasz WOJCIECH[2], Chin Wen Png[2], Yongliang Zhang[2], Nicholas R.J. GASCOIGNE[2]

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[2]Immunology Translational Research Programme and Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, Immunology Programme, Life Sciences Institute, National University of Singapore, 117597 Singapore

The associations between *Blastocystis* and gut microbiota have been extensively studied, but no study so far has examined the influence of different subtypes (STs) of *Blastocystis* on gut microbiota under experimentally controlled conditions. Furthermore, whether *Blastocystis*-altered gut microbiota affects the development of intestinal inflammatory diseases remains to be determined. In this study, we first investigated the effect of ST4, a common subtype in Europe, and ST7, a rare pathogenic subtype in humans, on the intestinal microbiota in normal healthy mice, and then explored the role of *Blastocystis*-altered gut microbiome in the development of dextran sulfate sodium (DSS)-induced colitis in antibiotic treated wild type and *Rag1*$^{-/-}$ mice. We showed that ST4 infection increased the bacterial diversity and abundance of *Clostridia*, whereas ST7 infection showed opposite effects. Transplantation of ST4-altered microbiota was able to prevent DSS-induced colitis by enhancing the Treg response (increased Foxp3 and IL-10) in the colon lamina propria of recipient mice, while ST7-altered microbiota exacerbated the severity of the colitis by inducing Th1 cell differentiation (increased IFN-γ, and TNF-α). Furthermore, the protective or exacerbating effects of *Blastocystis*-altered gut microbiota on colitis are adaptive immune cell dependent. Our data showed that ST4 and ST7 infections are associated with strikingly different microbiome features, and these alterations have significantly different effects on the severity of DSS-induced colitis. This study supports accumulating evidence that clinical outcomes of *Blastocystis* infection is subtype dependent.

Keywords: *Blastocystis*, gut microbiota, colitis, Treg response, Th1

Speaker's Profile

Kevin SW TAN is Associate Professor, Head, and Dean’s Chair of the Department of

Microbiology and Immunology, National University of Singapore. He is also Vice-Dean, Graduate Studies, at the Yong Loo Lin School of Medicine. His curiosity for parasites originated from his graduate student days at NUS and blossomed during his postdoctoral stint at The Rockefeller University, New York City. He was awarded tenure in 2011, and now devotes more time to academic and administrative service, apart from his research. Kevin's research focuses on understanding how parasites commit suicide and exploiting such knowledge to trigger death mechanisms as an anti-parasite strategy. He is also interested in the problem of drug resistance and his team has developed new ways to find drugs that overcome resistance. More recently, his team has embarked on projects focusing on the role of single cell eukaryotes (SCEs) in the host microbiome. He hopes that the research from his team will accelerate the finding of new cures for parasitic diseases.

Session 3-4
July 11th (THU) 17:20-17:40

Methylene Blue Treatment Reveals Biomarkers Associated with Cerebral Malaria in Coatneyi-infected Macaque Model

Benoît MALLERET[1,3], Jing Wen HANG[1], Yew Wai LEONG[1,2], Vipin Narang[3], Piyanate SUNYAKUMTHORN[4], Shihui Foo[3], Josephine LUM[3], Bernett LEE[3], Arthur E. Brown[5], Laurent RENIA[2,6,7], Gareth D. H. TURNER[8,9], Samuel C. Wassmer[10], Eric D. LOMBARDINI[4], Bruce Russell[11,12]

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[11]Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand
[12]Department of Protozoology, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Japan

Plasmodium falciparum remains a major threat to the public health with its most common severe form of complication, cerebral malaria, which is fatal within 24 to 72 hours. *P. berghei* ANKA infection in CB57BL/6 mice has been widely used as the murine model for human cerebral malaria, but its relevance has been questioned due to their dissimilarity in histopathology. Hence, *P. coatneyi* – the malaria parasites of non-human primates – shares similar pathophysiological features with *P. falciparum* infection, and has been sporadically used as a model for severe malaria.

With the emergence of drug resistance malaria, methylene blue has shown to be effective against chloroquine-resistant *P. falciparum*. Furthermore, methylene blue treatment has improved survival and ameliorated experimental cerebral malaria in murine model. Here, we compared the gene expression profiling in different organs (brain, heart, kidney and liver) of uninfected, untreated and methylene blue-treated Rhesus macaques infected with *P. coatneyi*. We were able to cluster the infected samples from uninfected and treated samples in brain stem based on their genetic profile. Differential gene expression analysis revealed the effectiveness of methylene blue treatment as it reversed the effect of infection on the brain tissues. By comparing the differential expressed genes in three datasets (human infected peripheral blood, *Macaca mulata* infected brain stem and *Macaca mulata* infected blood), we have successfully identified several genes that are associated with cerebral malaria. These biomarkers would accelerate the prediction and diagnosis for cerebral malaria or other complicated infections by *P. falciparum*.

Speaker's Profile

Dr. Benoit MALLERET received his PhD in Immunology at the Alternative Energies and Atomic Energy Commission (CEA) in Paris, France. He subsequently moved from Paris to Singapore to take up a post-doctoral research position to study malaria parasites and host cell tropism at the Singapore Immunology Network (SIgN), A*STAR. In 2018, Benoit became an Assistant Professor in the Department of Microbiology and Immunology at National University of Singapore and his lab focuses on erythrocytic immunobiology and host-microbiome interactions in the gut. He has published over 80 articles with over 6,800 citations in journals such as Science, Immunity and Blood. He was awarded the 2020 Yong Loo Lin School of Medicine Young Researcher of the Year Award. He is also Director of the Electron Microscopy Unit in NUS Yong Loo Lin School of Medicine.

Keynote Lecture 5

Mitochondria as drug target: From parasites to virus

KITA Kiyoshi[1]

[1]School of Tropical Medicine and Global Health, Nagasaki University, Nagasaki 852-8523, Japan

Parasites have developed a variety of physiological functions necessary for their survival within the specialized environment of the host. Using metabolic systems that are very different from those of the host, they can adapt to low oxygen tension present within the host animals. Most parasites do not use the oxygen available within the host to generate ATP even they reside oxygen rich circumstance such as blood, but rather employ systems anaerobic metabolic pathways. In addition, all parasites have a life cycle. In many cases, the parasite employs aerobic metabolism during their free-living stage outside the host. In such systems, parasite mitochondria play diverse roles. In particular, dynamic changes in the morphology and components of the mitochondria during the life cycle are very interesting elements of biological processes such as developmental control and environmental adaptation. As mitochondrial function is essential for the survival of the parasites, it should be promising target of chemotherapy. Anti-malarial atovaquone, which targets cytochrome b of parasite mitochondrial respiratory chain, is a well-known example.

In this talk, uniqueness of parasite respiratory chains and discovery of anti-COVID-19 candidate as well as anti-malarial compound, 5-aminolevulinic acid (5-ALA) will be presented. In addition, recent progress of our study on Nodding Syndrome, an East African freak disease thought to be related to onchocerciasis will be reported.

Speaker's Profile

Dr. Kita was a professor (1998-2016) at The University of Tokyo (UT) and now is Dean of Nagasaki University, School of Tropical Medicine and Global Health (2015-). He was educated at Department of Biological Sciences, UT, and graduated in 1980. He joined Department of Biological Sciences, UT as assistant professor (1980-1983), and moved to Department of Parasitology, Juntendo University, School of Medicine (1983). Then, promoted to associate professor of Department of Parasitology, The Institute of Medical Science, UT (1991-1998).

He has been studying bacterial and mitochondrial respiratory chains from the viewpoint of oxygen homeostasis. After he moved to Juntendo University, he has expanded his research to anaerobic respiratory chain of parasite mitochondria as well as host human mitochondria,

and found that mitochondrial fumarate reductase plays an important role in the parasitic adaptation and cancer cells. Furthermore, he developed several promising anti-helmintics and trypanocidal drugs. He has been dispatched by JICA as a team leader of medical cooperation project to Paraguay (1984-1985). He is a councilor of Japanese Biochemical Society (1998-), Japanese delegate and Treasurer of FAOBMB (2002-2007). He was associate editor (2002-2006) of Journal of Biochemistry. He was executive board of Japanese Society of Tropical Medicine (1994-1996) and Secretary General of Japanese Society of Parasitologist (2000-2003) and President of the Society (2003-2006) and was President of Japanese Biochemical Society (2009 - 2011). He is a winner of the Duke of Edinburgh Award in 2020.

Keynote Lecture 6

Cancer Theranostics

Xiaoyuan CHEN[1,2,3,4,5]

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Theranostics, the combination of ther(apy) and (diag)nostics, aims to develop molecular diagnostic tests and targeted therapeutics with the goals of individualizing treatment by targeting therapy to an individual's specific disease subtype and genetic profile. It can be diagnosis followed by therapy to stratify patients who will likely respond to a given treatment. It can also be therapy followed by diagnosis to monitor early response to treatment and predict treatment efficacy. It is also possible that diagnostics and therapeutics are co-developed (sonotheranostics, immunotheranostics, magnetotheranostics, optotheranostics, radiotheranostics, etc.). This talk will highlight the forms of radiotheranostics and immunotheranostics, especially new mRNA nanovaccines formulas that are therapeutic for cancer and infectious diseases.

Speaker's Profile:

Prof. Xiaoyuan (Shawn) Chen is Nasrat Muzayyin Chair Professor in Medicine and Technology, National University of Singapore. His current research focuses mainly on different forms of theranostics that can be clinically translatable. He has published over 1000 papers and numerous books (total citations ca. 140,000, H index 198 based on Google scholar). He is the founding editor of journal Theranostics (IF 12.4). He was elected as AIMBE Fellow (2017), SNMMI Fellow (2020), and Member of Academia Europaea (MAE, 2024), received JBN Trailblazer Award (2023), SNMMI Michael J. Welch Award (2019), ACS Bioconjugate

Chemistry Lecturer Award (2016), NIH Director's Award (2014), and NIBIB Mentor Award (2012). He became a member of the Advanced Materials Hall of Fame (2023). He is also the Past President of Chinese American Society of Nuclear Medicine and Molecular Imaging (CASNMMI), Past President of the Radiopharmaceutical Science Council (RPSC), Society of Nuclear Medicine and Molecular Imaging (SNMMI), and Past President of the Chinese American Society of Nanomedicine and Nanobiotechnology (CASNN).

Keynote Lecture 7

Current status and response to drug-resistant bacterial infections

Katsunori YANAGIHARA[1]

[1]Departments of Laboratory Medicine, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki 852-8523, Japan

Drug-resistant bacteria are one of the most important public health issues. Methicillin-resistant Staphylococcus aureus and drug-resistant Pseudomonas aeruginosa, which have traditionally been major problems, are on the decrease in Japan because appropriate countermeasures based on many research findings have been implemented.

On the other hand, multidrug-resistant and highly resistant Enterobacteriaceae have become serious. Extended-spectrum β-lactamase-producing (ESBL) Enterobacteriaceae have rapidly increased since 2000, accounting for 20-30% of Escherichia coli and more than 10% of Klebsiella pneumoniae. ESBLs expand the range of drugs that can be degraded, and ESBL-producing bacteria are resistant to many β-lactam drugs, making them an important drug-resistant organism in infection control.

Carbapenem-resistant Enterobacteriaceae (CRE) are a future concern. Enterobacteriaceae are causative agents of sepsis, peritonitis, urinary tract infections, respiratory tract infections, etc. CRE are resistant to carbapenems, which are the specific antimicrobial agents for these infections. Although the number of CRE isolates in Japan is still small, there are concerns about its spread in the future.

In my presentation, I will discuss the current status of drug-resistant bacteria as well as advances in testing equipment and antimicrobial agents.

Speaker's Profile

Katsunori YANAGIHARA is a professor of Department of Laboratory Medicine, Graduate School of Biomedical Sciences, Nagasaki University. He has been researching drug-resistant bacteria for more than 20 years. He is also the head of a research group of the Ministry of Health, Labor and Welfare. He is also involved in the development of new testing equipment and new drugs.

Keynote Lecture 8

Indole metabolites made by a parasite control T cell differentiation in the gut

Nicholas RJ GASCOIGNE[1]

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The microbiome of the large intensity plays a unique role in host physiology. Host-microbiome coexistence can be beneficial or detrimental to the host. This depends largely on the balance between specialized regulators and responders within the intestinal CD4+ T cell population. We found that ulcerative colitis-like changes in the large intestine after infection with the protist Blastocystis ST7 are associated with reduction of anti-inflammatory Treg cells and simultaneous expansion of pro-inflammatory Th17 responders. This reorganization of the CD4+ T cell compartment depended on the tryptophan metabolite indole-3-acetaldehyde (I3AA) produced by this single cell eukaryote. I3AA negatively impacted the Treg subset in vivo and iTreg development in vitro by modifying signals from recognition of TGFβ by these cells, which affects recognition of self-flora antigens by conventional CD4+ T cell clones. Stronger than normal TCR signaling (as manifested by increased TCR-dependent CD69 expression and down-regulation of the co-inhibitory molecule PD-1), was caused by parasite-derived I3AA. This is a new mechanism of control of CD4+ fate decisions, and shows the ability of protist members of the microbiome, and tryptophan metabolites derived from them (or other sources), to modulate the adaptive immune compartment, particularly in the context of gut inflammatory disorders.

Speaker's Profile:

Professor Nicholas Gascoigne joined the Department of Microbiology at Yong Loo Lin School of Medicine as Head in August 2013. Prior to joining NUS, he was a Professor at The Scripps Research Institute, La Jolla, California, USA, where he joined the faculty in 1987. He performed postdoctoral work in the lab of Mark Davis at Stanford University, and was a Ph.D. student of Prof Av Mitchison at UCL in the early 1980's. He has established himself as a leading researcher in cellular and molecular immunology, focusing on the regulation of signalling strength in T cell activation and development. His current interests are in T cell signaling, activation, development, and the ways in which microbiome influences T cell differentiation. He is also focused on signaling pathways in CAR-T cells and how they can be manipulated to improve CAR-T function in cancer immunotherapy.

