The 13th Nagasaki-Singapore Medical Symposium / Leading Program International Symposium 2017

Time	Day 1: 18th May 2017 (THU)	Time	Day 2: 19th May 2017 (FRI)
8:20	Registration	8:40	Registration
8:50–9:10	**Opening Remarks** Shigeru Katamine (President, Nagasaki University) Kenji Hirayama (Dean, Institute of Tropical Medicine, Nagasaki University) Nicholas R.J. Gascoigne (Provost's chair, Head of Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore)	9:10–9:50	**Keynote lecture 5** Sohkichi Matsumoto (Graduate School of Medical and Dental Sciences, Niigata University, Japan)
9:10–9:50	**Keynote lecture 1** Nicholas R.J. Gascoigne (Yong Loo Lin School of Medicine, National University of Singapore)		
9:50–10:30	**Keynote lecture 2** Osamu Nakagomi (Graduate School of Biomedical Sciences, Nagasaki University)	9:50–10:30	**Keynote lecture 6** Koji Nakayama (Graduate School of Biomedical Sciences, Nagasaki University)
10:30–10:45	Tea Break @1F Sensai Hall	10:30–10:45	Tea Break @1F Sensai Hall
10:45–11:25	**Keynote lecture 3** Kiyoshi Kita (School of Tropical Medicine & Global Health, Nagasaki University)	10:45–11:25	**Keynote lecture 7** Laurent Rénia (Singapore Immunology Network, A*STAR, Biopolis, Singapore)
11:25–12:05	**Keynote lecture 4** John M. Kelly (London School of Hygiene and Tropical Medicine, UK)	11:25–12:05	**Keynote lecture 8** Cevayir Coban (Immunology Frontier Research Center, Osaka University, Japan)
12:05–13:00	Lunch Time	12:05–13:00	Lunch Time
13:00–13:25	**Session 1** Vincent T.K. Chow (Yong Loo Lin School of Medicine, National University of Singapore)	13:00–13:25	**Session 4** Kevin S.W. Tan (Yong Loo Lin School of Medicine, National University of Singapore)
13:25–13:50	Sylvie Alonso (Yong Loo Lin School of Medicine, National University of Singapore)	13:25–13:50	Richard Culleton (Institute of Tropical Medicine, Nagasaki University)
13:50–14:15	Jiro Yasuda (Institute of Tropical Medicine, Nagasaki University)	13:50–14:15	Daisuke Kimura (Graduate School of Biomedical Sciences, Nagasaki University)
14:15–14:40	Yoshinao Kubo (Graduate School of Biomedical Sciences, Nagasaki University)	14:15–14:30	Tea Break @1F Sensai Hall
14:40–14:55	Tea Break @1F Sensai Hall	14:30–14:55	**Session 5** Bruce Russell (Department of Microbiology and Immunology, University of Otago, New Zealand)
14:55–15:20	**Session 2** Paul MacAry (Yong Loo Lin School of Medicine, National University of Singapore)	14:55–15:20	Masahito Asada (Institute of Tropical Medicine, Nagasaki University)
15:20–15:45	Meng Ling Moi (Institute of Tropical Medicine, Nagasaki University)	15:20–15:45	Cynthia Y. He (Centre for BioImaging Sciences, National University of Singapore)
15:45–16:10	Justin Jang Hann Chu (Yong Loo Lin School of Medicine, National University of Singapore)	15:45–16:00	Tea Break @1F Sensai Hall
16:10–16:25	Tea Break @1F Sensai Hall	16:00–16:25	**Session 6** John Chen (Yong Loo Lin School of Medicine, National University of Singapore)
16:25–16:50	**Session 3** Yongliang Zhang (Yong Loo Lin School of Medicine, National University of Singapore)	16:25–16:50	Volker Patzel (Yong Loo Lin School of Medicine, National University of Singapore)
16:50–17:15	Lay Myint Yoshida (Institute of Tropical Medicine, Nagasaki University)	16:50–17:15	Philip Gothard (London School of Hygiene and Tropical Medicine, UK)
17:15–17:40	Koichi Izumikawa (Graduate School of Biomedical Sciences, Nagasaki University)	17:15–17:20	**Closing Remarks** Katsuyuki Yui (Chair of the organizing committee, Graduate School of Biomedical Sciences, Nagasaki University)
18:00–18:45	Poster presentation I (Odd number) at1F Sensai Hall (with light refreshments)		
18:45–19:30	Poster presentation II (Even number) at1F Sensai Hall		

Day 1: 18th May 2017 (THU)

Bauduin Lecture Hall, Ryojun Auditorium 2F

Registration
08:20-09:10

Opening remarks
08:50-09:10 Shigeru Katamine (President, Nagasaki University, Japan),
Kenji Hirayama (Dean, Institute of Tropical Medicine, Nagasaki University, Japan)
Nicholas R.J. Gascoigne (Provost's chair, Head of Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore)

(Chair: Katsuyuki Yui, Graduate School of Biomedical Sciences, Nagasaki University)
Keynote lecture 1
09:10-09:50 Nicholas R.J. Gascoigne (Yong Loo Lin School of Medicine, National University of Singapore)
"Control of T cell receptor signaling during activation and development"

Keynote lecture 2
09:50-10:30 Osamu Nakagomi (Graduate School of Biomedical Sciences, Nagasaki University)
"Transmission of animal rotaviruses to humans: distinguishing its evolutionary implication from contemporary events"

10:30-10:45 **Tea Break @1F Sensai Hall**

(Chair: Kenji Hirayama, Institute of Tropical Medicine, Nagasaki University)
Keynote lecture 3
10:45-11:25 Kiyoshi Kita (School of Tropical Medicine & Global Health, Nagasaki University)
"Mitochondria as drug target: From parasites to cancer cells"

Keynote lecture 4
11:25-12:05 John M. Kelly (London School of Hygiene and Tropical Medicine, UK)
"Tracking *Trypanosoma cruzi* parasites to tissue-specific reservoir sites during chronic murine infections"

12:05-13:00 **Lunch Time @Pompe Hall (for guests)**

Session 1
(Chair: Noriyuki Nishida, Graduate School of Biomedical Sciences, Nagasaki University)
13:00-13:25 Vincent T.K. Chow (Yong Loo Lin School of Medicine, National University of Singapore)
"NETs and Leaks in pneumonia caused by Influenza virus and pneumococcus"

13:25-13:50 Sylvie Alonso (Yong Loo Lin School of Medicine, National University of Singapore)
"Novel *in vitro* model to study Enterovirus 71 neurovirulence reveals autophagy-mediated exit without lysis (AWOL) for this lytic virus."

13:50-14:15 Jiro Yasuda (Institute of Tropical Medicine, Nagasaki University)
"Tetherin/BST-2 as a cellular antiviral factor"

14:15-14:40 Yoshinao Kubo (Graduate School of Biomedical Sciences, Nagasaki University)
"Identification of novel host anti-HIV-1 factors that are induced by interferon γ"

14:40-14:55 **Tea Break @1F Sensai Hall**

Session 2

(Chair: Kouichi Morita, Institute of Tropical Medicine, Nagasaki University)

14:55-15:20 Paul MacAry (Yong Loo Lin School of Medicine, National University of Singapore)
"T-lymphocyte and Antibody responses in patients infected with or recovered from Dengue Virus"

15:20-15:45 Meng Ling Moi (Institute of Tropical Medicine, Nagasaki University)
"Determination of the role of flavivirus cross-reactive antibodies during Dengue and Zika infection by using common marmosets"

15:45-16:10 Justin Jang Hann Chu (Yong Loo Lin School of Medicine, National University of Singapore)
"System biology approaches for antiviral strategies development against human enteroviruses that cause HFMD"

16:10-16:25 **Tea Break @1F Sensai Hall**

Session 3

(Chair: Katsunori Yanagihara, Graduate School of Biomedical Sciences, Nagasaki University)

16:25-16:50 Yongliang Zhang (Yong Loo Lin School of Medicine, National University of Singapore)
"MAP kinase phosphatase 2 regulates innate immune response to intracellular bacterial infection"

16:50-17:15 Lay Myint Yoshida (Institute of Tropical Medicine, Nagasaki University)
"Role of viral and bacterial pathogens on pediatric pneumonia in Vietnam"

17:15-17:40 Koichi Izumikawa (Graduate School of Biomedical Sciences, Nagasaki University)
"Outbreak of carbapenem-resistant enterobacteriaceae in neonatal intensive care unit and growing care unit in Nagasaki University Hospital"

18:00-18:45 **Poster presentation I (Odd numbers) @1F Sensai Hall (with light refreshments)**

18:45-19:30 **Poster presentation II (Even numbers) @1F Sensai Hall**

Day 2: 19th May 2017 (FRI)

Bauduin Lecture Hall, Ryojun Auditorium 2F

Registration
08:40-09:10

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

Keynote lecture 5
09:10-09:50 Sohkichi Matsumoto (Graduate School of Medical and Dental Sciences, Niigata University, Japan)
"From biology of *Mycobacterium* to the development of the control strategies against tuberculosis"

Keynote lecture 6
09:50-10:30 Koji Nakayama (Graduate School of Biomedical Sciences, Nagasaki University)
"The type IX secretion system, gliding motility and the type V pilus in the Bacteroidetes phylum bacteria"

10:30-10:45 **Tea Break @1F Sensai Hall**

(Chair: Osamu Kaneko, Institute of Tropical Medicine, Nagasaki University)

Keynote lecture 7
10:45-11:25 Laurent Rénia (Singapore Immunology Network, A*STAR, Biopolis, Singapore)
"Identification of reticulocyte receptors for *Plasmodium vivax* merozoite invasion"

Keynote lecture 8
11:25-12:05 Cevayir Coban (Immunology Frontier Research Center, Osaka University, Japan)
"Tissue-specificity during malaria infection"

12:05-13:00 **Lunch Time @Pompe Hall (for guests)**

Session 4
(Chair: Sharon Cox, School of Tropical Medicine & Global Health, Nagasaki University)
13:00-13:25 Kevin S.W. Tan (Yong Loo Lin School of Medicine, National University of Singapore)
"Drug-mediated digestive vacuole disruption in malaria parasites: What's knobs got to do with it?"

13:25-13:50 Richard Culleton (Institute of Tropical Medicine, Nagasaki University)
"Genetic analysis of *Plasmodium malariae* and *Plasmodium ovale* from asymptomatic adolescents in South-East Nigeria."

13:50-14:15 Daisuke Kimura (Graduate School of Biomedical Sciences, Nagasaki University)
"Interleukin-27 inhibits the generation of memory $CD4^+$ T cells after treatment with anti-malarial drug."

14:15-14:30 **Tea Break @1F Sensai Hall**

Session 5
(Chair: Mana Miyakoda, Graduate School of Biomedical Sciences, Nagasaki University)
14:30-14:55 Bruce Russell (Department of Microbiology and Immunology, University of Otago, New Zealand)
"Zoonotic *P. cynomolgi* switches preference for red cell tropism and Duffy dependence"

14:55-15:20 Masahito Asada (Institute of Tropical Medicine, Nagasaki University)
"Ungulate malaria parasites"

15:20-15:45 Cynthia Y. He (Centre for BioImaging Sciences, National University of Singapore)
"CryoET of *Trypanosoma brucei*"

15:45-16:00 **Tea Break @1F Sensai Hall**

Session 6
(Chair: Hideki Hayashi, Graduate School of Biomedical Sciences, Nagasaki University)

16:00-16:25 John Chen (Yong Loo Lin School of Medicine, National University of Singapore)
"Genome hypermobility by co-lateral transduction"

16:25-16:50 Volker Patzel (Yong Loo Lin School of Medicine, National University of Singapore)
"A *trans*-splicing based suicide gene therapy approach targeting virus infection or cancer"

16:50-17:15 Philip Gothard (London School of Hygiene and Tropical Medicine, UK)
"How should we train doctors in tropical medicine?"

Closing remarks

17:15-17:20 Katsuyuki Yui (Chair of the organizing committee, Graduate School of Biomedical Sciences, Nagasaki University)

Reception for guests **@ The Hotel Nagasaki 15F "The Kitchen"**
19:00-21:00

Abstracts

Keynote Lecture 1

Control of T cell receptor signaling during activation and development

Nicholas R.J. Gascoigne

Department of Microbiology and Immunology, National University of Singapore, Singapore 117545

The developmental programme of thymocytes involves both positive and negative selection of the T cell receptor repertoire. A careful balance between these selection events is required to enable recognition of invading pathogens but to avoid autoimmunity. TCR recognition is an analog event, but the outcome of signaling during development, after transduction into several signaling cascades, is digital – either life (and differentiation) or death. Themis is a T cell-specific protein that is required at the positive selection checkpoint. It associates with Grb2 and is thus recruited to the LAT signalosome after TCR stimulation. Themis controls the strength of TCR signals from positive-selecting ligands. It interacts with the phosphatase Shp1 (Ptpn6), regulating its phosphatase activity, which increases after TCR stimulation. In this way, Themis negatively regulates the strength of TCR signaling to positive selecting ligands, but responses to strong, negative selecting ligands are unaffected. In the absence of Themis, signaling in response to positive selecting ligands results in increased apoptosis or lineage-deviation to “agonist-selected” cell types. Themis is downregulated after positive selection, but remains expressed at an intermediate level in mature thymocytes and in peripheral T cells. We have made a conditional knockout mouse to assess Themis’ role in mature T cells. New results from these mice will be presented.

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.

Keynote Lecture 2

Transmission of animal rotaviruses to humans: distinguishing its evolutionary implication from contemporary events

Osamu Nakagomi

Department of Molecular Epidemiology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

Rotavirus A (RVA), a virus species within the *Reoviridae* family, is a leading cause of severe acute gastroenteritis in infants and young children, but its host species is not limited to humans. RVA has been recovered from many mammalian species as well as birds. While there are RVA strains inherent to each animal host species, the host species barrier can be breached. Animal RVA strains, albeit infrequently, infect and cause disease in humans. Such transmission of RVA beyond the host species barrier was conceptualised by the sharing of genomic RNA constellation termed as "genogroup" which, in practice, was defined by high stringency RNA-RNA hybridization assays. Genogrouping by RNA-RNA hybridization assays distinguished two types of interspecies transmission events; (1) transmission as a whole genome and (2) transmission of partial genome as genetic reassortment. When whole genome sequencing of RVA strains became a reality in the 2000s, the concept of genogroup was replaced by "genotype constellation" in which a genotype is defined by a nucleotide sequence identity cut-off value set for each gene. Whole genome sequence information has made it possible to reconstruct phylogenetic relationships and estimate the evolutionary rates of the genes. Thus, molecular epidemiologists are now able to distinguish (1) human RVA strains that acquired animal rotavirus genes over the course of evolutionary history from (2) animal RVA strains having recently transmitted to children but died out without establishing a human-to-human transmission chain. This has tremendous implication in our understanding of how RVA evolves over time. A few examples will be presented and discussed including our hypothesis that all contemporary human G1 VP7 genes are virtually descendants from a single, successful transmission event of a porcine G1 rotavirus to humans.

Speaker's Profile

Together with his wife, Toyoko Nakagomi, Professor Nakagomi provided, for the first time, robust molecular evidence for interspecies transmission of rotaviruses. It was a heretical idea in the 1980s and received strong scepticism. He has devoted on this issue over the ensuing three decades. He co-authored >250 original and review articles on rotaviruses which were cited >7000 times to date. Scopus ranks him the 5th most productive author in the world under the keyword of "rotavirus".

Keynote Lecture 3

Mitochondria as drug target : From parasites to cancer cells

Kiyoshi KITA

School of Tropical Medicine and Global Health, Nagasaki University, Nagasaki 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, marked 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 (Siregar et al., Science, 2016).

Our studies on respiratory chain of the parasitic helminth, *Ascaris suum* has shown that the mitochondrial NADH-fumarate reductase system (fumarate respiration), which is composed of complex I (NADH-rhodoquinone reductase), rhodoquinone and complex II (rhodoquinol-fumarate reductase) plays an important role in the anaerobic energy metabolism of adult parasites inhabiting hosts as well as unique features of the developmental changes that occur during their life cycle (Ōmura et al., PNAS, 2001). Recently, we found "fumarate respiration" system plays an important role not only in *A. suum* but also other parasites including cestoda, *Echinococcus multilocularis* protoscoleces. Furthermore, we found "fumarate respiration" is essential for the growth of some cancer cells in hypoxic and low nutrition condition, suggesting a promising target for chemotherapy. In addition, other novel parasite respiratory chains including Trypanosomes will be discussed.

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 gradated 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).

Dr. Kita 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). Dr. Kita 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).

Keynote Lecture 4

Tracking *Trypanosoma cruzi* parasites to tissue-specific reservoir sites during chronic murine infections

John M. Kelly

Department of Pathogen Molecular Biology, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, UK

Chagas disease is caused by the insect-transmitted protozoan *Trypanosoma cruzi*, and is the most important parasitic infection in Latin America. As a result of migration, it is also emerging as a public health issue in non-endemic regions. Infections with *T. cruzi* are life-long, and lead to cardiomyopathy in 20-30% of cases. A causal link between cardiac infection and pathology has been difficult to establish because of a lack of robust methods to detect scarce focally distributed parasites within tissues. By combining highly sensitive bioluminescence imaging and fluorescence technology, we have developed procedures which have allowed us to track infection dynamics, quantify tissue-specific parasite loads, and provide new insights into parasite biology in predictive murine models. We have established that the gut is the major reservoir site during chronic infections, found evidence for asynchronous amastigote replication within individual host cells, and identified new morphological forms of the parasite at a variety of tissue sites. We propose a model of cardiac pathogenesis driven by periodic trafficking of parasites into the heart during the chronic stage, occurring at a frequency determined by host and parasites genetics.

NETs and Leaks in Pneumonia Caused by Influenza Virus and Pneumococcus

Vincent TK Chow[1], L Li[2], AN Moorthy[1], Y Zhang[1], T Narasaraju[3], N Sakamoto[4], K Izumikawa[4], Andrew NS Tan[2]

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

[2]School of Biological Sciences, Nanyang Technological University, Singapore

[3]Center for Veterinary Health Sciences, Oklahoma State University, Oklahoma, USA

[4]Department of Infectious Diseases, Graduate School of Biomedical Sciences, Nagasaki University, Japan

Excessive host inflammatory responses negatively impact disease outcomes of pneumonia. In view of the rapid evolution of respiratory pathogens, it is challenging to develop more effective vaccines and antimicrobial agents. To better understand host-pathogen interactions during the critical inflammatory and repair phases of pneumonia, we studied the role of neutrophils, neutrophil extracellular traps (NETs) and c-angiopoietin-like 4 (cANGPTL4) in the pathogenesis of influenza and pneumococcal pneumonia. The presence of NETs and the effects of cANGPTL4 were studied in mouse models of primary infection with influenza virus H1N1 and/or H3N2, and secondary pneumococcal pneumonia. cANGPTL4 antibody treatment and knockout mice were also investigated. Lung biopsy and clinical samples from patients with or without pneumonia were also analyzed for cANGPTL4 expression. Excessive infiltration of neutrophils and significant formation of NETs were associated with severe influenza and pneumococcal pneumonia. Intense pulmonary NETs generation, elevated myeloperoxidase activity, cytokine dysregulation, pneumococcal capsule thickness determined the disease severity. Influenza infection stimulated the expression of cANGPTL4 via a direct IL6-STAT3-mediated mechanism. cANGPTL4 enhanced pulmonary tissue leakiness and exacerbated inflammation-induced lung injury. Treatment of infected mice with neutralizing anti-cANGPTL4 antibody significantly accelerated lung recovery and enhanced lung tissue integrity. The cANGPTL4-deficient mice also displayed diminished lung damage and recovered more rapidly from influenza pneumonia compared to their wild-type counterparts. Retrospective examination of lung biopsies and clinical samples from patients with infection-induced pneumonia with tissue damage revealed elevated expression of cANGPTL4 compared to normal or uninfected samples. These observations highlight the important roles that NETs and cANGPTL4 play in pulmonary infection and damage, and may facilitate the development of novel biomarkers and intervention strategies to improve the management of pneumonia.

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 Infectious Diseases Research Group, Singapore-MIT Alliance for Research & Technology. Since 1996, he established the Human Genome Laboratory in the Department of Microbiology 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 ~250 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 at the 6th Asia-Pacific Congress of Medical Virology). His research interests in the past several years have focused on the molecular genetics and infectomics of influenza pneumonia and of hand, foot and mouth disease, specifically on the cellular, molecular, and viral pathogenesis of severe influenza and enterovirus 71 infections.

Novel *in vitro* model to study Enterovirus 71 neurovirulence reveals autophagy-mediated exit without lysis (AWOL) for this lytic virus.

Sylvie ALONSO

Department of Microbiology & Immunology, Yong Loo Lin School of Medicine, NUS; and Immunology program, Life Sciences Institute, NUS.

Abstract

Enterovirus 71 (EV71) causing Hand, Foot and Mouth Disease, is regarded as the most important neurotropic virus worldwide. EV71 is believed to replicate in muscles and infect motor neurons to reach the central nervous system (CNS). To further investigate the mechanisms involved, we have employed the motor neuron cell line NSC-34. NSC-34 cells were permissive to EV71 and virus production yields were strain-dependent with differential efficacy at the entry, replication and egress steps. Furthermore, unlike all the other cell lines previously reported, EV71-infected NSC-34 cells neither displayed cytopathic effect nor underwent apoptosis. Instead, autophagy was markedly up-regulated and virus-containing autophagic vacuoles were isolated from the culture supernatant, providing the first experimental evidence that EV71 can adopt a non-lytic exit pathway. Finally, the ability of EV71 to infect productively NSC-34 cells correlated with its ability to invade the CNS *in vivo*, supporting the relevance of NSC-34 cells to study the intrinsic neurovirulence of EV71 strains.

Speaker's biography:

A/P Alonso has completed her PhD in France (University Claude Bernard Lyon I) and pursued her postdoctoral studies at the Pasteur Institute of Lille (France) and Cornell University (NY, USA). She has established her laboratory at NUS, Department of Microbiology and Immunology since 2005. She is also a member of the Immunology programme at the Life Sciences Institute (LSI), NUS. Her main interests lie in host-pathogen interactions with a focus on Dengue, Tuberculosis and Enterovirus 71. She has published more than 70 peer-reviewed papers in reputed journals and has been serving as an editorial board member of PLOSone and Frontiers in Immunotherapies and Vaccines.

Tetherin/BST-2 as a cellular antiviral factor

Jiro Yasuda

Department of Emerging Infectious Diseases, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan

Human Tetherin/BST-2 was first identified as a cellular restriction factor that blocks the release of HIV-1 in the absence of the viral accessory protein, Vpu. Subsequent studies have shown that Tetherin/BST-2 is encoded in the genome of most animal species and also inhibits the release of various enveloped viruses including other retroviruses, filoviruses, arenaviruses, and herpesviruses (Sakuma *et al*., 2009, Fukuma *et al*., 2011, Takeda *et al*., 2012, Abe *et al*., 2014).