Keynote Lecture 9

Cytoadhesion and rosette formation of Plasmodium knowlesi-infected red blood cells

Osamu KANEKO[1,2,3],Stephen C. EZENWANNE[1,2], Huai CHUANG[1,3], Yuko KATAKAI[4], Satoru KAWAI[5], Bruce RUSSELL[1,6], Miako SAKAGUCHI[7]

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Cytoadherence of Plasmodium falciparum-infected red blood cells (iRBCs) is a hallmark of malaria pathogenesis implicated in developing severe malaria during human infections. The binding of iRBCs has two forms: one to vascular endothelial cells and the other to uninfected RBCs, which is called "rosetting". Both types of binding are involved in the occlusion in the microvasculature in the organs of falciparum malaria. However, the binding of Plasmodium knowlesi-iRBCs was not well understood until recently. In this seminar, I will introduce the current understanding of the cytoadherence properties of P. knowlesi-iRBCs, present our recent findings on the cytoadhesion and rosetting of this parasite, and discuss their possible involvement in the disease severity.

Speaker's Profile

Osamu KANEKO graduated from Osaka City University Medical School, Japan in 1990 and then trained as an Orthopedic Surgeon for 2 years. Following that, he decided to spend 4 years studying malaria at Osaka City University's Graduate School of Medicine. After he received his Ph.D., he worked at NIAID, NIH, USA (Louis H. MILLER Lab), Ehime University (Motomi TORII Lab), and then joined Nagasaki University as a full-time professor in 2007. He is most interested in the molecular mechanisms of the erythrocyte invasion by malaria parasites. He has recently become more involved in the monkey malaria business.

Host adaptation and virulence mechanisms of *Vibrio parahaemolyticus*

Toshio KODAMA[1], Sarunporn TANDHAVANANT[1, 2]

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Some Gram-negative bacterial pathogens have sophisticated secretion systems to transport virulence proteins such as exotoxins and effectors. Exotoxins are exported via the type II secretion system (T2SS). In contrast, the type III secretion system (T3SS) directly translocates effectors into host cells to modify their functions. As these secretion systems and virulence proteins are essential virulence determinants for pathogens, secretion and transcriptional regulation, particularly in response to host cell contact, are considered critical steps in the establishment of infection. Therefore, these secretion systems are strictly regulated by several mechanisms in a series of pathogenic processes. This presentation focuses on the host adaptation and pathogenicity of *Vibrio parahaemolyticus*, which is known as a causative agent of food poisoning and wound infection. This pathogen possesses virulence factors, including an exotoxin, a thermostable direct hemolysin (TDH), and/or a TDH-related hemolysin (TRH), and two sets of gene clusters for T3SSs (T3SS1 and T3SS2). Our recent functional analyses revealed that this pathogen has an outstanding mechanism for sensing the intestinal environment and host cell contact to regulate the precise expression and secretion of T3SS effectors and exotoxins. Herein, we discuss how this organism recognizes contact with host cells and regulates the gene expression of virulence factors.

Speaker's Profile

Toshio KODAMA is a professor of the Department of Bacteriology, Institute of Tropical Medicine Nagasaki University. He studies the pathogenicity of enterobacterial pathogens (e.g. *Vibrio* spp. and *Salmonella* spp.) and believes that understanding the detailed mechanisms of bacterial pathogenicity gives a clue to the development of effective vaccines and the establishment of new treatment strategies without antibiotics.

New findings obtained from analysis of virulence factors of periodontal pathogens

Mariko NAITO[1], Mikio SHOJI[1]

[1]Department of Microbiology and Oral Infection, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki 852-8523, Japan

Periodontal disease, which is caused by bacterial groups in the oral cavity, greatly affects the patient's QOL, including not only oral functions (eating, mastication, swallowing, and speech), but also facial appearance and aesthetics. In addition, various relationships have been shown not only with oral diseases but also with systemic diseases. Our group has been analyzing the pathogenic mechanism of *Porphyromonas gingivalis*, a major causative agent of chronic periodontitis. For the comprehensive analysis, we determined the complete whole genome sequence of the P. *gingivalis* type strain ATCC33277. Based on this information, we found a new secretion system (named type 9 secretion system: T9SS) that transports various virulence factors (e.g., gingipains, powerful proteolytic enzymes) across their cell membranes. This system consists of approximately 33 genes and constitutes a higher-order structure. Furthermore, it was revealed that the pili of this bacterium have a different structure and polymerization mechanism from those of other bacteria (named type V pili). The type V pili are present only in periodontal pathogens and their closely related bacterial species. We revealed that common oral bacteria actually have a different mechanism from other pathogenic bacteria.

On the other hand, analysis of another periodontal pathogen, *Prevotella intermedia*, has been significantly delayed due to the inability to create mutant strains. We succeeded in establishing genetic manipulation technology for P. *intermedia*. By creating T9SS mutant strains, we revealed that T9SS is essential for multiple physiological activities that are involved in the pathogenicity of P. *intermedia*. Our findings will lead to the development of drug discovery that targets periodontal pathogens

Speaker's Profile

Mariko NAITO is a professor of Department of Microbiology and Oral Infection, Graduate School of Biomedical Sciences, Nagasaki University. Her area of research encompasses the periodontal pathogens and their virulence mechanisms.

Session 4-3
July 12th (FRI) 13:40-14:00

Pathogenicity islands uncouple prophages from intra-host competition to promote their reproductive success during polylysogeny

John CHEN[1]

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Abstract: Staphylococcal pathogenicity islands (SaPIs) are bacteriophage parasites that encode superantigens and toxins. They are genetic elements that reside quiescently in bacterial chromosomes, awaiting certain phages to activate their life cycles and provide mobility. Helpers are phages that express antirepressors to release SaPIs from repression; however, it is not known if SaPIs can likewise induce prophages to enter the lytic cycle. Here we report the discovery that SaPIs promote their promiscuous transmission by inducing resident prophages. We found that SaPIs express a highly conserved protein that binds to phage master repressors to disable their function. This results in a pseudo-lytic cycle wherein SaPIs infect, replicate, and lyse from lysogenic host cells. By doing so, SaPIs circumvent the requirement for a helper, expanding the range of phages they can exploit for their dissemination. Moreover, this mechanism bypasses the bacterial SOS response, giving SaPI-activated prophages an advantage in strains with multiple prophages induced by DNA damage. Our results unveil complex interaction between SaPIs and phages, with important consequences for the evolution of both elements and their bacterial hosts.

Speaker's Profile

Dr. John Chen is an assistant professor in the Department of Microbiology and Immunology at the National University of Singapore. He received his undergraduate degree from Princeton University and his post-graduate degrees from the Columbia University College of Physicians and Surgeons. Following his graduate studies, he conducted his post-doctoral work at the New York University School of Medicine. Dr. Chen is interested in the molecular basis of *Staphylococcus aureus* pathogenesis. His research program currently focuses on staphylococcal pathogenicity islands and bacteriophages, towards a deeper understanding of how they interact and counteract each other, and of their roles in shaping pathogen genomes.

Non-viral vectors for gene therapy, mitochondrial gene therapy and genetic vaccination

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Genetic therapies and vaccination approaches require efficient, specific, safe, and affordable genetic vectors. While viral vectors are expensive and harbor risks linked to immunogenicity and genomic integration, mRNA has a very short half-life. We have been developing advanced minimalistic non-viral dumbbell-shaped DNA vectors termed SPRING DNA™. The unique sequence and structure of SPRING helps to overcome major limitations of alternative DNA vectors such as plasmids, minicircles and conventional dumbbells. By employing facilitated nuclear diffusion and active nuclear import, SPRING DNA™ exhibits up to 6- or 95-fold more efficient nuclear targeting yielding up to 60- or 160-fold higher levels of gene expression compared with conventional dumbbells or plasmids, respectively[1]. As opposed to any alternative DNA based vectors or mRNA, SPRING DNA™ is substantially more stable and does not exhibit any signs of disintegration at 50 °C for up to 3 months or longer *in vitro*. *In vivo* and in primary cells, SPRING DNA™ does not suffer from transgene silencing and triggers long-lasting gene expression. A single intramuscular (i.m.) or intravenous injection (mice) of naked or LNP-formulated SPRING DNA™ triggered continuous gene expression for 6 months (ongoing) in the muscle or liver. This finding suggests SPRING can efficiently be delivered i.m. without LNPs. Opposed to plasmid DNA, naked and LNP-formulated SPRING DNA™ was found to trigger only insignificant levels of innate immune sensing in mice as indicated by a 32-plex cytokine profiling assay. Via its single-stranded loops, SPRING DNA™ was conjugated with helper functions such as tri-antennary GalNAc residues or aptamers for targeted delivery into hepatocytes or distinct cancer cells[2]. We developed the first genetic 3-in-1 CRISPR/Cas editing vectors delivering the Cas9 enzyme, one or two single-guide RNAs, and

a single-stranded donor template resembling one of the dumbbells' hairpin-loops. Such designed vectors triggered up to 10-fold higher HDR compared with conventional plasmid-based vectors[3]. To achieve cell type-specific gene expression, the SPRING technology can be combined with the RNA *trans*-splicing technology[4]. In that case, the endogenous pre-mRNA biomarker profile of the target cell determines if gene expression will be activated or not. To interfere with mitochondrial gene expression, we developed a highly modular and scalable RNA-based vector that effectively delivers functional nucleic acids, RNA and/or DNA, into the mitochondria of human cells without any size limitation. Using this vector, mitochondria-targeted delivery of antisense RNA or mRNA tiggered 96% knockdown of mitochondrial gene expression or intra-mitochondrial transgene expression. SPRING DNA™ has the potential to go beyond the limitations of mRNA and viral vectors. The vectors are produced in a novel PCR-based process[5]. We are currently upscaling this PCR to litre volumes, therefore building a novel manufacturing device. We are developing SPRING for gene complementation therapy of rare liver diseases, for suicide gene therapy to selectively kill cancer or virus-transduced cells, for mitochondrial gene therapy targeting optic neuropathies, and for genetic vaccination.

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Speaker's Profile

Dr. Volker Patzel, German chemist, received a Ph.D. from the Ruprecht Karls University in Heidelberg and an MBA from the Steinbeis University in Berlin. Worked as postdoc at the German Cancer Research Center in Heidelberg, then as research group leader at the Max Planck Institute for Infection Biology in Berlin. Joined the National University of Singapore (NUS) in 2009 under the NUS-Cambridge Scheme and is now Senior Lecturer at the NUS Department of Microbiology and Immunology. Has >50 publications, filed 15 patent families and is founder of AVECRIS Pte Ltd in Singapore. Research focusses on RNA technologies and non-viral delivery and the exploration for genetic therapy and vaccination. Teaches graduate and undergraduate students in biotechnology and entrepreneurship.

Neuropathogenesis of tick-borne encephalitis virus infection: dendritic transport of tick-borne flavivirus RNA by neuronal granule

Kentaro YOSHII[1], Minato HIRANO[1]

[1]Department of Viral Ecology, Research Center for the Control and Prevention of Infectious Diseases, Nagasaki University, Nagasaki 852-8523, Japan

Tick-borne encephalitis virus (TBEV) is a member of the genus Flavivirus within the family Flaviviridae, and causes fatal encephalitis in humans with severe sequelae. TBEV is prevalent over a wide area of the Eurasian continent including Japan. Neurological diseases caused by encephalitic flaviviruses are severe and associated with high levels of mortality. However, little is known about the detailed mechanisms of viral replication and pathogenicity in the brain. In this study, we tried to investigate the specific mechanism of TBEV replication in neuron which affects development of neurologic disease.

Accumulations of viral antigen and double-stranded RNA produced by viral RNA replication were detected in the neuronal dendrites of primary neurons infected with TBEV. Further, electron microscopic observation confirmed the characteristic ultrastructural membrane alterations containing virion-like structures in the dendrites. Specific sequences of the 5' untranslated region of TBEV genomic RNA were identified as a cis-acting element for RNA transport in the dendrites. Mutated TBEV with impaired RNA transport in dendrites caused a reduction in neurological symptoms in infected mice. TBEV genomic RNA bound a RNA-binding protein of neuronal granules, which regulate the transport and local translation of dendritic mRNAs, and disturbed the transport of dendritic mRNAs.

These results demonstrated that TBEV hijacked the neuronal granule system for the transport and replication of viral genomic RNA in dendrites, resulting in severe neurological disease. Our findings of this unique virus-host interaction will improve further understanding of the molecular mechanisms of viral replication and the pathogenicity of neurotropic viruses.

Speaker's Profile

Kentaro YOSHII is a professor of Department of Viral Ecology, Research Center for the Control and Prevention of Infectious Diseases, Nagasaki University. His area of research encompasses highly pathogenic viral zoonosis with focus to study the virus-host cell interaction, and understand the molecular and pathogenic mechanism of virus infection, developing diagnosis for emerging viral diseases and also understand the ecology of virus transmission and evolution around the world.

Epidemiological and ecological studies of Severe Fever with Thrombocytopenia Syndrome in our laboratory.

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Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne disease caused by the SFTS virus (SFTSV), a member of the order *Bunyavirales*, family *Phenuiviridae*, and genus *Bandavirus*. In 2010, the first SFTS case was reported in China and subsequently in Japan and South Korea in 2013. The distribution of SFTS has recently been expanding into Southeast Asia. The mortality rate for SFTS ranges from 5% to 28%, with the elderly at a higher risk of a fatal clinical outcome. Clinical manifestations include acute fever with thrombocytopenia and leukopenia. Currently, no antiviral drugs or vaccines are available to treat SFTSV.

The main route of SFTSV transmission is tick bites, although virus isolation from tick specimens has seldom been reported, and information on tick-human association has been limited in Japan.

Recently, cats have shown similar clinical manifestations to human cases, and transmission from cats to humans has been reported. The incidence of feline SFTS in Nagasaki is among the highest in Japan. Cat-to-human transmission has also been reported in Nagasaki in recent years. However, currently, no detailed information is available on the clinical manifestations and laboratory parameters for SFTS diagnosis or prognostic factors in cats.

In our laboratory, we obtained clinical specimens from human patients in the university hospital and animal specimens from veterinarians. In this presentation, two research topics related to SFTS will be introduced. One is an interesting SFTS case transmitted by a tick non-native to Japan, marking the first identification of its potential as a vector of SFTSV. Another is the study for comprehensive information on feline SFTS, contributing to the protection of cat owners, community members, and veterinarians from the risk of cat-transmitted SFTSV infection.

Speaker's Profile

Yuki TAKAMATSU is an associate professor in the Department of Virology at the

Institute of Tropical Medicine, Nagasaki University (Nekken-Virology). His research focuses on the epidemiology, clinical aspects, and molecular characteristics of arthropod-borne viruses and highly pathogenic viral infections. Nekken-Virology has conducted field studies in Vietnam and Brazil, concentrating on eco-epidemiological, serological, and molecular phylogeny to elucidate the background of arbovirus epidemics. Additionally, he investigates the molecular mechanisms of replication of highly pathogenic viruses and conducts microstructural analysis of filoviruses using live cell imaging microscopy and electron microscopy.

Deficiency of IRF3 promotes a beneficial neutrophil response to severe pulmonary bacterial infection

ZHANG Yongliang[1]

[1]Department of Microbiology and Immunology, Young Loo Lin School of Medicine, National University of Singapore, 117597 Singapore

Pneumonia is the most common cause of sepsis and is responsible for the majority of cases of the acute respiratory distress syndrome (ARDS), for which no specific drugs or therapeutics available for direct treatment or prevention. Neutrophils play a pivotal yet paradoxical role in the development of severe pneumonia. The interferon regulatory factor 3 (IRF3) is known as the master regulator of type I interferons. However, the regulatory function of IRF3 in neutrophil recruitment and function in pneumonia is unclear.

In this study, IRF3 wildtype (WT) and knockout (KO) mice were infected with Klebsiella pneumoiae (Kp) or Burkholderia thaliandensis (Bt) to examine the IRF3 function in bacterial pneumonia. The results showed that IRF3 KO mice were resistant to lethal Kp and Bt pulmonary infection, which was associated with reduced bacterial burden and lung injury compared to WT mice. The resistance of IRF3 KO mice to lethal infection was independent of type I IFNs, but dependent on neutrophils. Further investigation on cellular and molecular mechanisms showed that IRF3 was important for the expression of molecules that control neutrophil recruitment to the lung upon bacterial infection. Furthermore, the changes of the immune landscape in the lung in the absence of IRF3 was investigated. These findings suggest that IRF3-regulated neutrophil recruitment and function could be targeted to develop therapeutic intervention for treatment of severe bacterial pneumonia such as ARDS.

Speaker's Profile

Dr. Zhang Yongliang obtained his PhD in Molecular Microbiology in 2002 from the National University of Singapore (NUS), Singapore. He performed his postdoctoral research in the Department of Immunology, University of Washington, and the Department of Immunology, the University of Texas MD Anderson Cancer Center, USA. He joined the Department of Microbiology and the LSI Immunology Programme, NUS, as an Assistant Professor in 2009, and was promoted to Associate Professor with Tenure in 2017. Research in his laboratory focuses on understanding the physiological function of dual-specificity phosphatases (DUSPs). He utilizes multiple

approaches, including gene overexpression, knockdown and knockout, and animal models, in join with human studies to investigate their roles in immunity, metabolism and cancer. Findings made by his group have unveiled novel roles played by DUSPs in diseases including infectious diseases, cancer and metabolic disorders. Targeting DUSPs for the development of novel therapeutic methods to improve patient outcomes is one of his major research interests currently and in the future.

Cell death during bacterial infection

CHEN Kaiwen[1]

[1]Department of Microbiology and Immunology, Young Loo Lin School of Medicine, National University of Singapore, 117597 Singapore

Macrophages express a repertoire of pattern recognition and cytokine receptors that mediate proinflammatory signal transduction pathways to combat microbial infection. To retaliate against such responses, pathogenic microorganisms have evolved multiple strategies to impede innate immune signalling. Recent studies have demonstrated that suppression of TAK1 and IKK signalling by YopJ during Yersinia pseudotuberculosis infection promotes the assembly of a RIPK1 kinase-dependent death-inducing complex that enables caspase-8 to directly cleave gasdermin D (GSDMD) to trigger pyroptosis. However, whether and how macrophages respond to Yersinia infection in the absence of YopJ or caspase-8 activity remains unclear and will be discussed in this talk.

Speaker's Profile

CHEN Kaiwen is an Assistant Professor, Department of Microbiology and Immunology, Young Loo Lin School of Medicine, National University of Singapore. His laboratory studies the mechanisms by which inflammation is initiated and terminated by the innate immune system. A major interest of the lab is to understand how various programmed cell death pathways including apoptosis, pyroptosis and necroptosis are initiated during microbial infection and consequently how these death pathways promote host immunity *in vivo.* His lab also uses a range of microbial mutants to define the corresponding microbial subversion mechanisms. The knowledge gained from these studies will improve our understanding of host-pathogen interactions and may unravel novel antimicrobial therapeutics and regulations of inflammation.

Induction of liver-resident memory CD8$^+$ T cells and protection against malaria at extraerythrocytic stage by mRNA-containing lipid nanoparticles

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Malaria is a febrile disease caused by *Plasmodium* parasites, and one of the most important life-threatening infectious diseases in the world. Right after entering the human body by mosquito bites, *Plasmodium* sporozoites invade hepatocytes and proliferate (liver-stage), followed by the development of symptomatic blood-stage. Recent studies showed that resident memory T cells (T_{RM}) in liver play a critical role in liver-stage malaria protection. Although RTS,S/AS-01 and R21/Matrix-M were prequalified by WHO as malaria vaccines, still further malaria vaccine development is required. On COVID-19 pandemic, mRNA-containing lipid nanoparticles (LNPs) was approved as a new vaccine platform. In this study, we aimed to develop liver T_{RM} inducing malaria vaccine based on mRNA contained LNPs (mRNA-LNPs).

We utilized pH-sensitive lipid LNPs, which contains third generation SS-cleavable pH-Activated Lipid-like Material (ssPalm) that enhances endosome disruption and mRNA release to the cytosol and promotes efficient protein production. Single dose intravenous injection of ovalbumin (OVA) mRNA LNPs induced antigen-specific cytotoxic T lymphocytes (CTLs) efficiently in a dose-dependent manner in the liver. Furthermore, five weeks after the immunization, T_{RM} were generated in the liver. In addition, we immunized mice intramuscularly

twice with LNPs containing mRNA encoding *P. berghei* ANKA (PbA) circumsporozoite protein (CSP), and examined protection against PbA sporozoite. As a result, strong protection was observed on the immunized mice even when the sporozoites were inoculated thirteen weeks after the immunization. These results demonstrate that mRNA-LNPs is a promising liver-stage malaria vaccine platform. Based on the finding, our vaccine development study is on-going.

Speaker's Profile

Shusaku MIZUKAMI is an Associate Professor / Principal Investigator of Department of Immune Regulation, Institute of Tropical Medicine (NEKKEN), Nagasaki University. His research field is immunology and vaccine development on infectious diseases, especially for malaria.

Analysis of chronic host-aspergilloma interactions using a novel mouse model

Masato TASHIRO[1,2], Ryosuke HAMASHIMA[1,3], Yuichiro NAKANO[1], Hotaka NAMIE[1], Yuya ITO[4], Tatsuro HIRAYAMA[4,5], Kazuaki TAKEDA[4], Naoki IWANAGA[4], Kodai NISHI[6], Hong Liu[7], Takahiro TAKAZONO[1,4], Takeshi TANAKA[2], Akira WATANABE[8], Yoshihiro KOMOHARA[9], Akitsugu FURUMOTO[10], Katsunori YANAGIHARA[11], Hiroshi MUKAE[4], Scott G Filler[7,12], Koichi TAKAYAMA[3] & Koichi IZUMIKAWA[1,2]

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[12]David Geffen School of Medicine at UCLA, Los Angeles, 90502, CA, United States of America

Chronic aspergillosis poses a formidable challenge as an infection caused by *Aspergillus* spp. Hemoptysis, which occurs in approximately half of patients, adds complexity and occasionally leads to fatal outcomes. Despite its grim prognosis, with reported 5-year mortality

rates ranging from 38% to 52%, chronic aspergillosis remains less recognized than invasive aspergillosis. Unlike the invasive form, chronic aspergillosis manifests in immunocompetent patients without *Aspergillus* tissue invasion and presents with unique features such as persistent aspergilloma within air-filled cavities for more than 3 months.

We investigated aspergilloma, a critical element of chronic aspergillosis, using a novel mouse model. Implanted in our model was an *A. fumigatus* fungus ball into an air-filled subcutaneous cavity. Initially, a live fungus ball was introduced into the cavity of a healthy mouse, expecting no tissue invasion due to the immunocompetent nature of the mice. Unexpectedly, however, *Aspergillus* invaded the tissues. Based on earlier clinical findings showing dead hyphae in aspergilloma, we attempted to implant an autoclaved, killed fungus ball.

Remarkably, a fungus ball of entirely dead hyphae persisted in the mouse cavity for over 3 months without clearance. Cellular analysis revealed an initial predominance of neutrophils around the fungus ball, later transitioning to foamy macrophages accumulating lipids. *Aspergillus* fragments were detected within the cells of these foamy macrophages. In vitro experiments further confirmed macrophage damage induced by dead hyphae, suggesting a potential barrier to aspergilloma clearance. In addition, elevated levels of vascular endothelial growth factor in the dead fungus ball and increased vascularity around it were observed in our mouse model. Even in the scenario where all *Aspergillus* within the aspergilloma is deceased, the persistent presence of a substantial number of fungal bodies could contribute to hemoptysis.

Our findings emphasize the need for innovative treatments that target fungal clearance and challenge the limited efficacy of antifungal agents against deceased fungal bodies. This research marks a substantial advancement in our comprehension of chronic aspergillosis, particularly in unraveling the interactions between dead hyphae and host cells.

Speaker's Profile

Dr. Masato Tashiro, M.D., Ph.D., is a highly accomplished Senior Assistant Professor in the Department of Infectious Diseases at Nagasaki University Graduate School of Biomedical Sciences. With a strong background in pulmonology, infectious diseases (including mycology and respiratory diseases), and infection control, Dr. Tashiro has made significant contributions to the medical field. He obtained his M.D. from Oita University School of Medicine in 2004, followed by a Ph.D. from Nagasaki University Graduate School of Biomedical Sciences in 2012. He is actively engaged in research and teaching in the Department of Infectious Diseases, with a focus on infectious diseases, mycology, and respiratory diseases.

Session 6-2
July 12th (FRI) 17:10-17:30

Candida auris: An Emerging Threat of Multidrug-Resistant Fungal Infections

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Bloodstream infections caused by fungi are primarily attributed to *Candida* spp. and are associated with high mortality rates. Approximately 750,000 cases of invasive candidiasis are reported annually worldwide, resulting in a mortality rate of 46-75% among infected individuals and approximately 40% among those receiving antifungal therapy. The limited availability and quality of therapeutic agents for candidiasis, primarily echinocandins or azole antifungals, with liposomal amphotericin B as an alternative, has led to the emergence of multidrug-resistant *Candida* species, which pose a critical threat in clinical settings. In recent years, multidrug-resistant *Candida auris* has caused global outbreaks. *C. auris* is listed as an Urgent Threat group in "Antibiotic Resistance Threats in the US, 2019" by the Centers for Disease Control and Prevention (CDC), and as a Critical Priority Group in " WHO fungal priority pathogen List, 2022". Based on tentative breakpoints suggested by the CDC, approximately 90, 30, and < 5% of isolates in the USA have been reported to be resistant to fluconazole, amphotericin B, and echinocandins, respectively. *C. auris* is particularly problematic due to its multidrug resistance, rapid global emergence, and high mortality rates, making it a significant concern for the public, medical community, and basic researchers. In this presentation, we outline the issues surrounding candidiasis and describe our research with a focus on drug resistance in *C. auris*.

Speaker's Profile

Tatsuro HIRAYAMA is an associate professor at the Department of Pharmacotherapeutics, Nagasaki University Graduate School of Biomedical Sciences. His primary research focus is mycology, specifically regarding drug resistance mechanisms in *Candida* species.

Nontuberculous Mycobacterial Pulmonary Disease: An Overview and Insights from Nagasaki, Japan

Shotaro IDE[1]

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Nontuberculous mycobacteria (NTM) are ubiquitous present in the environment, including soil and water, and in recent years, the number of patients with nontuberculous mycobacterial pulmonary disease (NTM-PD) has been increasing worldwide. The frequency of causative organisms varies by region, with *Mycobacterium avium* complex (MAC) accounting for more than 80% of isolates in Japan, and *M. abscessus* is most frequent in Singapore. Factors known to cause NTM-PD include underlying diseases associated with structural changes in the lungs, such as bronchiectasis, immune-suppressing diseases and drugs, and genetic polymorphisms. NTM-PD requires long-term observation and treatment, and poor prognostic factors have been reported, including older age, male sex, low body mass index, comorbidities, cavitary lesions, chronic pulmonary aspergillosis, and erythrocyte sedimentation rate. Co-existence with chronic pulmonary aspergillosis is refractory and has a poor prognosis; however, it is often difficult to treat owing to drug interactions. In contrast, some mild cases of NTM-PD may not worsen, even after a long period of watchful waiting. This presentation aims to provide an overview of NTM-PD and our challenges and research in Nagasaki, Japan.