Tetherin/BST-2 is a type II integral membrane protein consisting of an N-terminal cytoplasmic tail, a transmembrane domain, followed by an extracellular domain important for dimerization, and a glycophosphatidyl inositol (GPI) lipid anchor at its C-terminus. It appears to inhibit virus release by physically tethering viral particles to the cell surface by means of its N-terminal transmembrane domain and C-terminal GPI anchor. The extracellular domain has two putative *N*-linked glycosylation sites, which are highly conserved at the same positions among human, rhesus monkey, dog, pig, rat, and mouse, and orthologs have been identified that are actually glycosylated heterogeneously. Previously, we showed that *N*-linked glycosylation is dispensable for the antiviral activity of human Tetherin/BST-2 against Lassa and Marburg viruses (Sakuma *et al*., 2009).

Human Tetherin/BST-2 is constitutively expressed in terminally differentiated B cells, bone marrow stromal cells, and plasmacytoid dendritic cells, and is upregulated in various cell types on treatment with type I and type II interferon (IFN). Therefore, Tetherin/BST-2 is thought to be involved in antiviral host defense as an innate immunity mechanism. It has also been reported that several viruses encode antagonists, which antagonize the antiviral activity of Tetherin/BST-2.

Here I will introduce our recent studies on Tetherin/BST-2 as a cellular antiviral factor.

Speaker's Profile

1991 DVM, Hokkaido Univ, 1994 Ph.D, Grad Univ Adv Studies, 1994- Postdoc. Univ Alabama (UAB) (Prof. Eric Hunter), 1996- Assis Prof. Inst Med Sci, Univ Tokyo, 2000- Assoc Prof. Inst Genetic Med, Hokkaido Univ, 2004- Chief, Nat Res Inst Police Sci, 2010- Prof. Inst Tropical Med, Nagasaki Univ

Identification of novel host anti-HIV-1 factors that are induced by interferon γ

Yoshinao Kubo[1,2], Mai Izumida[1,3], Hideki Hayashi[1,4], Toshifumi Matsuyama[1,5]

[1]Department of Molecular Microbiology and Immunology, [2]Program for Nurturing Global Leaders in Tropical and Emerging Communicable Diseases, [3]Department of Clinical Medicine, Institute of Tropical Medicine, [4]Medical University Research Administrator (MEDURA), Graduate School of Biomedical Science, Nagasaki University, Japan and [5]Department of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, USA

Interferons (IFNs) are cytokines that have anti-virus activity. IFNs are divided into 3 types according to their receptors. Mechanism of the anti-virus activity of type I IFN (IFN-α and -β) has been vigorously studied, and several anti-virus host factors induced by the type I IFN have been already identified. However, the mechanism by which type II IFN (IFN-γ) restricts virus replication is still unknown. We found that IFN-γ treatment of HeLa cells significantly restricted the HIV-1 vector infection. To identify host factors induced by IFN-γ, microarray analysis was performed. Host factors that have been already reported to inhibit virus infection were not induced by the IFN-γ-treatment. To identify cellular factors involved in the IFN-γ-mediated restriction of HIV-1 vector infection, cDNA clones of host factors that were increased by IFN-γ more than 10 times were isolated, and their effects on HIV-1 vector infection were comprehensibly analyzed. As the result, we found that FAT10, IDO1, and IFI6 dramatically suppressed HIV-1 vector infection. IDO1 (indoleamine 2,3-dioxygenase 1) digests an essential amino acid, tryptophan. Addition of excess tryptophan recovered the HIV-1 vector infection in IDO1-expressing cells. IDO1 elevated the LC3-II level. Silencing of Atg3 that is required for induction of autophagy restored the HIV-1 vector infection in IDO1-expressing cells. These results suggested that IDO1 restricts HIV-1 infection by inducing autophagy through the depletion of tryptophan. This study discovered that FAT10, IDO1, and IFI6 function as host restriction factors against HIV-1.

Speaker's Profile (Yoshinao Kubo)

Dr. Kubo obtained PhD degree from Institute for Virus Research, Kyoto University, and then got assistant professor position in Kyoto University. He completed postdoctoral training at Clinical Research Institute of Montreal, Canada, and RIKEN, Japan. He studied on the pathogenesis of murine leukemia virus. He moved to Institute of Tropical Medicine, Nagasaki University, and started HIV-1 research. Now, he is an associate professor in Program for Nurturing Global Leaders in Tropical and Emerging Communicable Diseases, Nagasaki University.

T-lymphocyte and Antibody responses in patients infected with or recovered from Dengue Virus

Paul MacAry

Department of Molecular Microbiology and Immunology, Yong Loo Lin School of Medicine; LSI Immunology Programme, the Life Science Institute, National University of Singapore

Dengue virus (DENV) is a member of the family Flaviviridae and the genus Flavivirus. DENV includes four related although antigenically-distinct serotypes (DENV1, 2, 3 and 4) and all four serotypes can be found throughout the tropical and sub-tropical regions of the world. DENV is the etiological agent of dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue is the most significant mosquito-borne viral disease afflicting human populations with over one hundred million estimated new infections each year and current treatment is supportive in nature-it is based on fluid replacement and the provision of analgesics. Recently, we discovered that patients recovering from a natural dengue infection develop a broad antibody repertoire directed against both neutralizing and non-neutralizing determinants on the virus. We also found that natural infection induces dengue-specific CD8 (+) T lymphocytes that are highly activated and proliferating, exhibit antiviral effector functions. In my presentation, I will detail our recent findings on the form and functionality of T-lymphocyte and antibody responses in patients infected with or recovered from Dengue Virus.

Speaker's Profile

Dr Paul MacAry is currently an Associate Professor in the Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, NUS and the Director of the Immunology Programme, Life Science Institute, NUS. He received his BSC Hons in Molecular Genetics from Glasgow University and his PhD in Immunology from GKT, University of London. He has been an independent investigator in the Department of Microbiology and Immunology Programme at the National University of Singapore (NUS) since 2005. The MacAry laboratory covers a broad spectrum of antibody-related scientific endeavour, from basic B-cell research to industrial applications with an emphasis on antibody biology, immune repertoire mapping and protein engineering.

Determination of the role of flavivirus cross-reactive antibodies during Dengue and Zika infection by using common marmosets

Meng Ling Moi

Department of Virology Institute of Tropical Medicine, Nagasaki Japan

Antibodies are considered central in the protective immunity to Dengue, Zika and other flaviviruses. Infection with one dengue virus (DENV) serotype confers life-long protection to infection with the same serotype whereas cross-protective immunity to other DENV serotypes wanes after a limited period. After the cross-protective period, individuals are at risk of developing severe dengue upon secondary infection with a heterotypic serotype. During secondary infection, these cross-reactive, non-neutralizing antibodies are hyphothesized to enhance virus infection of the Fcgamma receptor (FcγR) bearing cells. In flavivirus infection, the ability to mount an early and vigorous antibody response is associated with better clinical outcomes, indicating that anamnestic anti-flavivirus immune response induced by prior flavivirus infection is important in conferring protection. This implicates that antibodies play a central role in modulating disease outcome. In this study, we developed an animal model using marmosets for the studies of secondary flavivrus infection. Using marmosets and patient serum samples, we confirmed the presence of flavivirus cross-reactive neutralizing and infection-enhancing antibodies. These antibody-immune complexes are infective only to FcγR-bearing cells and contributes to higher viremia titers during secondary flavivirus infection.

The study has several implications; (1) non-neutralizing, infection-enhancing activity hampers flavivirus neutralizing activity, contributing to an immune profile that fails to offer protection, (2) during secondary flavivirus infection, non-neutralizing antibodies form infectious virus-immune complexes, leading to higher infectivity of virus target cell in vivo, the FcγR-bearing cells, and (3) in comparison to conventional neutralizing assays, assays using the FcγR-expressing cells may better reflect the biological properties of antibodies *in vivo*.

Speaker's Profile

Meng Ling Moi is an Associate Professor at the Institute of Tropical Medicine, Nagasaki University. She is currently working on mosquito-borne diseases research, with particular interest in immunopathogenesis and animal models.

System Biology Approaches for Antiviral Strategies Development against Human Enteroviruses that cause HFMD

Justin Jang Hann Chu

Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University Health System, National University of Singapore

The Hand, Foot and Mouth Disease (HFMD) is a febrile illness, which can result from infections by a plethora of human enteroviruses, including Enterovirus 71 (EV71). Although HFMD is generally a mild and self-limiting affliction in young children and immunocompromised adults, a subset of patients develop severe neurological, poliomyelitis-like symptoms, which can result in fatality. By using integrated system-wide approaches including human genome-wide gene silencing profiling, miRNA profiling and proteomics via high-throughput techniques, combined with bio-imaging and computational biology, we attempt to understand the biological complexity of virus-host interactions and translating it into antiviral strategies against human enteroviruses. Given the compact genome of EV71, many cellular proteins are likely to be required for its successful replication. To date, only a handful of these factors have been identified. Here we report the identification of host susceptibility and resistance factors affecting EV71 infection from a genome-wide RNAi screen coupled with miRNAome and proteome profiling in human cell lines and clinical samples. Bioinformatics analyses revealed the involvement of a plethora of cellular processes including transcription regulation, cell cycle, calcium signaling, translation initiation and membrane biogenesis etc. A number of host susceptibility (MINK kinase, NGLY1) and resistance (CDK6 and AURKB) factors were selected for detailed analysis due to its strong inhibitory or enhancement profiles upon gene silencing in EV71-infected cells. Through proteomic analysis and infection inhibition assay, we have mapped out the detail pathway/mechanism on the interaction of host-viral interactions. This study provides a comprehensive map of cellular components involved in EV71 replication that can form the basis for antiviral targeting and our understanding of virus pathology.

Speaker's Profile

Dr Justin Chu is currently an Associate Professor in the Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore. He is also holding a joint appointment as a Principal Investigator in the Institute of Molecular and Cell Biology, A*STAR. Dr. Chu is actively engaged in the study of the molecular biology of mosquito-borne viruses including Dengue, Zika, West Nile/Japanese Encephalitis, Chikungunya as well as human enteroviruses that cause Hand, Foot and Mouth Disease (HFMD). The outcome of these studies have helped to pave the roadmap towards the development of a number of antivirals and strategies that are now in the process of clinical translational evaluation for these medically important viruses. Four patents have also been generated from these current research.In addition, Dr Chu has also developed innovative molecular diagnostics and proteomics techniques for the diagnosis of clinical samples from patients infected with mosquito-borne viruses including Zika, Dengue and Chikugunya viruses. Dr. Chu has published over 80 international peer-reviewed scientific publications, book chapters and over 100 conference papers. A number of these scientific papers are published in prestigious journals including Nature Communications, PNAS, PLoS Pathogens, Biomaterials, Journal of Biological Chemistry, Journal of Virology and Antiviral therapy. Dr Chu is currently serving as the associate editor and reviewer for a number of peer-reviewed journals and international grant agencies in the areas of medical virology and anti-viral strategies.

MAP kinase phosphatase 2 regulates innate immune response to intracellular bacterial infection

Yongliang Zhang

Department of Molecular Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore; LSI Immunology Programme, Life Science Institute, National University of Singapore

MAP kinase phosphatases (MKPs), also known as dual-specificity phosphatases (DUSPs), are cysteine-based protein tyrosine phosphatases that dephosphorylate phosphotyrosine, phosphothreonine, and phosphoserine residues in their substrates. MKP2 or DUSP4 has been previously shown to play an important role in host innate immune responses to parasitic infections. However, the role of MKP2 in response to bacterial infections remains elusive. In this study, MKP2 deficient bone marrow-derived macrophages (BMDMs) and mice were infected with the gram positive facultative intracellular bacterium, *Listeria monocytogenes.* Expression of pro-inflammatory cytokines and type-I interferons were found to be increased in MKP2 KO BMDMs, associated with increased activation of MAPK/ ERK, IRF3 and TBK1. Interestingly, the MKP2 deficiency increases susceptibility of cells and mice to *L. monocytogenes* infection through reduced bacterial clearance. This suggests that MKP2 is critical in regulating both MAPK and TBK1-IRF3 pathways in response to infections which in turn influences the outcome of the infection.

Speaker's Profile

Dr Yongliang Zhang is currently an Associate Professor in the Department of Microbiology & Immunology, National University of Singapore. He received his PhD degree from the National University of Singapore. Following his graduate studies, he conducted his post-doctoral work at the University of Washington, Seattle and The University of Texas M.D. Anderson Cancer Center. He joined the Department of Microbiology, NUS as an Assistant Professor in 2009. Research in his laboratory focuses on deciphering the physiological and pathophysiological functions of signalling molecules, mainly a group of proteins known as MAPK phosphatases (MKPs) or dual-specificity phosphatases (DUSPs). Findings made by his group unveiled novel and important roles played by MKPs/DUSPs in diseases including infectious diseases, cancer and metabolic disorders. Targeting MKPs/DUSPs for the development of novel therapeutic methods to improve patient outcomes is one of his major research interests currently and in the future.

Role of Viral and Bacterial Pathogens on Pediatric Pneumonia in Vietnam

Lay Myint Yoshida[1*], Michiko Toizumi[1], Le Minh Nhat[1,3], Keisuke Yoshihara[1], Nguyen Thi Hien Anh[3], Chihiro Iwasaki[1], Noriko Kitamura[1], Mizuki Takegata[1], Masahiro Hashizume[1], Hiroyuki Moriuchi[2], Dang Duc Anh[3], Koya Ariyoshi[4]

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Background: Acute respiratory infections (ARI) remain a main cause of morbidity and mortality in children. The World Health Organization (WHO) estimates about 156 million new pneumonias each year in children aged less than 5 years, of which 151 million episodes occurs in developing countries. Acute respiratory infections are caused by a broad range of viruses and bacterial pathogens. We utilize our ongoing population based pediatric acute respiratory infection surveillance established at Khanh Hoa General Hospital (KHGH) in Nha Trang, Vietnam in 2007 to investigate to role of viruses and bacterial on pediatric pneumonia in Vietnam.

Method: All children from our target population hospitalized at KHGH which is the only hospital in the region were enrolled to the study. Clinical-epidemiological information, nasopharyngeal samples were collected, routine blood testing and chest Xray were taken to all enrolled cases. Multiplex PCR assays were performed to determine the common respiratory viruses and bacteria. Periodical cross section viral and bacterial carriage surveys were perform to the healthy children in the community.

Results: We found that respiratory viruses were associated with 65 to 70% of hospitalized ARI cases, and Rhino, RSV and influenza A viruses were major viral pathogens. Multiple viral infection were detected in 12 to 15% of the cases, and RSV and HMPV infections independently increased the risk of pneumonia.

Streptococcal pneumoniae (SP) is the major bacterial pathogen for pneumonia and commonly colonize in the nasopharynx of children. We investigate the association of pneumococcal bacteria load and viruses in healthy, children with radiologically confirmed pneumonia (RCP), lower respiratory tract infection (LRTI) and healthy children. We found that SP load was higher in children with RCP compared with healthy controls or other LRTIs. SP load was 15-fold higher in pneumonia children with viral co-infection compared with those without ($1.4x10^7$/ml versus $9.1x10^5$/ml; P =0.0001). SP load was over 200-fold higher in serotypeable SP compared with non-typeable SP ($2.5 x10^6$/ml versus $1x10^4$/ml; P < .0001).

Vietnam introduced Hib vaccine into the national immunization program in 2010 so we investigated the role of Hib vaccine on hospitalized pneumonia cases on Vietnam. We found a substantial decline (17-29%) of RCP following Hib vaccination by statistical model. Reduction in healthy carriage was

also observed. Our ongoing ARI surveillance has allowed us to determine the minimal clinical impact of 2009 pandemic influenza and high impact RSV ON-1 emergence in central Vietnam.

Conclusion: RSV, rhino, and influenza viruses and SP play important role among hospitalized ARI cases in Vietnam. Population based ARI surveillance study plays a crucial role to monitor newly emerging viruses.

Speaker's Profile

Lay-Myint Yoshida is a Professor at the Pediatric Infectious Diseases Department, ITM, NU and leads the clinical research group of NU-Vietnam research project in Vietnam. He received his medical doctor degree, MBBS (MD) from Medical University 1, Yangon, Myanmar and obtained his PhD at Department of Infectious Diseases, Institute of Medical Science, University of Tokyo, Japan. His research area covers abroad area including HIV drug resistance, viral and bacterial pathogens on pneumonia (ARI), congenital infection, dengue, etc. He has been working for more than 11 years on the population based cohort study in Nha Trang, Vietnam founded by J-GRID, AMED, Japan. He also leads a PCV reduced dosing schedule trial in Vietnam funded by the Bill & Melinda Gated foundation.

Outbreak of carbapenem-resistant enterobacteriaceae in neonatal intensive care unit and growing care unit in Nagasaki University Hospital

Koichi IZUMIKAWA

Department of Infectious Diseases, Unit of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Carbapenem-resistant enterobacteriaceae (CRE) as known as “night mare bacteria” is an emerging drug-resistant bacteria. Some CRE bacteria have become resistant to most available antibiotics. Infections with CRE are difficult to treat and can be fatal - one report cites they can contribute to death in up to 50% of patients. It is required to report to health authority office since September 2014 in Japan, if CRE infection occurred. We encountered an outbreak of CRE infection in neonatal intensive care unit (NICU) and growing care unit (GCU) in Nagasaki University Hospital (NUH). Total of 16 new born babies were infected with CR-*Enterobacter cloacae* complex. Antibiotics were administered for two babies and successfully cured. Infection control team intervenes mainly milk bottle management, waste management and hand hygiene, however, the team was not able to terminate outbreak. NICU and GCU were temporarily closed and two external supports from outside of NUH were engaged. Team of Field Epidemiology Training Program Japan and three experts from National University Infection Control Association visited NUH and investigated. They concluded that incomplete of hand-hygiene, errors of zooning in the ward and inappropriate manipulation of tube-feeding were possible causes of outbreak. We improved all of these posted causes and reopened NICU and GCU after 30 days of closure. Although definite cause of outbreak was not found, comprehensive approach for improving infection measures was effective and no new CRE have been isolated in NICU and GCU up to now. This is the first CRE outbreak in NICU and GCU in Japan and successfully controlled by comprehensive approach. We would like to share our experiences and the countermeasures against CRE outbreak in tertiary hospital.

Speaker’s Profile

Koichi Izumikawa is a professor of Department of Infectious Diseases, Unit of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan and the director of Infection Control and Education Center, Nagasaki University Hospital, Nagasaki, Japan. Prior to his current appointment, he worked in Nagasaki University Hospital, Nagasaki, Japan, Toranomon Hospital, Tokyo, Japan and other institutes as a M.D. and studied in the Clinical Mycology Section and Molecular Microbiology Section of National Institute of Allergy and Infectious Diseases, National Institute of Health, U.S.A. He is a member of the American Society for Microbiology, The Japanese Society of Internal Medicine, The Japanese Association for Infectious Diseases, and The Japanese Society of Chemotherapy. He obtained his M.D. and later his Ph.D. from the Nagasaki University School of Medicine, Nagasaki, Japan. His area of research encompasses the drug resistant mechanism of fungus and antimicrobial treatment of infections, and he has published more than 200 papers on this research topic in peer-reviewed local and international journals such as *Journal of Infectious Diseases*, *Japanese Journal of Infectious Diseases*, *Medical Mycology*, and *Antimicrobial Agents and Chemotherapy*.

Keynote Lecture 5

From biology of *Mycobacterium* to the development of the control strategies against tuberculosis

Sohkichi Matsumoto

Department of Bacteriology, Niigata University School of Medicine, Niigata, Japan
Department of Bacteriology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Mycobacterium tuberculosis is now a top human killer among pathogens. Over 10 million people newly developed tuberculosis and 1.8 million people died from it in 2015 (2016 WHO Report). *M. tuberculosis* is an intracellular bacterium that can survive even after engulfed by the phagocytic cells. After infection is established, *M. tuberculosis* is rarely eliminated, and nowadays approximately infects one third of the world's human population.

At latent state, most of *M. tuberculosis* down-shifts the metabolisms and enter into non-replicating dormant state. Dormant *M. tuberculosis* lives for a long term and resists from drug treatments. Latent tuberculosis is the major reservoir of disease, since *M. tuberculosis* has no environmental reservoir and reactivation arises in 5-10% of asymptomatically infected individuals during the lifetime. Thus preventing disease progression or the eradication of persisting bacilli in human being should be efficient action against tuberculosis. To achieve that, we should develop 1, diagnosis that detects disease progression, 2, new drugs that sterilize dormant *M. tuberculosis*, and 3, vaccines that prevent disease progression.

Understanding growth control of *M. tuberculosis* is important because replication itself causes tuberculosis. I identified a mycobacterial DNA-binding protein, MDP1, which controls the growth of mycobacteria. MDP1 is an essential protein in slow growers, and is up-regulated in iron or oxygen-limited condition and participates in the regulation of gene expression. KatG, an essential enzyme for activation of isoniazid, is one of the genes regulated by MDP1 and therefore up-regulation of MDP1 confers tolerance to isoniazid. Besides regulation of gene expression, MDP1 has iron storage and ferroxidase activities like ferritin. Iron is an essential metal for almost all living organisms and ferroxidase activity prevents Fenton reaction, which generates the most aggressive oxygen radical, hydroxyl radical.

Taken together, MDP1 is involved in growth coordination and prolonging the life of *M. tuberculosis*, and thus the possible drug target. And immune response to MDP1 is prominent in latent TB, suggesting the potential use of it diagnosis and vaccine.

Speaker's Profile

1986-1992, Nagasaki University, School of Dentistry. Education.
1992-1999, Instructor of Oral Bacteriology, Nagasaki University, School of Dentistry, Research about BCG and mycobacteria
1999-2001, Fellow, Tuberculosis Research Section, NIAID/NIH, Research about *M. tuberculosis.*
2002-2013, Assistant and Associate professors Osaka City University Graduate School of Medicine, TB and other mycobacterial diseases.
2013-current, Professor of Bacteriology, Niigata University School of Medicine. TB and other mycobacterial diseases.

Keynote Lecture 6

The type IX secretion system, gliding motility and the type V pilus in the Bacteroidetes pylum bacteria

Koji Nakayama

Division of Microbiology and Oral Infection, Department of Molecular Microbiology and Immunology, Graduate School of Biomedical Science, Nagasaki University, Nagasaki, Japan

The Type IX secretion system (T9SS) is a new secretion system, which was originally found in secretion of gingipain proteinases of the oral pathogen *Porphyromonas gingivalis* and is now found in many species belonging to the Bacteroidetes phylum. Most of the T9SS component proteins are also required for bacteroidete gliding motility.

We proposed a helical loop track model for gliding motility in the bacteroidete gliding bacterium *Flavobacterium johnsoniae*, where SprB filaments are propelled along a left-handed closed helical loop on the cell surface by proton motive force and attachment of SprB to the substratum generates translation of the cell. Immunofluorescent labeling of SprB on a gliding cell revealed that SprB sometimes overtook another SprB that moved at the same direction, suggesting the presence of multiple lanes in the helical loop track. Several electron microscopic analyses revealed the presence of multi-rail structure underneath the outer membrane, which was associated with the SprB filaments. The multi-rail structure was immunoreacted with anti-GldJ antibody and a *gldJ* mutant exhibited no multi-rail structure, suggesting that the GldJ lipoprotein is a component of the structure. A similar structure was observed in a bacteroidete marine gliding bacterium, *Saprospira grandis*.