Speaker's Profile

Shotaro IDE is a senior assistant professor of Infectious Diseases Experts Training Center, Nagasaki University Hospital. His research area encompasses respiratory medicine and infectious diseases, especially pneumonia, mycobacteriosis, and fungal infections.

Poster Presentation

Day 1: 11th June (THU)
1F Sensai Hall

No.	Name	University
P01	FUKUSHIMA Koki	Department of Respiratory Medicine, Graduate School of Biomedical Sciences, Nagasaki University
P02	ASHIZAWA Hiroki	Department of Respiratory Medicine, Graduate School of Biomedical Sciences, Nagasaki University
P03	ENDO Akira	Saw Swee Hock School of Public Health, National University of Singapore
P04	EZENWANNE Chukwuma Stephen	School of Tropical Medicine and Global Health, Nagasaki University
P05	Thant Zin TUN	Graduate School of Biomedical Sciences, Nagasaki University
P06	FURUYAMA Wakako	Department of Virus Infection Dynamics, National Research Center for the Control and Prevention of Infectious Diseases, Nagasaki University
P07	CHUANG Huai	Department of Protozoology, Institute of Tropical Medicine, Nagasaki University
P08	OSAKO Hiromu	Department of Virology, Institute of Tropical Medicine, Nagasaki University (ITM-NU)
P09	UEDA Mika	Department of Virology, Institute of Tropical Medicine, Nagasaki University (ITM-NU)
P10	KHONGYOT Thanawat	School of Tropical Medicine and Global Health, Nagasaki University
P12	Morakot KAEWTHAMASORN	Veterinary Parasitology Research Unit, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University
P13	NARUSE Taeko	Department of Protozoology, Institute of Tropical Medicine, Nagasaki University
P14	MIYAZAKI Shinya	Department of Cellular Architecture Studies, Institute of Tropical Medicine (NEKKEN), Nagasaki University
P15	KAGAYA Wataru	1Department of Eco-epidemiology, Institute of Tropical Medicine, Nagasaki University
P16	MIYAZAKI Yukiko	Department of Protozoology, Institute of Tropical Medicine (NEKKEN), Nagasaki University
P17	IBRAHEEM Yarob	Department of Molecular Microbiology and Immunology, Graduate School of Biomedical Sciences, Nagasaki University
P18	YAMADA Kento	Department of Virus Infection Dynamics, National Research Center for the Control and Prevention of Infectious Diseases, Nagasaki University
P19	YAJIMA Misako	National Research Center for the Control and Prevention of Infectious Diseases (CCPID), Nagasaki University
P20	ASAKURA Toshiaki	Department of Infectious Disease Epidemiology and Dynamics, London School of Hygiene & Tropical Medicine
P21	TASHIRO Masato	Department of Infectious Diseases, Graduate School of Biomedical Sciences, Nagasaki University
P22	Augustin KABONGO	School of Tropical Medicine and Global Health, Nagasaki University
P23	SAKURA Takaya	School of Tropical Medicine and Global Health, Nagasaki University

No.	Name	University
P24	KAKIUCHI Satoshi	Infection Control and Education Center, Nagasaki University Hospital
P25	NAMIE Hotaka	Department of Infectious Diseases, Nagasaki University
P26	MIYAUCHI Yasushi	School of Tropical Medicine and Global Health, Nagasaki University
P27	KARUNAWEERA Nadira D.	Department of Parasitology, Faculty of Medicine, University of Colombo
P28	NG'ETICH japheth Kibet	Institute of Biomedical Sciences, Nagasaki University
P29	OTA Kenji	Nagasaki University Hospital
P30	HAYASHISHITA Mizuki	School of Tropical Medicine and Global Health, Nagasaki University

The effect of *Prevotella intermedia* Culture Supernatant on *Mycobacterium avium* infection.

Koki FUKUSHIMA[1,2], Naoki IWANAGA[2], Hiroki ASHIZAWA[2], Nana NAKADA[2], Tatsuro HIRAYAMA[2], Kazuaki TAKEDA[2], Masataka YOSHIDA[2], Shotaro IDE[2], Masato TASHIRO[3], Takahiro TAKAZONO[2,3], Kosuke KOSAI[4], Koichi IZUMIKAWA[3], Katsunori YANAGIHARA[4], Hiroshi MUKAE[1,2]

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Rationale: The bacterial flora analysis of 16S rRNA gene sequence in bronchoalveolar lavage fluid from NTM-PD patients has reported a higher prevalence of the *Prevotella* spices compared to non-NTM patients with similar shadows. *Prevotella intermedia* culture supernatant (*P.int* sup) has been reported to contribute to the exacerbation of pneumonia caused by *Streptococcus pneumoniae* in a mouse model. In this study, we investigated the impact of *P.int* sup. on the infection of NTM.

Methods: In vitro, we infected alveolar macrophage-like cells (AMLC) with *Mycobacterium avium* and assessed phagocytic ability as the intracellular *M.avium* by colony forming unit (CFU) at 4 hours with or without *P.int* sup and bactericidal ability as the increase from 4 hours to 48 hours. Furthermore, we collected AMLC at 4 and 24 hours after co-culture initiation and evaluated mRNA expression real-time RT-PCR. Additionally, in vivo, we compared the survival rate and lung bacterial burden in a mouse model of NTM lung disease with or without *P.int* sup.

Results: The CFU at 4 hours significantly / (CFU at 48 hours - CFU at 4 hours) decreased in the P.int sup. group (bactericidal ability, P=0.0002). The expression of mRNA related to bactericidal activity (Atg5) significantly increased in the P.int sup. group(P=0.0303). In vivo, the *P.int* sup. group exhibited a decreased mouse survival rate and an increasing trend in lung bacterial burden.

Conclusions: In NTM-PD, P.int sup. may regulate alveolar macrophage functions, creating an environment favorable for NTM survival.

P2

Exacerbation of oral *Streptococci*-induced pneumonia by *Prevotella intermedia*

Hiroki ASHIZAWA[1,2], Naoki IWANAGA[2], Nana NAKADA[2], Tatsuro HIRAYAMA[2], Kazuaki TAKEDA[2], Masataka YOSHIDA[2], Shotaro IDE[2], Takahiro TAKAZONO[2,3], Koichi IZUMIKAWA[3], Katsunori YANAGIHARA[4], Hiroshi MUKAE[1,2]

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Rationale: *Prevotella intermedia* is an anaerobic and one of the periodontal pathogens. To date, a bacterial flora analysis using the 16S ribosomal RNA gene in bronchoalveolar lavage fluid has revealed a co-infection of oral *Streptococcus* spp. and *P. intermedia* of community-acquired pneumonia. In this study, we investigated the mechanism underlying the exacerbation of oral *Streptococcus* spp. (*S.* spp.)-induced pneumonia by *P. intermedia* using a mouse model.

Methods: *P. intermedia* and *Fusobacterium nucleatum* were cultured anaerobically in a modified GAM medium, separately, and the supernatants were collected. 1×10^8 CFU of *S. anginosus* and *S. mitis* were oropharyngeally administered to seven-week-old female C57BL/6J mice together with the *P. intermedia* or *F. nucleatum* culture supernatants. Survival and body weight change of these mice were compared with those administered with *S.* spp. and control media. In addition, we analyzed the number of viable bacteria, inflammatory cells, cytokines, and pathological changes in the lungs 24 hours post-challenge.

Results: Mice co-administered with oral *S.* spp. and culture supernatants of *P. intermedia* showed a significant decrease in survival and weight change, compared to *S.* spp. with control media (*S. anginosus*; $p<0.01$, *S. mitis*; $p<0.05$) or *F.nucleatum*. Furthermore, the number of viable bacteria in the lungs showed an aggravating trend ($p<0.01$), and pathological findings (HE staining) showed a robust clustering of inflammatory cells, mainly neutrophils, in the co-administered group. Real time RT-PCR of the lungs in the group co-administered with *P.*

intermedia supernatants exhibited a significant increase in *Ly6g*, *Cxcl15* and *Il17a* mRNA expression compared to the control and *F. nucleatum* media groups, suggesting that neutrophilic inflammation was induced. Since *Elane* was, however, decreased, neutrophil function was reduced. Ontology analysis by bulk RNA-seq showed a significant decrease in NADP binding and NADPH in the *P. intermedia* supernatants group.

Conclusions: These data support *P. intermedia* exacerbated oral *S.* spp induced pneumonia. Elucidation of the detailed role of *P. intermedia* might open a novel therapeutic strategy to overcome refractory pneumonia.

P3

Modelling vaccine decisions and within-household transmission

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Background: Individual-level decision making for paid vaccines are often discussed in the context of microeconomics including the game theory, where individuals attempt to maximise the utility weighing the utility weighing between benefit and cost of vaccination. However, the benefit of vaccines against endemic and seasonal infections such as influenza (and COVID-19 in the near future) can be stochastic and unpredictable, preventing individuals from accurately comprehending actual benefit of receiving vaccination. Moreover, as vaccine uptake may be a household-level decision making process, especially that for children, within-household transmission can further complicate the perception of both individuals' and households' utility. How vaccine uptake is characterised in different households and how it is related to the conferred protection within households remains an open question.

Method: Using a seasonal influenza dataset of over 10,000 primary school students from Matsumoto city, Japan, in the 2014/15 season, we identified determinants of influenza vaccine uptake between households of different compositions. We then used a Longini-Koopman model, fitted to the influenza outcome from the same dataset elsewhere, to analyse the relationship between the estimated utility of vaccination (measured by individual- and household-level risk reduction) and vaccine uptake.

Results: We found that higher vaccine uptake among primary school children is associated with a number of factors related to household compositions, including having fewer siblings, the presence of multiple adults in the household and being the first child among siblings. Coupling such patterns with the estimated within-household transmission dynamics of seasonal influenza, we found that vaccine uptake is lower in some of the households that potentially benefit more from vaccination than other households, presumably because of financial burden.

Conclusion: Vaccine promotion policies targeting specific types of households may address the disparity in health benefits from vaccination.

Type I rosetting of *Plasmodium knowlesi*-infected red blood cells

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Malaria continues to be the most lethal parasitic disease, responsible for over 608,000 deaths in 2022. The rise of zoonotic forms of malaria, particularly *Plasmodium knowlesi*, presents a significant challenge to the eradication efforts in Asia. A critical feature of primate malarias, including *P. knowlesi*, is their capacity for rosetting. This process, where infected Red Blood Cells (iRBCs) adhere to uninfected RBCs (uRBCs), leads to the blockage of microvasculature in vital organs and hampers the clearance of the parasite, which can result in severe malaria cases. While extensively studied in *P. falciparum*, our understanding of rosetting in other types of malaria, specifically those not caused by the *Laverania* subgenus, is still limited. In this study, we reveal that *P. knowlesi* is capable of forming stable Type I rosettes with *Macaca mulatta* RBCs in a laboratory setting, without the need for antibodies or serum factors. Using a cloned line of *P. knowlesi*, we observed that the ability to form rosettes can be sustained through a process of enrichment, but interestingly, this trait reverses approximately 53 days after enrichment. This suggests a mechanism of epigenetic regulation similar to that seen in *P. falciparum*. Additionally, we've developed a new imaging flow cytometric technique to measure rosette formation quantitatively and efficiently. These discoveries enhance our comprehension of the pathogenesis of severe knowlesi malaria and establish a foundation for future *in vivo* studies on rosetting, a critical step in developing new chemotherapeutic treatments.

P5

Essential role of Pleckstrin Homology domain-containing protein 1 (PH1) in AMA1 secretion during erythrocyte invasion by *Plasmodium yoelii*

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Malaria, caused by *Plasmodium* parasites, is a significant public health concern. In the mammalian host, parasites must multiply within erythrocytes; thus, the invasion into erythrocytes is a critical step. The proteins secreted from the micronemes play essential roles in orchestrating this intricate process; therefore, unraveling the molecular mechanism underlying such secretion holds the promise of elucidating novel targets for drug intervention. Our previous investigations in *Plasmodium yoelii* demonstrated that deleting APH, one of the Pleckstrin Homology (PH) domain-containing proteins, impaired asexual growth and erythrocyte invasion. These phenotypes were likely linked to impaired secretion of MTRAP and AMA1. Based on the hypothesis that other PH domain-containing proteins are also involved in microneme secretion, we generated a transgenic *P. yoelii* 17XL line wherein the PH1 gene locus can be inducibly excised. Moreover, PH1 deletion impaired the translocation of AMA1 to the merozoite surface but did not affect that of MTRAP. Indirect fluorescence assay revealed Myc-tagged APH colocalized with MTRAP, while Myc-tagged PH1 did not colocalize with AMA1 and MTRAP. Our observation for PH1 is consistent with *P. falciparum* PH2, which was required for the secretion of EBL, another microneme protein, but did not colocalize with EBL (Ebrahimzadeh et al. 2019).

Characterization of the release pathway of Ebola virus soluble glycoprotein

P6

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Introduction: The Ebola virus (EBOV), a member of the family *Filoviridae*, causes severe Ebola virus disease (EVD) in humans, with a fatality rate of up to 90%. Although approved countermeasures exist for EVD, their efficacy remains limited. The EBOV glycoprotein (GP) gene encodes three different GPs and its primary product, soluble GP (sGP), is abundantly secreted during infection. In previous studies, we demonstrated that EBOV sGP acts as a pathogenicity factor. To date, the mechanisms whereby synthesized sGP is released into the extracellular space remains unknown. Herein, we investigated the molecular mechanism underlying the intracellular transport of *de novo*-synthesized sGP through its secretory pathway.

Methods: Human embryonic kidney HEK293 cells were transfected with an expression plasmid encoding EBOV sGP (pCA-EBOV GP) or inoculated EBOV-expressing GFP (EBOV-GFP). Cells were harvested at various time points and the intracellular dynamics of sGP were analyzed by immunofluorescence staining. Additionally, 141 knockout cell lines were generated using a lentiviral CRISPR/Cas9 library targeting major membrane-trafficking genes. Each knockout cell line was transfected with pCA-EBOV GP or inoculated with EBOV-GFP, and the amounts of released sGP were quantitated using sandwich ELISA.

Results and conclusion: Immunofluorescence staining revealed that sGP partly co-localizes markers associated with the endoplasmic reticulum (ER), cis-Golgi, and trans-Golgi networks in transfected and infected cells. Furthermore, CRISPR/Cas9 screening identified several host factors involved in protein trafficking via Golgi apparatus; their roles in sGP secretion are currently being investigated. Our findings suggest that sGP is released via the ER-Golgi secretory pathway.

The work is partly supported by the Intramural Research Program of the NIAID.

P7

Development of a system evaluating expression of an exogenously introduced SICA protein on infected red blood cell surface by *Plasmodium knowlesi*

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Zoonotic malaria caused by *Plasmodium knowlesi* has been increasingly reported in Southeast Asia. *P. knowlesi* infection can be fatal, and it is necessary and important to understand the molecular basis of its virulence and pathogenicity to develop better intervention strategies. A post-mortem examination of human *knowlesi* malaria cases has shown sequestration of *P. knowlesi*-infected red blood cells (iRBCs) in the blood vessels. This phenomenon has been proposed to be associated with the severity. Sequestration is likely mediated by the cytoadhesion of iRBCs to vascular endothelial cells. Recently, we discovered one member of the Schizont Infected Cell Agglutination (SICA) protein family mediated the cytoadhesion of *P. knowlesi*-iRBCs to human umbilical vein endothelial cells (HUVECs), and we named it SICA-HUVEC. To evaluate if the exogenously introduced SICA-HUVEC is exposed on the iRBCs, we added an HA-tag at the N-terminal of SICA-HUVEC and Myc-tag at its C-terminal (termed HA-SICA-HUVEC-Myc). HA-SICA-HUVEC-Myc on the iRBC surface was detected with anti-HA antibodies and retained the binding activity to HUVECs. The established system is useful to further characterize SICA-HUVEC and to screen chemicals to inhibit the expression of parasite-derived molecules on the iRBC surface, which reduces the binding activity of the iRBCs, thereby reducing the parasite virulence. We present the results of one Proof of Principle experiment.

Clinical factors associated with SFTS diagnosis and severity in cats

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Severe fever with thrombocytopenia syndrome (SFTS) is a potentially fatal tick-borne zoonosis caused by SFTS virus (SFTSV). Although primarily transmitted through tick bites, there have been reports of animal-to-human transmission of SFTSV. However, little is known about feline SFTSV infection. This study aimed to provide comprehensive information on feline SFTS. Serum and swab specimens were obtained from 221 cats with suspected SFTS from animal hospitals in Nagasaki between 2018 and 2024. Data from 187 cats were analyzed to identify biomarkers for SFTS diagnosis and clinical outcomes.

A comparison between SFTSV-positive and SFTSV-negative cases revealed that positive cases had significantly higher body weight ($p = 0.046$), red blood cell count (RBC: $p = 0.004$), aspartate aminotransferase (AST: $p = 0.034$) and total bilirubin (TBil: $p = 0.005$) levels, as well as significantly lower white blood cell (WBC: $p < 0.001$) and platelet (PLT: $p = 0.014$) counts. Although no significant difference was observed, mortality in the SFTSV-positive group (46.8%) was higher than in the SFTSV-negative group (20.0%) ($p = 0.063$). Clinical symptoms and lifestyle were not associated with SFTSV infection in cats. Based on these results, a scoring model by integrating the five factors (body weight, RBC, WBC, AST and TBil) was developed, predicting SFTSV infection with 88.2% sensitivity and 64.5% specificity (AUC = 0.828). Regarding the outcome of the SFTS-positive group, increased ALT, AST and higher serum RNA levels were identified in fatal cases (ALT: $p = 0.021$, AST: $p = 0.008$, serum RNA: $p = 0.019$). Additionally, phylogenetic analysis did not elucidate the severity of the disease based on differences in viral genetic diversity in cats with SFTSV infection.

In conclusion, body weight, RBC, WBC, PLT, AST and TBil were found to be useful in diagnosis, while AST, ALT and serum RNA levels were associated with the clinical outcomes. Managing SFTS cases in cats is important for reasons: protecting cats from infection and safeguarding owners and surrounding individuals. The scoring model may be helpful in identifying SFTS infection in cats. These findings provide valuable insights for protection cats and people around cats from SFTSV infection.

Understanding the Dengue Epidemic Situation in Vietnam (1998-2014)

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Dengue fever is a mosquito-borne febrile disease caused by dengue virus (DENV). Numerous phylogenetic analyses of DENV epidemic strains have been reported. However, a comprehensive analysis has yet to been conducted to determine whether the patient profile inferred from DENV phylogenetic analysis matches actual epidemiological data, such as the number of patients and hospitalization records. This study aims to focus on dengue fever patient count obtained from the Vietnamese Ministry of Health, visualizing its epidemic status to analyze potential pattern or periodicity in dengue fever epidemics. Additionally, we will examine factors that may influence the prevalence of dengue fever by referencing past literature.

To depict epidemic situation, we plotted circles whose sizes are defined by the number of patients on a map of Vietnam and created an animation displaying a series of monthly graphs from 1998 to 2014. Additionally, graphs were generated by region and by time period. Based on the results, approximate curves were developed for each region to compare prevalence patterns and overall and regional homogeneity. We also examined the number and serotypes of registered dengue virus strains at the National Center for Biotechnology (NCBI) and compared them with the present data. The approximation curves revealed that epidemic patterns closely resembled those of 1998 and 2010, corresponding to pandemic occurrences. Notably, the southern region exhibited a gentle increase, resembling a gradual incline, while other regions experienced sharp peaks in epidemic occurrences. Moreover, the epidemic situation in the Mekong Delta region and the southeastern region, including Ho Chi Minh City, significantly influenced the epidemic across Vietnam. Thus, it was suggested that monitoring the situation in the Mekong Delta and Southeast regions is crucial. Additionally, it was observed that the number of registered strains at NCBI did not accurately reflect the actual number of patients.

The Effect of Mental Health from COVID-19 Vaccine Hesitancy in University Students: A Multi-Center Pilot Study.

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P10

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Introduction: The coronavirus pandemic has brought societal issues, including mental health. Many studies have highlighted elevated levels of anxiety, depression, and stress, particularly among younger individuals. This pandemic can impact students, including reduced social interactions due to online learning, which may induce more mental problems. The COVID-19 vaccine was an intervention for all eligible people, while hesitant to get vaccinated has emerged as another potential challenge. We hypothesize that mental issues may affect the decision to get the COVID-19 vaccine.

Objectives: This pilot study explored mental health issues and vaccine hesitancy among undergraduate students in Thailand, Laos, and Japan.

Methodology: We recruited undergraduate students for an online survey in 2011-2022 in Laos PDR, Thailand, and Japan's universities. The questionnaire gathered sociodemographic information, while the second part utilized standardized scales, including the Patient Health Questionnaire-9 (PHQ-9), Generalized Anxiety Disorder Questionnaire-7 (GAD-7), Perceived Stress Scale (PSS-10), and Vaccine Hesitancy related information. Descriptive and logistic regression analyses were employed.

Results: We recruited 841 participants from Japan, Thailand, and Laos. Participants were mainly 18-24 years old and predominantly female across all countries. Stress score (very high) was reported highest in Thailand (14.2%), followed by Laos (9.09%) and Japan (4.26%). Depression score (moderate to severe) was higher in Laos (37.88%) and Thailand (25.34%) than Japan (17.55%). Anxiety scores (moderate to high) were highest in Laos (28.79%), followed by Thailand (19.00%) and Japan (9.04%). Regarding COVID-19 vaccine hesitancy, 47.8% of participants expressed reluctance toward COVID-19 vaccination, with Thai students exhibiting the highest hesitancy. Moreover, among students who reported higher mental health scores, they were more reluctant to take the COVID-19 vaccine. Females were three times more likely to be hesitant than males. Fear of coronavirus disease and disbelief in vaccine effectiveness were also associated with increased hesitancy.

Conclusion: Our results highlighted mental health scores elevated among students, which correlated with heightened vaccine hesitancy. Designing strategies to improve vaccine acceptance should expand more than disseminating information about the vaccine. Psychological support to at-risk groups should also be prioritized.

Malaria parasites *Plasmodium inui* and *P. cynomolgi* in long-tailed macaques and their possible *Anopheles* vectors in Ratchaburi Province, Thailand

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Abstract: Thailand is one of the malaria-endemic countries, with a total of 16,675 indigenous malaria cases reported in 2023, up 24.3% from 10,155 in 2022. However, it is experiencing significant challenges in eliminating malaria due to naturally occurring human infections by zoonotic species of malaria parasites: *Plasmodium knowlesi, P. cynomolgi, P. inui,* and *P. fieldi.* Thus, investigations into simian malaria in macaques and *Anopheles* vectors involved in the transmission cycle should be conducted. In early April 2018, a photographer filming a documentary at the Tenasserim Mountain Range, straddling Ratchaburi Province and Myanmar, tested positive for *P. knowlesi.* Between April and June 2018, blood samples were taken from 59 wild long-tailed macaques (*Macaca fascicularis*) in Ban Kha District, Ratchaburi, where a human knowlesi case was reported, as well as 47 *Anopheles* mosquitoes captured close to the macaque colony. Using a PCR assay targeting the mitochondrial cytochrome b gene coupled with DNA sequencing, 21 out of 59 macaques (35.59%) tested positive for simian malaria parasites: 20 with *P. inui* and 1 with *P. cynomolgi. Anopheles* mosquitoes were morphologically identified as *An. dirus* s.l. (34), *An. minimus* (8), and *An. barbirostris* s.l. (5) and subsequently divided into 10 pools. *P. inui* was detected in one pool of *An. dirus* s.l., but the others tested negative. Although *P. knowlesi* was not found among the sampled macaques, the presence of *P. inui* and *P. cynomolgi* should not be ignored as they can potentially cause zoonotic infections. *An. dirus* s.l., belonging to the Leucosphyrus Group, might be the possible vector for simian malaria in the investigated area.

Keywords: macaque, *Anopheles*, *Plasmodium inui*, *Plasmodium cynomolgi*

Strong equilibrium selection pressure on the Plasmodium falciparum *surface-associated interspersed gene (surf) 13.1* gene

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Among the molecules found in Plasmodium falciparum (Pf), those that are subject to equilibrium selective pressure are likely to be targets of human protective immunity, and we identified them as potential vaccine targets. Therefore, we obtained whole-genome resequencing data from Pf-cloned strains established from 28 patient blood samples collected in the Mbita district of Kenya and 30 from the Mae Sot district of Thailand over a short period of time. We have annotated the whole-genome sequences using the genome dataset of the 3D7 strain (approximately 23.3 Mb in length) as a reference. The obtained sequences were then analyzed using Tajima's D, a statistic used to estimate selection pressure across a genome. This resulted in a list of 11 genes that showed high D values in both populations, indicating molecules that may be under equilibrium selective pressure. In this study, we focused on the surf13.1 gene, which showed D values greater than 1 in both the Kenya and Thailand isolate populations. We extracted the nucleotide sequence data (6.5-8 kb) corresponding to the surf13.1 gene from the resequencing data of each sample, and some missing parts were filled in using Sanger sequencing. A total of 234 single nucleotide polymorphism (SNP) sites were detected in the Kenyan isolate population and 222 sites in the Thai population, with more than 95% of polymorphisms found in the coding extracellular regions. Furthermore, the sliding window analysis showed a significantly higher D value ($P < 0.01$) in the extracellular region just before the transmembrane region in both populations, and a significantly higher D value ($P < 0.05$) was detected in the N-terminal cysteine-rich region in the Thai isolates. These results indicate that the polymorphism of the surf13.1 gene evolved to evade host immunity.

A platform for evaluating cytoadhesion of *Plasmodium falciparum*-infected erythrocytes to diverse human cells

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Plasmodium falciparum infection causes diverse malaria symptoms, such as cerebral malaria, which is attributed to the cytoadhesion of *P. falciparum*-infected erythrocytes to different human cells. As specific cytoadhesion inhibitors could be a practical adjunctive therapeutic approach combined with existing antimalarial drugs, a robust drug assay system to identify anti-cytoadhesion compounds is desired. However, the cytoadhesion assay requires primary or semi-primary endothelial cells, which significantly reduces the study throughput. In this study, we developed a robust cytoadhesion assay using immortalized human endothelial cells and bone marrow-derived mesenchymal stem cells. We used brefeldin A (BFA), an inhibitor of protein transport that can eliminate the adhesion ability of parasitized erythrocytes, as a positive control for the cytoadhesion assay. We also discuss an ongoing project that focuses on the interactions between *P. falciparum*-infected erythrocytes and human bone marrow-derived mesenchymal stem cells to study the mechanisms underlying asymptomatic malaria infection. Comparative transcriptomic analysis using cytoadhesive and control strains identified specific parasite genes upregulated in the cytoadhesive population, providing potential cytoadhesive ligands for bone marrow cells. We envisage the platform we developed in this study as a valuable approach for identifying anti-cytoadhesion compounds and investigating malaria parasite biology associated with unique parasite-host interactions.