We investigated a set of 20 Bacteroidia pilins including *P. gingivalis* pilins. Crystal structures and biochemical data revealed a diverse protein superfamily with a common Greek-key β sandwich fold with two transthyretin-like repeats that polymerize into a pilus through a strand-exchange mechanism. The assembly mechanism of the central, structural pilins involves proteinase-assisted removal of their N-terminal β strand, creating an extended hydrophobic groove that binds the C-terminal donor strands of the incoming pilin. Bacteroidia pilins are translocated to the cell surface by the lipoprotein transport system.

Speaker's Profile

Dr. Nakayama is the Professor and Chair of Division of Microbiology and Oral Infection, Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences. He graduated from School of Dentistry, Kyushu University and obtained his Ph.D. from Kyushu University. He mainly studies pathobiology of the periodontal pathogen *Porphyromonas gingivalis*.

Keynote lecture 7

Identification of reticulocyte receptors for Plasmodium vivax merozoite invasion

Benoît Malleret[1,2], Rossarin Suwanarusk[1], Shanshan Wu Howland[1], Ameya Sinha[3], Trang TT Chu[3], Cindy S Chu[4,5], Rajesh Chandramohanadas[3], Francois Nosten[4,5], Bruce Russell[2*], **Laurent Rénia[1*]**

[1]Laboratory of Pathogen Immunobiology, Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Biopolis, Singapore

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

[3]Pillar of Engineering Product Development, Singapore University of Technology & Design, Singapore

[4]Shoklo Malaria Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand

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

* equal contribution

The co-evolution between the *Plasmodia* genus and their human hosts has driven different erythrocyte tropisms for each *Plasmodium* species. *P. vivax* has a specific tropism for immature red blood cells (reticulocytes). *P. vivax* reticulocyte binding protein (PvRBP) family is believed to mediate specific invasion of reticulocytes by *P. vivax*. The invasion of vivax is also DARC (Duffy antigen/chemokine receptor) dependent and involved the interaction with DBP (Duffy binding protein) at the surface of vivax merozoites. Interestingly, DARC is also expressed by mature red blood cells (normocytes) therefore the presence of this receptor is not solely responsible for reticulocyte tropism of *P. vivax*. We have recently demonstrated that *P. vivax* invades preferentially CD71+ reticulocytes. Using with mass spectrometry and flow cytometry approaches, we identify different proteins specifically express at the surface of CD71+ reticulocytes and investigated whether they were directly involved in vivax merozoites invasion. The identification of merozoite ligands associated to these reticulocyte receptors was performed using a RBP protein library in cell to cell binding assays.

P. vivax is still responsible for almost half the number of malaria cases outside Africa. Thus identification of a new ligand-receptor for *P. vivax* receptors may lead to new therapeutic approach, an important step for malaria eradication in South-East Asia.

Speaker's Profile

Laurent Renia is currently the executive director of the Singapore Immunology Network (SIgN). Laurent Renia obtained his PhD in 1991 Universite Pierre et Marie Curie in Paris, France where he studied the immune response against the pre-erythrocytic phase of malaria. He continued to work on

malaria immunology in New York University (1991-1992). He then returned to Paris in 1993 where he obtained a permanent position as junior research scientist at the French national of Institute of Health (INSERM) working on malaria immunology in the INSERM Unit 313 at the Hopital Pitie-Salpetriere in Paris. He moved to the INSERM Unit 445 at the Institut Cochin in Paris where he started his own group in 1997. Between 2001-2006, he became research director at INSERM, co-director and director of the Department of Immunology at the Institut Cochin. He joined SIgN in 2007. He holds adjunct Professorships with the Department of Microbiology, Yong Loo Lin School of Medicine at the National University of Singapore, at the School of biological Sciences, Nanyang technological University in Singapore, and is an associated laboratory to the French National Institute of Health (INSERM). He has published more than 200 articles and book chapters. He is an Academic Editor for Infection and Immunity, *PLoS ONE, Infection and Immunity, Microbial Pathogenesis and Frontiers in Immunology.*

Keynote Lecture 8

Tissue-Specificity During Malaria Infection

Cevayir COBAN

Laboratory of Malaria Immunology, Immunology Frontier Research Center (IFReC), Osaka University
http://malimm.ifrec.osaka-u.ac.jp/

Malaria is still one of the most devastating infectious diseases of the world, causing huge numbers of mortality each year. The death from malaria infection occurs due to the organ-specific immunopathology caused by parasites that the detailed understanding of this immunopathology remains unknown. In my laboratory, by using mice models, we have utilized several imaging technologies such as ultra-high field MRI and intra vital multi-photon microscopy to understand immunopathology caused by *Plasmodium* parasites, their interaction with host at the cellular as well as organ level. We have recently made a progress on the understanding of the disease pathology particularly in brain and bone. Our studies has great implications for designing novel therapeutics against malaria and improve human lives who suffer from it.

Speaker's Profile

Dr. Coban is a Professor and the Head of Laboratory of Malaria Immunology at Immunology Frontier Research Center (IFReC), Osaka University, JAPAN. She obtained her M.D. from the Hacettepe University Medical School in Ankara, TURKEY and completed her postdoctoral training at FDA and Johns Hopkins University School of Public Health, Maryland, USA. She moved to Osaka University to study innate immune responses to infectious agents including *Plasmodium* parasites. Her lab's interests include the investigation of host-*Plasmodium* parasite interactions and the development of vaccines against infectious diseases.

Drug-mediated digestive vacuole disruption in malaria parasites: What's knobs got to do with it?

Kevin S. W. Tan,[1] Yan Quan Lee,[1] Benoit Malleret[2]

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Malaria parasite reside and replicate within host red blood cell (erythrocytes). Human erythrocytes exhibit a mode of programmed cell death unlike apoptosis. In senescent erythrocytes, influx of Ca2+ activates the cysteine protease μ-calpain, resulting in cytoskeletal degradation. Parasite invasion of the erythrocyte causes the erythrocyte membrane to be permeable to Ca2+. However, Ca2+ activity in the erythrocyte cytosol is suppressed as the parasite sequesters Ca2+ in various organelles, one of which is the digestive vacuole. *P. falciparum* also expresses knobs on the erythrocyte surface in order to adhere to the endothelial lining and remove itself from the peripheral circulation. This enables the parasite to avoid phagocytic clearance by the spleen. Formation of these knobs require the parasite protein KAHRP and its attachment to the host cytoskeleton. It was discovered that rupture of the digestive vacuole appears to induce the μ-calpain death pathway of the erythrocyte, resulting in the degradation of KAHRP and the loss of parasite cytoadherence. The implications of this unexpected observation are discussed.

Speaker's Profile

Kevin SW TAN is Associate Professor at the Department of Microbiology & Immunology, National University of Singapore. He is also Head, Innovation in Graduate Studies and Assistant Dean for 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 is relieved to be awarded tenure in 2011, and can now spend more time on social issues, such as public science education. 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 recently come up with new ways to find drugs that overcome resistance. He hopes that the research from his team would accelerate the finding of new cures for parasitic diseases.

Genetic analysis of *Plasmodium malariae* and *Plasmodium ovale* from asymptomatic adolescents in South-East Nigeria.

Medard Ernest[1], Muhydeen Abiodun Abdulraheem[1,2], Adebola Emmanuel Orimadegun[3] & Richard Culleton[1]

[1] *Malaria Unit, Department of Pathology, Institute of Tropical Medicine, Nagasaki University, Japan*

[2]*Department of Paediatrics, University College Hospital, Ibadan, Nigeria*

[3]*Institute of Child health, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria*

In malaria endemic regions, a certain percentage of the population will harbour parasites in their blood without succumbing to symptoms. These asymptomatic malaria parasite carriers do not seek anti-malarial treatment, and so infections are likely to persist for long periods. This is detrimental for two reasons; firstly, these individuals constitute a reservoir for the transmission of parasites within a population, and secondly, there may be underlying health risks to the individual associated with long term parasite carriage. We conducted a cross sectional survey of 1032 adolescents from eight schools located in Akinyele and Ibadan North Local Government Areas, a collection of semi-urban and urban communities in the southwest of Nigeria. A questionnaire was used to collect demographic, medical history and malaria prevention practices from participants. The prevalence of asymptomatic malaria was determined using microscopy, rapid diagnostic tests and PCR. Molecular species typing revealed surprisingly high rates of *P. ovale* and *P. malariae* infection. We analysed genes associated with drug resistance from these species, along with their *P. falciparum* orthologues. We were then able to compare the rates of mutations in these genes between parasite species collected from the same population, and in many cases, from the same co-infected individuals.

Speaker's Profile

Richard Culleton is a malaria researcher of relatively little importance at Nagasaki University's Institute of Tropical Medicine.

Interleukin-27 inhibits the generation of memory CD4$^+$ T cells after treatment with anti-malarial drug.

Daisuke Kimura[1], Mana Miyakoda[1], Masoud Akbari[1], Kazumi Kimura[1], Hiromitsu Hara[2], Hiroki Yoshida[3], Katsuyuki Yui[1]

[1]Division of Immunology, Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences

[2] Department of Immunology, Graduate School of Medical and Dental Sciences, Kagoshima University

[3] Department of Biomolecular Sciences, Faculty of Medicine, Saga University

People living in malaria endemic area acquire protective immunity after repeated infections in prolonged period, but this immunity can be lost once they move outside the endemic region and become free of parasite. Mechanisms underlying the loss of immunological memory is not clearly understood.

We previously reported Tr27 cells, malaria-specific Foxp3$^-$ CD4$^+$ T cells that produce IL-27, and their roles in regulating IL-2 production and proliferation of other CD4$^+$ T cells. We hypothesized that IL-27 contributes to the loss of memory responses against malaria infection, since IL-2 is critical for the survival and memory formation of activated CD4$^+$ T cells. To test this hypothesis, B6 and *Il27*$^{-/-}$ mice were infected with *Plasmodium berghei* ANKA (PbA), and were cured by the treatment with anti-malaria drug. After the cure, CD4$^+$ T cells from B6 mice did not produce IFN-□ in response to PbA antigens, while those from *Il27*$^{-/-}$ mice did. After re-challenge with PbA, B6 mice exhibited the response similar to their primary infection. However, *Il27*$^{-/-}$ mice showed the strong inhibition in parasitema, and their CD4$^+$ T cells exhibited enhanced IFN-□ response. The level of apoptosis in malaria-specific (CD11a^{hi}CD49d^{hi}) CD4$^+$ T cells was higher in B6 mice than those in *Il27*$^{-/-}$ mice after the drug-treatment. These results imply that IL-27 inhibits the generation of memory CD4$^+$ T cells by accelerating the apoptosis of specific CD4$^+$ T cells during contraction phase of the immune response.

Speaker's Profile

Dr. Daisuke served 12 years in Division of Immunology, Department of Molecular Microbiology and Immunology, Graduate School of Biomedical Sciences, Nagasaki University. Presently, he is dedicated to elucidate mechanism underlying the immunosuppression caused by malaria infection.

Zoonotic *P. cynomolgi* Switches Preference for Red Cell Tropism and Duffy Dependence

Varakorn Kosaisavee[1,2], Rossarin Suwanarusk[3,4], Adeline C. Y. Chua[4], Dennis E. Kyle[5], Benoit Malleret[2,3], , Eric Lombardini[6], Jessica Ong[4,7], François Nosten[8], Kevin S. W. Tan[2], Pablo Bifani[7], Georges Snounou[9], Laurent Rénia[3*], **Bruce Russell[4*]**

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Plasmodium cynomolgi, a parasite of south east Asian monkeys, has recently been implicated in zoonotic malaria. In this study we sort to identify key molecular barriers to the invasion of human erythrocytes by *P. cynomolgi* merozoites. Here we show for the first time that unlike monkey infections that *P. cynomolgi* merozoites exclusively target human reticulocytes expressing the Duffy antigen/chemokine receptor (DARC or CD234). Our characterization of the post invasion modifications (morphology, rheology and nanostructure) of human reticulocytes shows striking similarities to those of *P. vivax*. Our results suggest that zoonotic infections may be underreported due to confusion with *P. vivax* infections; and confirms its importance as a research model for developing vaccines against vivax malaria.