Evaluation of the protective efficacy of Olyset®Plus ceiling net on reducing malaria prevalence in children in Lake Victoria basin, Kenya

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The speed of reduction in global malaria transmission has slowed from around 2016 and new tools are needed to reverse the trend. In the Lake Victoria Basin of western Kenya, malaria remains highly endemic despite high coverage of interventions such as insecticide-impregnated long-lasting insecticidal nets (LLIN). The malaria-protective effect of LLINs is hampered by insecticide resistance in Anopheles vectors and its repurposing by the community. Screening the ceiling with long-lasting insecticide treated net (LLIN), especially the one incorporating the insecticide synergist piperonyl butoxide (PBO) such as Olyset®Plus, has a potential to combat these issues surrounding the current vector control. A cluster randomized control trial of a novel vector control tool, Olyset®Plus ceiling net were implemented in Mfangano Island in Lake Victoria. The preliminary results from the trial showed that the cluster-mean of the malaria RDT prevalence at six months after the intervention was 9.9% (95%CI: 3.4-16.3) in the control arm while it was 2.1% (0.5-3.6) in the intervention arm, resulted in prevalence ratio of 0.21 (0.09-0.78) ($p = 0.019$), suggesting the significant protective effects of the intervention. Wider adoption of Olyset®Plus ceiling nets to complement existing interventions may benefit other malaria-endemic counties and be incorporated as part of Kenya's national malaria elimination strategy.

A versatile *Plasmodium falciparum* reporter line expressing NanoLuc enables highly sensitive multi-stage drug assays

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Transgenic luciferase-expressing *Plasmodium falciparum* parasites have been widely used for the evaluation of anti-malarial compounds. Here, to screen for anti-malarial drugs effective against multiple stages of the parasite, we generate a *P. falciparum* reporter parasite that constitutively expresses NanoLuciferase (NanoLuc) throughout its whole life cycle. The NanoLuc-expressing *P. falciparum* reporter parasite shows a quantitative NanoLuc signal in the asexual blood, gametocyte, mosquito, and liver stages. We also establish assay systems to evaluate the anti-malarial activity of compounds at the asexual blood, gametocyte, and liver stages, and then determine the 50% inhibitory concentration (IC_{50}) value of several anti-malarial compounds. Through the development of this robust high-throughput screening system, we identify an anti-malarial compound that kills the asexual blood stage parasites. Our study highlights the utility of the NanoLuc reporter line, which may advance anti-malarial drug development through the improved screening of compounds targeting the human malarial parasite at multiple stages.

Th1 priming of *Plasmodium*-specific CD4$^+$ T cells is orchestrated by γδ T cells in malaria via cDC1 activation

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Malaria, caused by *Plasmodium* parasites remains a significant global health challenge. IFN-γ produced by T helper 1 (Th1) cells plays a central role in the immune response to blood-stage *Plasmodium* infection. γδ T cells have been shown to promote IFN-γ production from CD4$^+$ T cells in response to *Plasmodium* infection mainly through interaction with conventional dendritic cells (cDCs). Among those cDCs, type1 (cDC1) is known for its role in promoting Th1 differentiation.

We utilized a blood-stage infection with *Plasmodium chabaudi chabaudi* (*Pcc*) and used *Plasmodium*-specific TCR-transgenic (PbT-II) mouse line to uncover the role of γδ T cells in influencing the fate of *Plasmodium*-specific CD4$^+$ T cells. We observed an impaired expansion and Th1 differentiation of PbT-II cells in mice lacking γδ T cells (TCRδKO mice), accompanied by impaired maturation of cDC1 in these mice. Furthermore, cDC1 showed impaired re-localization into the splenic white pulp in TCRδKO mice. We also observed γδ T cell activation and accumulation in the white pulp leading to increased cDC1/γδ T cell interactions in WT mice at the early phase of the infection. These interactions were dependent on CXCR3 signaling in γδ T cells and culminated in optimal priming for PbT-II cells to initiate Th1 differentiation. Th1 cells appeared early in the white pulp and would continue their differentiation in the red pulp at later timepoints. Finally, using CD11c^{DTR} bone marrow chimera mice, we found that cDC depletion led to impaired γδ T cell activation in Pcc infection. Reciprocal crosstalk between γδ T cells and cDC1s could be important for enhancing γδ T cell activation. Our results suggest that γδ T cells are key players in the immune response to Pcc infection, they promote cDC1 maturation which drives proper Th1 differentiation in PbT-II cells.

Marburg virus exploits the Rab11-mediated endocytic pathway in viral particle production

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Introduction: The Marburg virus (MARV), a member of the family *Filoviridae*, causes severe hemorrhagic fever in humans. Although it is well-established that the major viral matrix protein, VP40, is trafficked to the plasma membrane and contributes to large filamentous viral particle formation, the detailed molecular mechanisms remain unclear. To elucidate the mechanisms underlying this process, we examined studied the role of the small GTPase Rab11-mediated endocytic pathway.

Methods: The effect of MARV VP40 on the distribution of endogenous Rab11 was determined using immunofluorescence staining. The effects of co-expressed dominant-negative Rab11 and downregulation of Rab11 by siRNAs on VP40 distribution was assessed by immunofluorescence staining. The effect of down regulation of Rab11 on the release of MARV-like particles and authentic MARV was examined by performing western blot analysis and a median tissue culture infectious dose assay, respectively. Association between microtubules and VP40 was analyzed using immunoprecipitation and co-immunofluorescence staining.

Results and conclusion: VP40 expression promoted the diffuse cytoplasmic distribution of Rab11, which is normally distributed in the perinuclear region. Expression of the dominant-negative form and Rab11 knockdown reduced VP40 distribution to the cell periphery. Moreover, the release of MARV-like particles and authentic MARV was moderately reduced by Rab11 downregulation. Additionally, VP40 modulates the distribution of microtubules toward the cell periphery, which is often associated with Rab11. Depolymerization of microtubules reduced the accumulation of VP40 in the cell periphery and viral particle formation. Furthermore, VP40 physically interacts with α-tubulin, a major component of microtubules. Our findings indicate that MARV VP40 interacts with microtubules and facilitates their distribution towards cell periphery, leading to the trafficking of transiently tethered Rab11-positive vesicles towards the cell surface and release of virus particles to establish efficient viral egress.

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The first suit-type maximum containment laboratory in Japan

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In 2010, Nagasaki University launched a challenging project to construct the first suit-type maximum containment (biosafety level-4, BSL-4) laboratory in Japan. Construction of the BSL-4 laboratory started in January 2019 and completed in July 2021. The National Research Center for the Control and Prevention of Infectious Diseases (CCPID) was set up in 2022 to facilitate research on highly pathogenic viruses, including risk group 4 agents, and is also responsible for the operation and management of the BSL-4 laboratory. Through the operation of the laboratory, we are also cultivating the next generation of researchers with sophisticated understanding and experience in biosafety and biosecurity.

The Nagasaki BSL-4 laboratory has four suites for in vitro and in vivo experiments, chemical shower rooms, and a suit changing room. Single or double HEPA filter housings were installed on each suite's supply and exhaust ducts. Negative pressure in each room is ensured with automatic supply and exhaust airflow control. The laboratory has airtight doors and windows and a sealing system for the cables and pipes on the ceilings. Contaminated wastewater can be sterilized with autoclaves and a chemical treatment system on the lower floor of the laboratory. According to the National Building Standard Act, the building also has a seismically isolated structure for earthquake preparedness. We have also developed a positive-pressure suit with an external air supply by modifying a chemical protection suit in collaboration with a local company, aiming for their continuous supply and technical support.

The Nagasaki BSL-4 laboratory is currently undergoing test operation to evaluate each facility and the integrated control system to ensure safe and continuous laboratory operation. We should share lessons learned from the design, construction, and operation of the first suit-type laboratory with the national government and research community to improve the understanding of risk management of the maximum containment laboratory. The exchange of knowledge and experience through an international network of BSL-4 laboratories is also important for us to implement better operation of our new laboratory.

Setting-specific contact distribution-based clustering technique to identify population reactive to non-pharmaceutical interventions

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A transmission of respiratory diseases requires social contacts between individuals. Understanding contact patterns is therefore important for projections or scenario modelling to inform public health policy, such as non-pharmaceutical interventions (NPIs). Heterogeneity in social contact influences the disease-spread patterns and is introduced by various factors. Identifying clusters with similar contact patterns helps targeting a specific population. Considering a contact profile, defined as the distribution of contacts across different settings per individual, would improve identify population with similar contact patterns.

Here, we are developing a method to cluster population using reported contact behaviour of people of specific age, sex and socio-economic status (SES) to capture a contact profile and explore how contacts were changed through time. We used data from the multiple-cross-sectional social contact survey in the UK (the CoMix survey) in 2020 and 2021. We assumed that the setting-specific contact distribution (i.e., household, work, and school) follows a simple statistical distribution if contacts are not mixed with different populations or different settings. For a clustering algorithm, we employed the decision tree method to partition the population in a way that maximises Widely Applicable Information Criteria (WAIC). We calculated the WAIC for Weibull distributions fitted to the observed contact distribution regardless of settings and compared it to ones fitted to setting-specific contact distributions. We compared the clustering patterns during NPI and non-NPI periods. Our preliminary analysis showed the observed non-setting specific contact distribution was well described by the convolution of distributions fitted to observed household and non-household contact distributions. The shape of non-household distributions showed several change points, suggesting the distribution was mixed with population with different contact characteristics. We expected our study would contribute to capture contact profiles in different SES groups. The obtained clustering patterns are useful for the prioritising interventions.

Analysis of chronic host-aspergilloma interactions using a novel mouse model

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Chronic aspergillosis poses a formidable challenge as an infection caused by *Aspergillus* spp. Hemoptysis, which occurs in approximately half of patients, adds complexity and occasionally leads to fatal outcomes. Despite its grim prognosis, with reported 5-year mortality rates ranging from 38% to 52%, chronic aspergillosis remains less recognized than invasive

aspergillosis. Unlike the invasive form, chronic aspergillosis manifests in immunocompetent patients without *Aspergillus* tissue invasion and presents with unique features such as persistent aspergilloma within air-filled cavities for more than 3 months.

We investigated aspergilloma, a critical element of chronic aspergillosis, using a novel mouse model. Implanted in our model was an *A. fumigatus* fungus ball into an air-filled subcutaneous cavity. Initially, a live fungus ball was introduced into the cavity of a healthy mouse, expecting no tissue invasion due to the immunocompetent nature of the mice. Unexpectedly, however, *Aspergillus* invaded the tissues. Based on earlier clinical findings showing dead hyphae in aspergilloma, we attempted to implant an autoclaved, killed fungus ball.

Remarkably, a fungus ball of entirely dead hyphae persisted in the mouse cavity for over 3 months without clearance. Cellular analysis revealed an initial predominance of neutrophils around the fungus ball, later transitioning to foamy macrophages accumulating lipids. *Aspergillus* fragments were detected within the cells of these foamy macrophages. In vitro experiments further confirmed macrophage damage induced by dead hyphae, suggesting a potential barrier to aspergilloma clearance. In addition, elevated levels of vascular endothelial growth factor in the dead fungus ball and increased vascularity around it were observed in our mouse model. Even in the scenario where all *Aspergillus* within the aspergilloma is deceased, the persistent presence of a substantial number of fungal bodies could contribute to hemoptysis.

Our findings emphasize the need for innovative treatments that target fungal clearance and challenge the limited efficacy of antifungal agents against deceased fungal bodies. This research marks a substantial advancement in our comprehension of chronic aspergillosis, particularly in unraveling the interactions between dead hyphae and host cells.

Biochemical characterization and identification of ferulenol and embelin as potent inhibitors of malate:quinone oxidoreductase from *Campylobacter jejuni*

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The global threat posed by *Campylobacter jejuni* infection to public health is alarming, exacerbated by its rising incidence and antibiotic resistance. To combat this trend, diverse strategies are being adopted, notably the development of novel drugs with innovative mechanisms. Malate:quinone oxidoreductase (MQO) has emerged as a crucial enzyme for the

survival of various bacteria and parasites. Functioning as a peripheral membrane protein, MQO catalyzes the conversion of malate to oxaloacetate, a pivotal step in the tricarboxylic acid cycle. Additionally, MQO participates in reducing the quinone pool in the electron transport chain, thereby enhancing cellular bioenergetics. Notably, MQO's absence in mammals makes it an interesting drug target. As a preliminary exploration of MQO from *C. jejuni* (CjMQO) as a potential drug target, we purified active recombinant CjMQO and conducted its biochemical analyses, marking the first investigation of MQO from a pathogenic bacterium. Our study revealed that ferulenol, a mitochondrial MQO inhibitor with submicromolar potency, and embelin are nanomolar inhibitors of CjMQO. Interestingly, both inhibitors exhibited a mixed-type inhibition pattern against malate and noncompetitive inhibition against quinone, suggesting the presence of a third binding site to accommodate these inhibitors. This trait appears to be conserved between mitochondrial and bacterial MQOs. Remarkably, ferulenol and embelin also hampered the *in vitro* growth of *C. jejuni*, providing support for the hypothesis that MQO is indispensable for its survival and underscores its significance as a potential drug target. Currently, efforts are being made by our team to fully elucidate the role of CjMQO, validate it as a drug target in *C. jejuni,* and identify more specific inhibitors.

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Keywords: malate:quinone oxidoreductase; purification; biochemical characterization; drug target; enzyme inhibitor.

Analysis of liquid-liquid phase separation in *Plasmodium falciparum*

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Liquid-liquid phase separation (LLPS) is a physical phenomenon where a uniform liquid phase separates into two distinct liquid phases caused by changes in the physical properties of molecules that influence the strength of intermolecular interactions. LLPS is thought to have essential functions in biology, including serial enzymatic reactions and reaction specificity in cells, since it can immediately segregate specific molecules in liquid condensates at high density and provides a local reaction microenvironment. Glycolytic-body (G-body), formed through LLPS of glycolytic enzymes in hypoxia, was reported to increase the survival rate of human hepatocytes and yeast cells by reducing hypoxic stress through facilitated glycolysis and energy production. It was also reported that intrinsically disordered regions of glycolytic enzymes are important for G-body formation, and we confirmed the same regions in glycolytic enzymes of Plasmodium falciparum (Pf), a parasite that highly relies on glycolysis as the energy source in its asexual blood stage (ABS). Moreover, glycolytic activity should change quickly along with the intraerythrocytic development of the parasite, however, no clear evidence of the parasite's glycolysis regulation has been reported. Therefore, we hypothesized that the G-body is also formed in the ABS of the Pf parasite for regulating glycolytic activity spatiotemporally in a novel and rapid manner. We successfully generated several transgenic parasites expressing tagged glycolytic enzymes and observed G-body-like structures in the parasite under low glucose conditions. In addition, several G-body-related genes were identified by transcriptome analysis of parasites cultured under a low glucose medium. Here, we will discuss the G-body machinery and its possible functions in Pf.