Speaker's Profile

For the past 20 years, Bruce Russell has focused his research on the red cell biology of *Plasmodium vivax*. During this field-based work (Bougainville, Timor Leste, Indonesia, Myanmar and Thailand), he has developed a range of reliable assays and methods, which have enabled the quantitative study of *ex vivo* isolates of *P. vivax* (Collaboration with Laurent Renia, Georges Snounou, Kevin Tan, Nick Anstey, Francois Nosten and Ric Price). Of particular, note his drug susceptibility and reticulocyte invasion assays have not only resulted in the discovery of new blood stage drug and vaccine candidates; but have also revealed important aspects of *P. vivax* biology (i.e Specific Red Cell Tropism, Rheopathobiology and Stage Specific drug effect). Excitingly, his team's recent reinvigoration/ optimisation of the continuous culture of *P. cynomolgi* (collaboration with Pablo Bifani and Dennis Kyle) will provide a tractable model for better understanding *P. vivax*. Bruce's vivax malaria lab is currently based at the University of Otago, Dunedin, NZ.

Ungulate malaria parasites

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Haemosporida parasites of even-toed ungulates are diverse and globally distributed, but since their discovery in 1913 their characterization has relied exclusively on microscopy-based descriptions. In order to bring molecular approaches to bear on the identity and evolutionary relationships of ungulate malaria parasites, we conducted *Plasmodium cytb*-specific nested PCR surveys using blood from water buffalo in Vietnam and Thailand, and goats in Zambia. We found that *Plasmodium* is readily detectable from water buffalo in these countries, indicating that buffalo *Plasmodium* is distributed in a wider region than India, which is the only area in which buffalo *Plasmodium* has been reported. Two types of *Plasmodium* sequences were identified from water buffalo and a third type was isolated from goat. Morphology of the parasite was confirmed in Giemsa stained blood smears for the Type I sample. Complete mitochondrial DNA sequences were isolated and used to infer a phylogeny in which ungulate malaria parasites form a monophyletic clade within the Haemosporida, and branch prior to the clade containing bird, lizard and other mammalian *Plasmodium*. Thus it is likely that host switching of *Plasmodium* from birds to mammals occurred multiple times, with a switch to ungulates independently from other mammalian *Plasmodium*.

Presenter's profile

Assistant professor, NEKKEN

CryoET of Trypanosoma brucei

Cynthia Y. He

Department of Biological Sciences, Centre for BioImaging Sciences, National University of Singapore

Trypanosoma brucei contains a subpellicular microtubule array that is composed of >100 stable microtubules crosslinked to each other, forming a bird cage-like structure underneath the plasma membrane. Development of the subpellicular array is tightly linked to biogenesis of the flagellum and crucial for cell morphology, during the cell cycle as well as the life cycle development. In this study, we used cryo electron tomography to visualize the 3D organization of the subpellicular microtubule array in genetically engineered mini *T. brucei* cells. Our results provide an ultrastructural model on how the flagellum drives bihelical cell movement by modifying the arrangement of the subpellicular array.

Speaker's Profile

Dr. He Joined the Department of Biological Sciences at the National University of Singapore in 2007 and has since been using *Trypanosoma brucei* as a model to study organelle biogenesis, structure and functions. *T. brucei.* Using a combination of molecular genetics, biochemistry and microscopy methods, our lab focus on two highly conserved cellular functions, flagellum-driven motility and autophagy. In this talk I will discuss our recent progress in using cryo electron tomography to obtain high-resolution structural details on vitrified *T. brucei* cells captured in motion.

Genome hypermobility by co-lateral transduction

John Chen

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

All phage-mediated gene transfer is known to occur by one of two well-described mechanisms: generalized and specialized transduction. Here we introduce co-lateral transduction, which we propose to be the third and possibly most impactful universal mode of phage transduction. In co-lateral transduction, DNA packaging is initiated from the integrated prophage genome and proceeds in a unidirectional manner for up to several hundred kb. Large spans of the bacterial genome are packaged and transferred at frequencies that are extremely high and unprecedented for most of the previously described mechanisms of gene transfer. Since bacterial chromosomes often contain multiple prophages, this mode of transduction can potentially transmit a large portion of the bacterial genome at exceptionally high frequencies in a single lytic event. In summary, our results show that phage-mediated host gene transfer can occur on a scale that is many orders of magnitude greater than previously appreciated, leading to a shift in our perception of the impact that phages have on bacterial evolution.

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.

A *trans*-splicing based suicide gene therapy approach targeting virus infection or cancer

Volker Patzel

Department of Microbiology & Immunology, Yong Loo Lin School of Medicine, National University of Singapore. Department of Medicine, Addenbrooke's Hospital, University of Cambridge, UK.

In mammalian cells, spliceosome-mediated RNA *trans*-splicing may occur accidentally or be a mechanism used by distinct viruses to support the diversification of viral RNA and proteins. We employed molecular features of SV40 RNA *trans*-splicing together with computational RNA structure design to improve both on-target activity and specificity of *trans*-splicing RNA (tsRNA) in a Herpes simplex virus thymidine kinase (HSVtk)/ganciclovir (GCV) suicide gene therapy approach. As molecular targets we selected the □-fetoprotein (AFP), a marker of hepatocellular carcinoma (HCC), human papillomavirus type 16 (HPV-16), or human immunodeficiency virus type 1 (HIV-1) pre-mRNA. While unstructured mismatched target binding domains significantly improved 3' exon replacement, 5' exon replacement correlated with the thermodynamic stability of the tsRNA 3' end. Alternative on-target *trans*-splicing was found to be a prevalent event. The specificity of *trans*-splicing with the intended target splice site was improved 10-fold by designing tsRNA harbouring multiple target binding domains shielding alternative on-target and blinding non-target splicing events. Such optimised tsRNAs efficiently and selectively triggered death of HPV-16, HIV-1 or AFP-positive cells. Dual-targeting tsRNA simultaneously targeting AFP and a second HCC biomarker triggered enhanced cell death at 10-fold lower GCV doses. With regard to delivery *in vitro*, novel dumbbell-shaped DNA minimal vectors were found to be more potent than conventional plasmids. Our observations suggest RNA *trans*-splicing represents a promising approach to suicide gene therapy. Our suicide RNAs may be explored for topical non-invasive treatment of premalignant or cancerous HPV-16-associated lesions of the skin or mucous membranes or for therapy of HCC and AIDS. Alternatively, the suicide RNAs can be reprogrammed by replacing the BDs to target other diseased cell types that are characterised by the expression of one or multiple disease-specific biomarkers either *ex vivo* or *in vivo*.

Speaker's Profile

Dr. Volker Patzel, chemist, graduated from the Institute for Biochemistry, Technical University of Darmstadt, Germany. 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 Centre in Heidelberg and then as research group leader at the Max Planck Institute for Infection Biology in Berlin. Currently holds a dual appointment as Assistant Professor at the National University of Singapore and Assistant Director of Research at the University of Cambridge. Co-founder of a biotech company in Heidelberg as well as founder and director of the Steinbeis Transfer Centre for Nucleic Acids Design. Research focuses on the design and delivery of functional ribonucleic acids towards diagnostic and therapeutic applications.

How should we train doctors in tropical medicine?

Philip Gothard

Senior Lecturer, London School of Hygiene & Tropical Medicine
Consultant Physician, Hospital for Tropical Diseases, London

Phil Gothard takes an overview of the changing face of the specialty and shares his experience of setting up the East African Diploma in Tropical Medicine & Hygiene as a case study for future collaborations.

Speaker's Profile

Senior Lecturer, London School of Hygiene & Tropical Medicine
Consultant Physician, Hospital for Tropical Diseases, London

Poster Presentation

Day 1: 18th May (THU)
1F Sensai Hall

No.	Name	University
P01	Lim Ze Qin	Yong Loo Lin School of Medicine, National University of Singapore
P02	Huimin Yeo	Yong Loo Lin School of Medicine, National University of Singapore
P03	Nyo Min	Yong Loo Lin School of Medicine, National University of Singapore
P04	Meng Chee Phoon	National University of Singapore
P05	Miho Kaneko	Graduate School of Biomedical Sciences, Nagasaki University
P06	Bui Thu Thuy	Institute of Tropical Medicine, Nagasaki University
P07	Phu Ly Minh Huong	Graduate School of Biomedical Sciences, Nagasaki University
P08	DAO HUY MANH	Graduate School of Biomedical Sciences, Nagasaki University
P09	Mark Anthony D. Luz	Graduate School of Biomedical Sciences, Nagasaki University
P10	Aung Kyaw Kyaw	Graduate School of Biomedical Sciences, Nagasaki University
P11	Li Liang	Nanyang Technological University, Singapore
P12	Juliann Nzembi Makau	Graduate School of Biomedical Sciences, Nagasaki University
P13	Nguyen Co Thach	Graduate School of Biomedical Sciences, Nagasaki University
P14	SHASHIKA LAVANGI WIJESOORIYA	Institute of Tropical Medicine, Nagasaki University
P15	Olamide K. Oloniniyi	Graduate School of Biomedical Sciences, Nagasaki University
P16	Satoshi Shimada	Graduate School of Biomedical Sciences, Nagasaki University
P17	Keisuke Yoshihara	Graduate School of Biomedical Sciences, Nagasaki University
P18	Michiko Toizumi	Institute of Tropical Medicine, Nagasaki University
P19	Yuto Kegawa	Graduate School of Biomedical Sciences, Nagasaki University
P20	Takahiro Ishizaki	Graduate School of Biomedical Sciences, Nagasaki University
P21	Benoît MALLERET	Yong Loo Lin School of Medicine, National University of Singapore
P22	Asare Kwame Kumi	Graduate School of Biomedical Sciences, Nagasaki University
P23	Jie Xin TONG	Yong Loo Lin School of Medicine, National University of Singapore

No.	Name	University
P24	Mana Miyakoda	Graduate School of Biomedical Sciences, Nagasaki University
P25	Adeline C.Y. Chua	University of Otago, Dunedin, New Zealand
P26	Ganchimeg Bayarsaikhan	Graduate School of Biomedical Sciences, Nagasaki University
P27	Sayuri NAKAMAE	Graduate School of Biomedical Sciences, Nagasaki University
P28	Jiun Yu Jian	Graduate School of Biomedical Sciences, Nagasaki University
P29	Farhana Mosaddeque	Graduate School of Biomedical Sciences, Nagasaki University
P30	Awet Alem Teklemichael	School of Tropical Medicine and Global Health, Nagasaki University
P31	Taeko Moriyasu	Graduate School of Biomedical Sciences, Nagasaki University
P32	Caroline Kijogi	Graduate School of Biomedical Sciences, Nagasaki University
P33	Hassan Hakimi	Institute of Tropical Medicine, Nagasaki University
P34	Mitsuko Hasegawa	Graduate School of Biomedical Sciences, Nagasaki University
P35	Evans Chadeka	Graduate School of Biomedical Sciences, Nagasaki University
P36	Sharmina Deloer	Graduate School of Biomedical Sciences, Nagasaki University
P37	Risa Nakamura	Institute of Tropical Medicine, Nagasaki University
P38	Chin Wen Png	Yong Loo Lin School of Medicine, National University of Singapore
P39	Laura Victoria White	School of Tropical Medicine and Global Health, Nagasaki University
P40	Shuhei Ideguchi	Graduate School of Biomedical Sciences, Nagasaki University
P41	Mohammad Shah	Institute of Tropical Medicine, Nagasaki University
P42	Akintije Simba Calliope	Graduate School of Biomedical Sciences, Nagasaki University