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A New Perspective on Antibiotics Use Evaluation
~ Effectiveness on Antibiotics Stewardship Team's activity~

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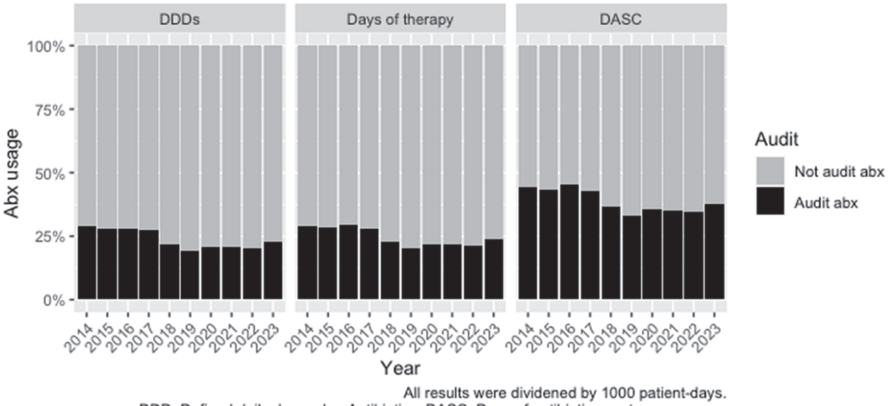
Abstract: Antibiotic use and management of the antibiotic spectrum are important indicators of appropriate antimicrobial use in hospitals. Nagasaki University Hospital (NUH) has been conducting an infectious disease consultation and assessment of broad-spectrum antibiotic use by an Antimicrobial Stewardship Team (AST) to evaluate the appropriate use of antibiotics. We evaluated the trends in antibiotic use from 2014 to 2023 using Defined Daily Dose /1000bed-days (AUD: Antibiotic Use Density), days of therapy (DOT), and Days of Antibiotic Coverage Spectrum (DASC), an evaluation method that considers the antimicrobial spectrum. The evaluation unit was 1000 patient-days. The proportion of broad-spectrum antibiotics (audit antibiotics) to the total antimicrobial use was also evaluated. Linear regression analysis was used to determine trends. The proportions of audit antibiotics were AUD 18.9~28.9%, DOT 20.1%~29.3%, and DASC 33.0~45.2% (Figure). Linear regression analysis showed an increasing trend in AUD and DOT but no significant change in DASC. In terms of the antibiotic spectrum, there was no significant change over approximately 10 years, suggesting that promoting the appropriate use of antibiotics was effective. However, the proportion of spectrum coverage by audit antibiotics remains high. Evaluating antibiotic use from different perspectives may be useful for more efficient AST activity by reflecting AST levels in more detail.

Figure. Proportion of audit abx 2014-2023



All results were dividened by 1000 patient-days.
DDD: Defined daily dose, abx: Antibiotics, DASC: Days of antibiotic spectrum coverage
Audit abx: piperacillin-tazobactam, meropenem, imipenem-cilastatin, and doripenem

Interaction between *Aspergillus fumigatus* and non-tuberculous mycobacteria

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Background: *Aspergillus* spp. is widely isolated from the environment, from soil to air. *Aspergillus* spp. cause fatal infections in immunocompromised patients. Patients with non-tuberculous mycobacteria- pulmonary disease (NTM-PD) complicates pulmonary aspergillosis in approximately 5%, which is associated with a poor prognosis. However, few reports have described the interaction between *A. fumigatus* and NTM. Therefore, we researched its interactions *in vitro* and *in vivo*.

Methods: The supernatants of *M. avium* (ATCC700737) and M. *abscessus* (ATCC19977) were added to *A. fumigatus* (Af 293) and other *Aspergillus* spp conidial suspension to evaluate the effect of metabolites of NTM on the biofilm formation of *Aspergillus* spp. In addition, we evaluated the effect of the supernatant of NTM on gene expression of *A. fumigatus* by RNA-seq. C57BL/6J mice were infected with NTM by aspiration of *M. avium* (ATCC 700737). The mice infected with NTM were also aspirated with *A. fumigatus* (Af293) after 11 weeks to evaluate the effect of prior infection with NTM on the clearance of *A. fumigatus*.

Results: The biofilms of *Aspergillus* spp. were significantly increased with the supernatant of NTM compared to the media control. The results of RNA-seq showed that the expression of genes involved in cell division and primary metabolism of *A. fumigatus* was increased by secondary metabolites of NTM. *In vivo* experiments, fungal burdens in the lungs have indicated that clearance of *A. fumigatus* was delayed compared with PBS control due to the prior infection with NTM.

Discussion: These results suggest that prior infection of NTM promotes the growth of *A. fumigatus* and may contribute to the colonization of *A. fumigatus* in the lung. We will analyze gene expressions and cytokine responses in animal models to elucidate the mechanism of *Aspergillus* colonization. We are also trying to identify causative agents and the molecular mechanisms of the interaction between *Aspergillus* spp and NTM.

P25

CXCITATORY AMINO ACIDS, POSSIBLE CAUSATIVE AGENTS OF NODDING SYNDROME AND NEW PATHOGENESIS

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Introduction: Nodding syndrome (NS) is one type of epilepsy and a progressive disease characterized by nodding symptoms with children in Sub-Saharan Africa. The burden for NS children is heavy, not only mentally but financially for themselves and their families, and yet, the cause and cure of NS remain unknown. The kainic acid-induced model in experimental animals is a well-known epilepsy model that is useful for studying human diseases. In this study, we examined similarities of clinical symptoms and histological brain changes between NS patients and kainic acid-treated rats. We argued for kainic acid agonist as one of the causes of NS and in addition, we introduce new pathogenesis of NS with kainic acid agonist.

Aim: We conducted kainic acid-induced rat model to validate kainic acid agonist as a new causative agent of NS.

Methods: Clinical signs in rats were studied after kainic acid administration, and histological lesions including the expression of tau protein and gliosis, were examined at 24 hours, 8 days, and 28 days after dosing. Additionally, estimation of human toxicity was conducted for the substance.

Results: Kainic acid-induced epileptic symptoms were observed in rats, including nodding accompanied by drooling and bilateral neuronal cell death in the hippocampus, piriform cortex, amygdaloid nucleus and thalamic nucleus of rat brain. In the regions that exhibited neuronal cell death, an increase in phosphorylated tau protein expression and gliosis were found immunohistochemically. The symptoms and brain histology were similar in the NS and kainic acid-induced rat models.

Conclusion: The results suggest that kainic acid agonist may be one of the causative substances for NS.

Current: Tricholomic acid contained in *Ustilago maydis*, known as maize disease and usually found in Northern Uganda, has an affinity for the kainic acid receptor. It is planned to analyze tricholomic acid contained in *Ustilago maydis* of maize and sorghum with Gulu University in Northern Uganda.

P26

Serological diagnostic assays for cutaneous leishmaniasis in Sri Lanka: rKMP-11-based ELISA

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Serology-based diagnostic tools for leishmaniasis can vary in terms of sensitivity, specificity, and reliability. The KMP-11 is a candidate diagnostic marker of leishmaniasis. This study aimed to compare the efficacy of recombinant KMP-11 antigen of *Leishmania donovani* and *Leishmania infantum*, along with crude *L. donovani* antigen, in detecting cutaneous leishmaniasis (CL) infections in Sri Lanka using enzyme-linked immunosorbent assay (ELISA). CL is the predominant clinical form observed in Sri Lanka, while cases of visceral leishmaniasis and mucocutaneous leishmaniasis are not widely recorded.

An optimized indirect ELISA was employed to determine the cut-off values, sensitivities, and specificities for the three selected antigens. The cut-off value for each test was determined using a receiver operating characteristic (ROC) curve based on the absorbance values of sera from 21 CL patients confirmed by microscopy and 21 healthy individuals from non-endemic areas.

The cut-off values for KMP-11 antigens were 0.169 for *L. donovani* and 0.162 for *L. infantum*, with sensitivities of 95.2% and 79.2%, and specificities of 100% and 71.4%, respectively. The cut-off for crude *L. donovani* antigen was 0.150, with a sensitivity of 98.0% and specificity of 90.3%. Positive ELISA results were seen in 95.2% of CL patient sera with KMP-11 for *L. donovani*, 81.0% with KMP-11 for *L. infantum*, and 100% with crude antigen. All antigens yielded negative results for non-endemic healthy controls.

Crude antigen showed the highest sensitivity, while the recombinant KMP-11 antigen of *L. donovani* displayed comparable performance in the detection of CL infections. Further validation, including a larger cohort from various settings, is needed to assess its potential for use as a candidate biomarker in future.

Key words: cutaneous leishmaniasis, *Leishmania donovani*, KMP-11 antigen, crude antigen, ELISA

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P27

High-throughput screening identifies compounds with nanomolar antiplasmodial activity against the asexual-stage parasites.

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The emergence of artemisinin-resistant malaria parasite highlights the need for new drugs with novel mechanisms of action against Plasmodium infection. To identify such compounds, we conducted a high-throughput screening campaign (HTS) using a robust fluorescent technique at a single-dose concentration of compounds included in the Nagoya Chemical Library. Primary HTS identified 1,365 hits (among 36,160 compounds) at a cut-off inhibition of 68.65%, established by the empirical rule of standard deviations (3×SD). A subset of 896 compounds was prioritized for dose-response assays following elimination owing to cytotoxicity against mammalian cells and the requirement to achieve a 1% hit rate for structure disclosure. This subset was subsequently tested for inhibitors of the mitochondrial electron

transport chain (MtETC), which is the most validated target, using the 3D7-yDHODH transgenic parasite strain alongside the wild-type 3D7. We have successfully identified 23 compounds showing complete parasite inhibition at nanomolar ranges, even at the lowest tested dose (12 nM) against 3D7, and seven compounds targeting MtETC showing > 100-fold shift in EC50 (3D7 vs. 3D7-yDHODH strains). A total of 435 compounds exhibiting ≤ 6.5 μM EC50 have been prioritized for further assays against a panel of Dd2 parasites harboring mutations in well-known drug target genes as an attempt to distinguish hit compounds with unknown or similar mechanisms of action. The MtETC-targeting compounds will be subjected to kinetic studies against the recombinant PfDHODH enzyme for further validation, and compounds that elicit unknown mechanisms of action will be used to raise resistant mutants, followed by whole-genome sequencing for target elucidation.

Comparative Analysis of SARS-CoV-2 Genetic Mutations before and after Antiviral Therapy

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Background: Recent guidelines endorse antiviral therapy for COVID-19 for specific indication. Although reports of antiviral resistance are increasing, detailed clinical evidence regarding the resistance mutation is limited. This study aims to assess the SARS-CoV-2 mutations in clinical samples from patients before and after antiviral treatment, exploring the potential risk of emerging resistance.

Samples and Methods: We conducted a retrospective analysis of paired upper respiratory samples from the patients with COVID-19, collected before and after antiviral therapy. A total of 19 pairs were analyzed, including 6 cases treated with remdesivir (Rem), 4 with nirmatrelvir/ritonavir (Nir), 3 with molnupiravir (Mol), and 6 controls (Ctr). Samples were sequenced using next generation sequencer (MiSeq, Illumina) and analyzed with CLC genomics workbench (QIAGEN), with the Wuhan Hu-1 strain as the reference.

Results: This study included 4 patients with a history of solid organ transplants (1liver, 1lung, 2kidney) and 8 with hematological disorders. Samples were collected at an average interval of 9.3 days apart. Viral RNA levels increased in 46.2% (6/13) of cases in the treatment groups. The mutation counts pre- and post- therapy were not statistically different in all groups; Nucleic acid mutations for Rem were 79.5 vs 80.2 (P=0.74), for Nir 81.5 vs 88.5 (P=0.23), for Mol 78.7 vs 107.3 (P=0.35), and for Ctr 88.8 vs 111.3 (P=0.13); Amino acid mutations were also similar; Rem 57.3 vs 57.0 (P=0.86), Nir 53.0 vs 55.0 (P=0.22), Mol 51.0 vs 58.0 (P=0.38), Ctr 54.0 vs 55.0 (P=0.15). No unique or shared amino acid mutations were associated with any treatment group, nor among cases with increased viral load.

Conclusion: Our findings indicate that short-term use of antiviral agents did not significantly alter the mutation profile of SARS-CoV-2, suggesting minimal concerns about resistance development during a single course of therapy, even among patients with increased viral load. These results support the continued use of these agents according to current guidelines, alleviating immediate fears of resistance emergence.

P29

Engineering Trypanosomal alternative energy metabolism pathways in human cells to investigate OXPHOS contributions in mitochondrial disease.

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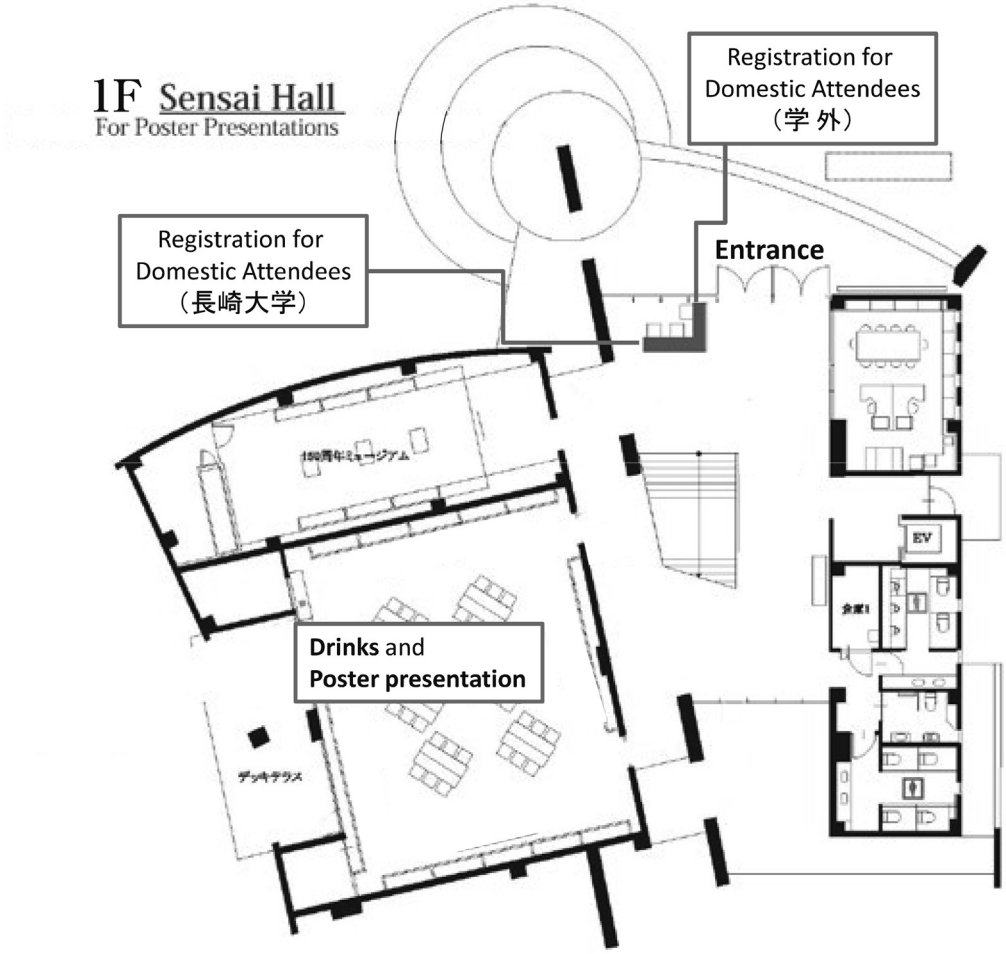
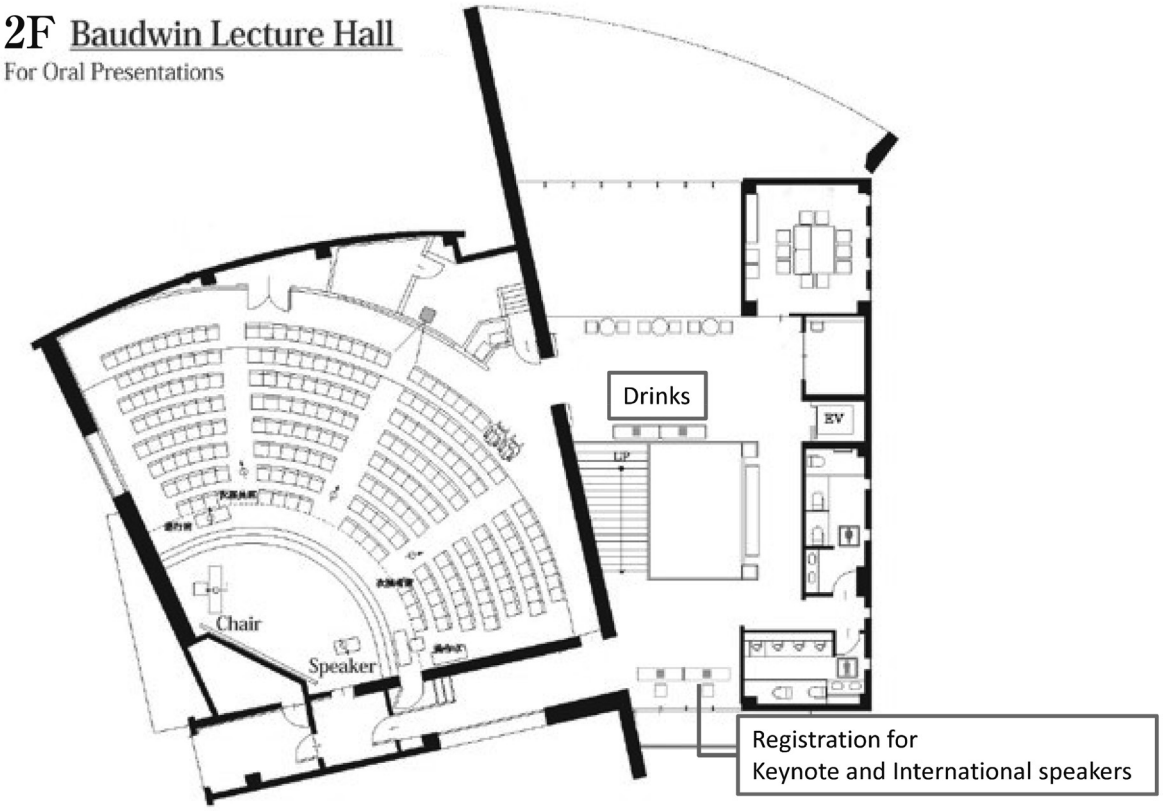
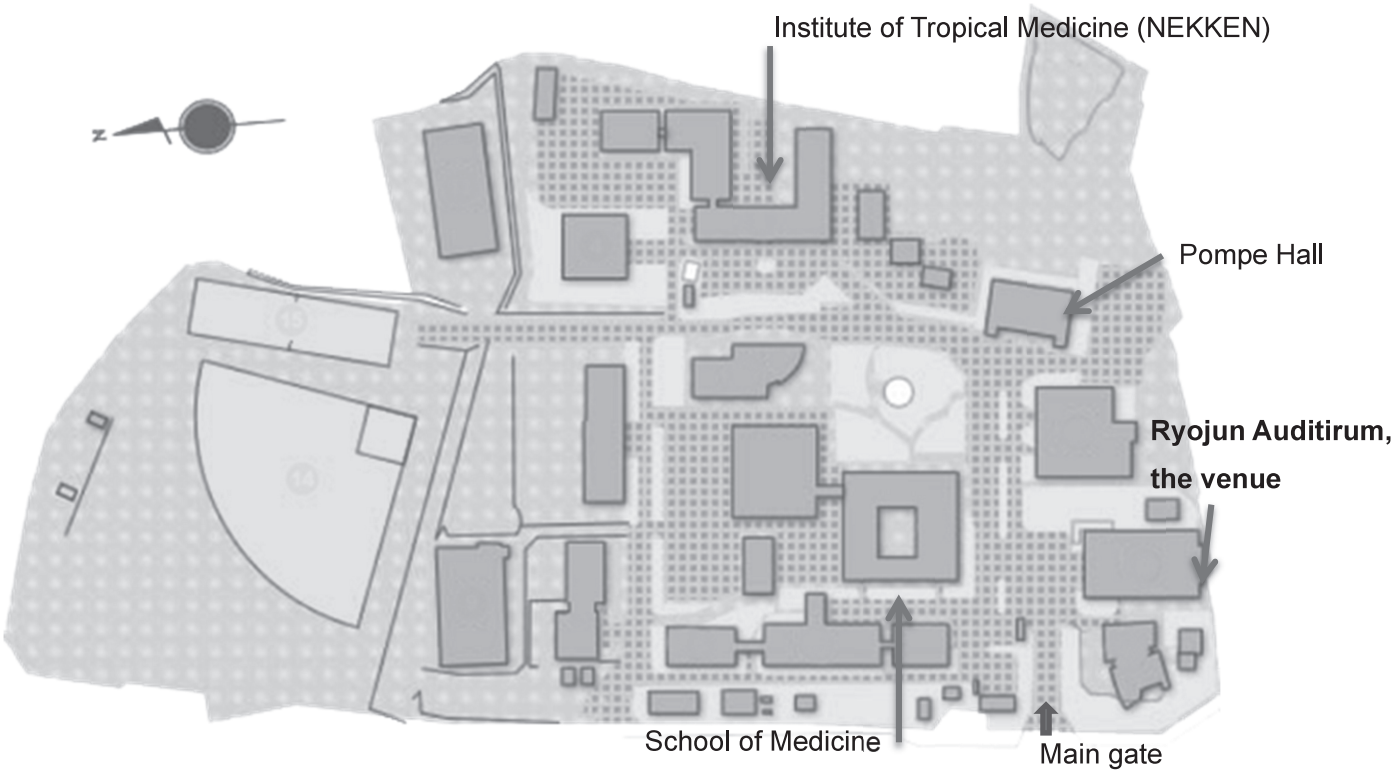
Trypanosomatids are parasitic protozoa responsible for several neglected tropical diseases, including sleeping sickness (Human African Trypanosomiasis), Chagas disease, and leishmaniasis. In the blood stream forms of *Trypanosoma brucei*, the alternative oxidase (TAO) serves as a terminal oxidase, while ATP is synthesized through oxidative phosphorylation (OXPHOS) and the acetate:succinate CoA transferase (ASCT)/succinyl-CoA synthase (SCS) cycle. In the mitochondria of eukaryotic cells, ATP synthesis predominantly occurs via ATP synthase through OXPHOS. Mutations in mitochondrial genes can lead to defective mitochondria, impairing OXPHOS and resulting in mitochondrial diseases. In this study, we aim to establish an "energy metabolic alternative pathway" in human cell mitochondria by utilizing TAO and/or ASCT and their ability to bypass respiration and OXPHOS defects induced by inhibitors. We overexpressed TAO and/or ASCT in HeLa cells and measured the oxygen consumption rate using oligomycin A (a complex V inhibitor), antimycin A (a complex III inhibitor), and ascofuranone (a TAO-specific inhibitor). The results indicated that cells expressing TAO or ASCT exhibited resistance to antimycin A or oligomycin A, respectively.

Ryojun Auditorium

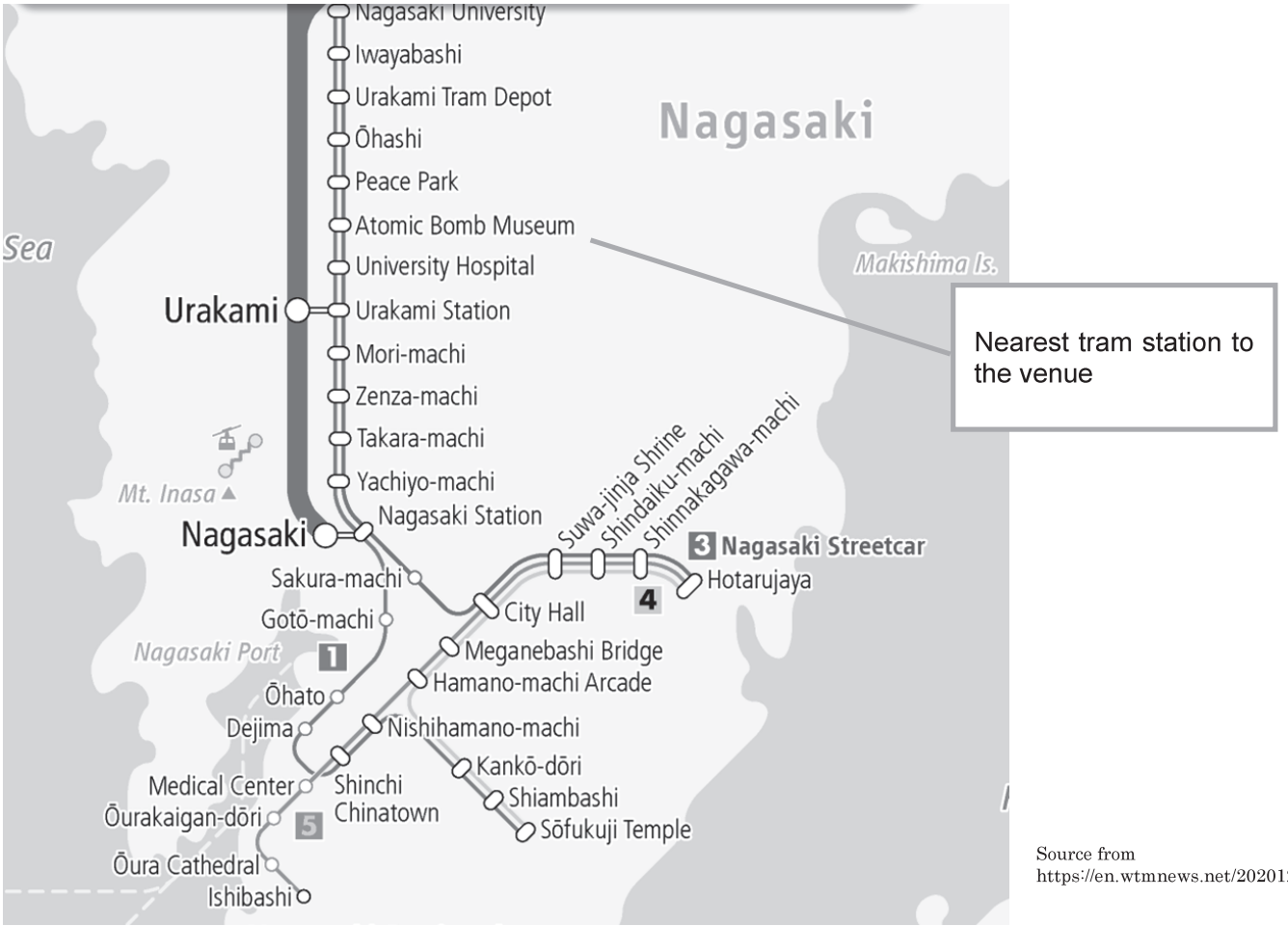




■Campus Map (**Sakamoto Campus 1**, Nagasaki University)



■Traffic Access to "**Sakamoto Campus 1**"



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