Characterization of Enterovirus 71 Infection in NSC-34 Motor Neuron Cells

Lim Ze Qin, Issac Too, Sylvie Alonso

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

Enterovirus 71 (EV71) is a non-enveloped, positive-stranded RNA virus that has been associated with severe neurological forms of Hand, Foot and Mouth Disease (HFMD). Although the route of EV71 neuroinvasion remains debatable, current studies support the retrograde axonal transport as a major route of EV71 to reach the central nervous system (CNS), whereby the virus infects motor neurons at the neuromuscular junctions and employs a retrograde axonal transport mechanism to eventually reach the brain. Here we report a novel *in vitro* model of EV71 infection using NSC-34 motor neuron cell line to investigate the intrinsic neurovirulence potential of EV71. NSC-34 cells are produced by fusion of mouse neuroblastoma cells with motor-enriched spinal cord cells. These cells exhibit high morphological and physiological resemblance to neurons at neuromuscular junctions, thus reflecting its applicability as an *in vitro* model to study EV71 neurovirulence. The results indicate that NSC-34 cells are permissive to EV71 infection with production of infectious viral particles in the culture supernatant. However, unlike in muscle RD cells, EV71-infected NSC-34 cells did not display cytopathic effect and did not undergo apoptosis, suggesting a non-lytic virus release process. In addition, up-regulation of autophagic markers was observed in EV71-infected NSC34 cells, which may suggest the ability of EV71 to hijack the autophagic pathway for its exit, similar to what has been previously described with poliovirus. As the mechanisms of EV71 neuroinvasion remains unclear, study of EV71 infection cycle in this model may provide further understanding of viral pathogenesis in the CNS.

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UNDERSTANDING THE NEUROVIRULENCE OF ENTEROVIRUS 71

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[1]Department of Microbiology and Immunology, National University of Singapore, [2]Program in Emerging Infectious Diseases, DUKE-NUS, [3]Department of Laboratory Medicine, National University Hospital, Singapore, Singapore

Two-week-old AG129 mice infected with clinical EV71 isolate S41 resulted in neurological symptoms and progressive limb paralysis associated with viral accumulation and neuronal damage in the central nervous system of infected mice, while infection with myotropic MS and C2 strains did not cause any clinical symptoms or disease. Upon alignment of the polyprotein sequences of the three clinical strains based on their virulence in AG129 mice, several mutations of interest were found in VP2, 3A, 3C and 3D regions. Site-directed mutagenesis was carried out based on these mutations on an infectious clone of S41. Growth kinetics of the mutants in various cell lines showed that the VP2149ItoK mutant (position 149 of the VP2 protein - isoleucine to lysine) had minimal replication in NSC-34 cells, a mouse motor neuron cell line. Reverse transfection and qRT-PCR revealed that the mutant was able to replicate in NSC-34 but was unable to establish a secondary infection, thus suggesting that the VP2149ItoK mutant was inhibited at the entry level. Infection with the infectious clone resulted in progressive limb paralysis and eventually death in AG129 mice. We observed a loss in virulence in the VP2149ItoK mutant where infected mice remained healthy, suggesting that the single mutation in the VP2 region was important in determining viral neurovirulence in AG129 mice.

Conclusion: A single mutation at the VP2 region at position 149 from isoleucine to lysine appeared to be critical for EV71 in establishing neurovirulence in AG129 mice and in NSC-34 cells. The inhibition appears to be at the entry level of the virus.

A Primary Tonsillar Crypt Cell Line for In Vitro Modelling of Enterovirus Infection and Pathogenesis

P3

Nyo Min, and Justin Jang Hann Chu

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BACKGROUND:

Major causative agents of hand-foot-mouth disease (HFMD) include human enterovirus type 71 (EV71) along with Coxsackievirus A16 (CA16) and Coxsackievirus A6 (CA6), belonging to human Enterovirus Species A of the family *Picornaviridae*. Predominantly, HFMD causes mild clinical manifestations such as fever and blisters in mouth, on palms or on sole of the feet. Occasionally, fatal cases were also reported presenting meningitis and polio-like flaccid paralysis. However, little information is yet known on pathogenesis of EV71, CA16 and CA6 in human. Recently, squamous epithelium lining of tonsillar crypt tissue was being identified as possible reservoir of EV71 infection which supports active viral replication and faecal-oral spreading. Here, we explored the mechanism of enterovirus infection in human primary tonsillar crypt cell (HtEpic) with EV71, CA16, CA6 and ECHO7 viruses together with *in vitro* role of miRNA during infection.

METHODS:

In this study, we performed *in vitro* characterization of the HtEpic cell line during EV71, CA16, CA6 and Echo7 infection. Active replication of the virus was confirmed by growth kinetic studies and viral plaque assays. Increase in viral RNA and protein levels were also observed across multiple time points via real-time polymerase chain reaction, western blotting and indirect immunofluorescence assay (IFA). Moreover, we also conducted next generation sequencing (NGS), detecting significant HtEpic miRNome changes during EV71 and CA6 infection at 6 and 12-hour post infection (HPI).

RESULTS:

Enterovirus viral titer in HtEpic cell line peaked at 48 HPI for EV71 ($2.1x10^6$ PFU/ml) and CA16 ($1.53x10^6$ PFU/ml). The peak in viral titer for CA6 ($1.3\ x10^4$ PFU/ml) was observed at 16 HPI and ECHO7 at 48 HPI ($2.9\ x10^7$ PFU/ml). Our real-time PCR unveiled active RNA strand synthesis for all three viruses across 48-hour time point. VP3 protein cleaved from the polyprotein were also detected in increasing manner from 12 to 24 HPI. Additionally, through IFA, punctate signal of enterovirus dsRNA was observed at the same time points. TUNNEL assay revealed increase in apoptosis rate caused by all 4 viruses from 0 to 24 HPI. Furthermore, NGS profiling of miRNome during infection revealed miRNA involving in an array of various cellular processes being dysregulated.

CONCLUSIONS:

We established for the first time, a primary cell line model that shows susceptibility to EV71, CA16, CA6 and ECHO7 infections. The optimal cell line may serve as a valuable tool in better understanding enterovirus mechanism of infection in non-cancerous cell model. Furthermore, the primary cell line could facilitate drug screening experiments as well as host factor interaction studies as proven by our miRNome screen as a proof of concept.

Comparative Seroepidemiology of Coxsackievirus A6, A16 and Enterovirus 71 Infections in Singapore Adults

P4

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[2]Saw Swee Hock School of Public Health, National University of Singapore, Singapore

Coxsackievirus A6 (CA6), coxsackievirus A16 (CA16) and enterovirus 71 (EV71) are the predominant causative agents causing periodic outbreaks of hand, foot and mouth disease (HFMD) in Singapore. We conducted a cross-sectional study to estimate and compare the seroprevalence of CA6, CA16 and EV71 infections in an adult cohort in Singapore. We also investigated the specificity of neutralizing antibodies against CA6, CA16 and EV71 in sera obtained from BALB/c mice immunized with the respective inactivated viruses. We tested 115 serum samples from healthy adults aged 21 to 78 years collected from Tan Tock Seng Hospital during the 2009 H1N1 pandemic in Singapore. The neutralizing antibody levels against CA6, CA16 and EV71 were analyzed by microneutralization test. The seroprevalence rate and geometric mean titer (GMT) of neutralizing antibodies against each of the enteroviruses were calculated. Young, female adult BALB/c mice were immunized intraperitoneally with monovalent, heat-inactivated CA6, CA16 or EV71, together with either PBS control, or an adjuvant (aluminium hydroxide or imiquimod, a toll-like receptor 7 agonist). Microneutralization tests were performed on the sera collected from immunized mice at various time-points. Overall, high seroprevalence rates were observed in the adult population in Singapore, with CA6 and CA16 seroprevalence being higher than that of EV71. While CA16 and EV71 seroprevalence rates were similar in both genders, CA6 seroprevalence was significantly higher in females than males. Infections of the three enteroviruses exhibited similar seroprevalence rates among different ethnic groups in Singapore. Overall, the GMT of the three enteroviruses displayed a declining trend as age increased. The GMTs for CA6, CA16 and EV71 were 44.2, 52.8 and 22.5 respectively among young parents aged 30 years, compared with 34.5, 24.1 and 17.9 in middle-aged adults aged 50 years. While CA16 and EV71 GMTs were not affected by the presence of children in the household, CA6 GMT was significantly higher for individuals with household members aged less than 5. A significant proportion of individuals possessed neutralizing antibodies against either both CA6 and EV71, or CA16 and EV71. This may imply either multiple infections of CA6, CA16 and EV71, or cross-reactivity of neutralizing antibodies. While this could not be ascertained from the medical history of the study participants, we addressed this question by mouse immunization models. Microneutralization tests on the sera collected from immunized mice revealed neutralizing antibodies against the specific enterovirus, but did not exhibit any non-specific cross-reactivity. In general, CA6 and CA16 infections are highly prevalent in the adult population in Singapore, and with seroprevalence rates even higher than that of EV71. It is likely that the high seroprevalence of CA6, CA16 and EV71 infections represent multiple enterovirus infections rather than cross-reactivity of enterovirus antibodies. In view of the potentially severe clinical complications of EV71 infection, EV71 candidate vaccines are currently in phase IV clinical trials to evaluate protective efficacy against this important causative agent of HFMD.

Whole-genome analysis of Vietnamese human P[6] rotaviruses provides evidence for independent, direct interspecies transmission of porcine rotavirus strains

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Interspecies transmission of animal *Rotavirus A* (RVA) to humans are often suspected by the detection of G or P types typical of animal RVA strains. However, G3P[6] and G4P[6] are commonly found in both human and porcine RVA strains. So, we determined by Sanger sequencing the whole genomes of six P[6] RVA strains detected from children with diarrhea in Vietnam in order to speculate their host species origin. The genotype constellations were G3-P[6]-I5-R1-C1-M1-A8-N1-T1-E1-H1 (for NT0001), G4-P[6]-I5-R1-C1-M1-A8-N1-T7-E1-H1 (for NT0077), G4-P[6]-I1-R1-C1-M1-A8-N1-T1-E1-H1 (for NT0042), and G4-P[6]-I1-R1-C1-M1-A1-N1-T1-E1-H1 (for NT0205, NT0599, and NT0621). Phylogenetic analysis of all genome segments showed that they belonged to the lineages consisting of porcine or porcine-like strains. Even the three strains possessing the same genotype constellation were unlikely transmitted from human to human as their genetic distances were larger than could occur during transmission. We hypothesized that each of six P[6] strains represented an independent, direct transmission of a porcine RVA to a child and died out without establishing a human-to-human spread.

Presenter's profile: Assistant Professor of Graduate School of Biomedical Sciences

Dengue viral genetic diversity in selected dengue patients

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Dengue virus (DENV), a *Flavivirus* belonging to *Flaviviridae* family, has four serotypes. The virus single positive- stranded RNA genome encodes for three structural proteins (Capsid, Envelope, and Membrane) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). Just like other arboviruses, the dengue virus cycles between vertebrate and invertebrate hosts, resulting in the additional selective constraints compared with single-host RNA viruses. Hence, intra-host genetic diversity may offer an advantage to survive in distinct environments. In this study, we evaluated the *in vitro* effects of viral mutations in intra-host adaptation and determined the fitness of DENV. Using DENV isolated from serum samples collected in the dengue epidemic in 2013 in Hue, Vietnam, the viruses were passaged serially in the mosquito (C6/36) and mammalian cells (Vero), or alternately between these two cell lines. Interestingly, after two or three passages in Vero cells, DENV with substitution of Valine (V) to Methionine (M) or Alanine (A) at position 115 of NS4B was isolated from these cells after inoculation by a single patient serum sample, while the parent type- Valine was maintained in C6/36 cells. The results suggest that some elements of the NS4B may be involved in viral replication and pathogenesis, as well as viral adaptability.

Presenter's profile: 3rd year Ph.D. student, Department of Virology, Institute of Tropical Medicine, Graduate School of Biomedical Sciences, Nagasaki University

Neurotropic characteristics of dengue serotype 3 virus isolated from a dengue encephalitis patient in Viet Nam

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Dengue encephalitis (DE) is characterized as unusual presentation of dengue infection. Despite the reports that DE accounts for only 1-5% of dengue cases, the number of cases reported worldwide is increasing. The molecular information on neurotropic dengue virus (DENV) in patients is limited, and the characterization of the disease is important in the determination of the pathogenesis of dengue-related neurological cases. Cerebrospinal fluid (CSF) sample from a DE patient and serum samples from two different patients with classical dengue fever were obtained during dengue endemic season in Vietnam in 2013. DENV serotype 3 (DENV-3) were isolated from these samples and were determined to belong to genotype III, which was confirmed for the first time in this country. The full genome sequence of the three isolates was determined to map out amino acid mutations. Phylogenetic analysis showed close relationship between the CSF-derived and the two serum-derived DENV-3. The envelope (E) sequence of the three isolates did not show any difference in nucleotide and amino acid substitutions. However, the full genome analysis revealed *Thr-1339-Ile* mutation in non-structural region 2A (NS2A) sequence in the CSF-derived DENV-3 strain. The characteristics of these virus strains were determined using human neuroblastoma cell line (SKNSH), glioblastoma cell line (T98G) and mouse neuroblastoma cell line (N2A). The infectivity of isolated DENV-3 strains was further characterized by using immunofluorescence assay (IFA), plaque forming assay as well as real-time polymerase chain reaction technique. These virus strains propagated in both mosquito cell line and human neural cell line, but virus growth was limited in mouse neuroblastoma cell line. Our data indicate that the CSF-derived DENV-3 has unique virulence features that might play a crucial role in the neuropathogenesis of DENV infection.

Presenter's profile: 3rd year Ph.D. student, Department of Virology, Institute of Tropical Medicine and Graduate School of Biomedical Sciences, Nagasaki University

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iPS cell derived dendritic cell like cell is infected with dengue virus, and acts as antigen presenting cell.

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Dengue virus infection pose a great threat to about 2 billion of people globally. Although several vaccine trials have been launched, there is no vaccine which is practically applicable to humans. The serotype specific humoral immunity has been reported to show a limited protection against infection, and this fact suggested the importance of the cellular immunity. Dendritic cell is defined as a professional cell to present antigenic peptide coupled with MHC to T cells, and also known as the first target cell of DENV infection. In this study, we tried to set up an *in vitro* antigen priming system for T cells using iPS cell derived DC like cells. We found that DENV efficiently infected iPS DC like cells. Moreover, the *in vitro* experiments showed clearly that the infected iPS DC like cells provoked massive T cell proliferation even from naïve donor. Our results indicates the possibility to identify important T cell antigenic epitopes using the iPS DC like cells and to enhance development of both T and B cell stimulating dengue vaccine.

Presenter's profile: 3rd year student of Nagasaki University Graduate School of Biomedical Sciences Doctoral Leadership Program.

Virological characterization of DENV circulating in Metro Manila, 2015-2016

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Dengue (DEN) is endemic in the Philippines. From January to August 2016, there were 84,085 reported cases of dengue and this number was 15.8% higher compared to that (72,627) reported in the same period in 2015. Out of the cases in 2016, 372 people died. DENV virus (DENV), the causative agent, has four phylogenetically and antigenically distinct serotypes (DENV1-4). Secondary infection with a heterologous DENV serotype has been shown to increase the risk of severe disease. In this study, we determined the circulating serotypes in the Philippines during the 2015-2016 outbreaks in Metro Manila. A total of 495 serum or plasma samples were collected from 427 DEN patients. DENV infection was confirmed by RT-PCR and anti-DENV IgM ELISA. A total of 212 patients had secondary DENV infection (193 of 332 outpatients [58.1%] and 19 of 55 inpatients [34.5%]). DENV was isolated from 120 out of 139 samples. Of the serotyped samples, the serotype distribution was 23.3% (n=28) DENV1, 22.5% (n=27) DENV2, 16.17% (n=20) DENV3, and 19% (n=23) DENV4. A total of 45% and 10% of inpatient samples were positive for DENV3 and DENV4 respectively; while there were none serotyped for DENV1 and DENV2. In contrast, 24.3%, 23.4%, 13.04% and 19.1% of outpatient samples were positive for DENV1, DENV2, DENV3 and DENV4, respectively. Interestingly, there has been an increase in the percentage of DENV4-infected patients. Phylogenetic analysis demonstrated that the isolated DENV4 belongs to genotype II, and is closely related to those DENV4 strains circulating in South East Asia. One sample had DENV3 and DENV4 serotypes. The result demonstrated that all four serotypes of DENV were co-circulating in Metro Manila in 2015-2016. This differs from prior reports in which a single serotype is dominant during outbreaks. The findings of this study suggest a changing pattern of circulating serotypes in the Philippines.

Presenter's profile: 2nd year Ph.D. student, Department of Virology, Institute of Tropical Medicine and Graduate School of Biomedical Sciences, Nagasaki University

Clinical, virological and epidemiological characterization of dengue outbreak in Myanmar, 2015

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Dengue is an endemic disease in Southeast Asian countries including Myanmar. In this study, hospital-based surveillance was conducted in 2015 when the largest dengue epidemic occurred in Myanmar. The aims of the study were to characterize the clinical manifestations and viremia pattern of dengue patients and to understand the molecular epidemiology of dengue viruses (DENV) in two selected regions of this country.Acute phase serum samples were collected from 332 clinically diagnosed dengue patients in Upper and Lower Myanmar from July-August, 2015. Of the 280 DENV-confirmed patients, 121(43.2%) and 111(39.6%) had primary and secondary infections, respectively. There was a high number of cases (24.5%; 12/49) characterized with severe dengue and primary infection. Patients with primary infection or negative for DENV IgM antibody demonstrated significantly higher viremia levels by plaque assays using FcγRIIA-expressing BHK and non FcγRIIA-expressing BHK cells. However, the mean viremia levels were not significantly different among the different severity groups but remained high up to day 6 among patients. A total of 106 DENV strains were isolated (76 DENV-1 genotype 1, 24 DENV-2 Asian 1, 1 DENV-3 genotype III, and 5 DENV-4 genotype 1) including two cases with dual serotype infection. Serotype (except DENV-3) and genotype distributions were similar in both areas. Phylogenetic analyses of the envelope gene of the epidemic strains revealed close similarity with the strains previously isolated in Myanmar and neighboring countries. High percentage of primary infection was noted among the patients and DENV-1 dominated the epidemic in 2015. Viremia levels among patients with primary infection were notably high for a long period. The high viral load with a long duration among the viraemic dengue patients could perhaps serve as the increased source of infection to support the transmission of DENV through the vector mosquitoes during this epidemic.

Presenter's profile: 4th year Ph.D. student, Department of Molecular Virology, Nagasaki University Graduate School of Biomedical Sciences

ANGPTL4 as a potential treatment target in influenza and associated bacterial pneumonia

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Excessive host inflammatory responses negatively impact disease outcomes in respiratory infection. Host-pathogen interactions during the infective phase of influenza are well studied, however little is known about the host's response during the repair stage. Influenza-associated secondary bacterial infection adds on more complication to the host immune responses after primary influenza infection. Designing effective vaccines and treatment options has proven challenging in view of the rapid evolution of the virus and bacteria. A better understanding of the host responses may facilitate innovative treatment strategies. We studied the detailed function of angiopoietin-like 4 (ANGPTL4) in influenza and associated bacterial pneumonia, which may open door to new intervention strategies. By injection of ANGPTL4 antibody or using transgenic mouse models, effect of ANGPTL4 was studied in mouse models infected with different strains of influenza virus and S. pneumoniae. Samples from patients with or without pneumonia were screened for ANGPTL4 characteristics.

Here we show that influenza and bacterial infection stimulated the expression of angiopoietin-like 4 (ANGPTL4) via a direct IL6-STAT3-mediated mechanism. ANGPTL4 enhanced pulmonary tissue leakiness and exacerbated inflammation-induced lung damage. The treatment of infected mice with neutralising anti-ANGPTL4 antibodies significantly accelerated lung recovery and improved lung tissue integrity. ANGPTL4-deficient mice also showed reduced lung damage and recovered faster from influenza and bacterial infection when compared to their wild type counterparts. Retrospective examination of human lung biopsies from infection-induced pneumonia with tissue damage showed elevated expression of ANGPTL4 when compared to normal lung samples.

These observations underscore the important role that ANGPTL4 plays in lung infection and damage, and may facilitate new therapeutic strategies for the treatment of pneumonia.

Targeting viral nucleoprotein in search of new influenza A virus inhibitors

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The emergence of drug resistance to neuraminidase-targeting influenza drugs necessitates the search for new drugs with novel mechanisms of action. Influenza A virus nucleoprotein is a highly conserved internal viral protein which plays critical roles in virus replication, presenting an attractive target for antiviral drugs. In this work (Makau *et al*), we used structure-based approach to find inhibitors of influenza A virus that target viral nucleoprotein. Our work employed an original docking algorithm termed Nagasaki University Docking Engine (Ishibashi *et al*) that ran on the Destination for GPU Intensive Machine supercomputer to select compounds that bind to nucleoprotein from a custom chemical library. We found a compound designated NUD-1 belonging to 4-hydroxyquinolinone family that effectively inhibited the replication of various influenza A virus strains including a clinical isolate of 2009 H1N1 pandemic in cell culture within a 50% inhibitory concentration range of 1.8 – 2.1 μM. Analysis of binding between nucleoprotein and NUD-1 using surface plasmon resonance assay and fragment molecular orbital calculations revealed that the compound could bind to nucleoprotein and inhibit protein-protein interactions essential for virus replication. Collectively, our data demonstrate that NUD-1 is a potential lead compound for anti-influenza drug development and highlights the usefulness of NUDE structure-based drug approach in selection of important drug candidates.

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Presenter's profile: 4th year student in the Leading Graduate School Program, Nagasaki University

Serological surveillance of Zika virus in Central Vietnam, 2016 – 2017

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Zika virus (ZIKV), a mosquito-borne flavivirus, was first identified in Uganda in 1947 in monkeys. ZIKV infection is hypothesized to be associated with microcephaly. In 2016, sporadic ZIKV outbreaks have been reported in Vietnam with 219 cases. Recently, a suspected case of microencephaly due to ZIKV in a 4-month old infant has been confirmed in Dak Lak region, Vietnam. In this study, a cross-sectional serological analysis of the region was performed to determine the size and geographical extent of ZIKV outbreak. A total of 48 serum samples (3 paired serum samples and 42 single serum samples) were collected from 45 persons in Central Vietnam by the National Institute of Hygiene and Epidemiology and NIHE-Nagasaki Friendship Laboratory, Nagasaki University. All serum samples were tested for anti-ZIKV antibodies using an in-house IgM ELISA kit for ZIKV and a commercial anti-DENV IgM antibody ELISA kit. To determine specific neutralizing antibodies to ZIKV, the Plaque Reduction Neutralizing Test (PRNT) was performed. Among the 48 serum samples from 45 cases, 16 of 45 (35.5%) cases were positive for ZIKV IgM whereas 5 of the 47 (10.6%) cases were positive for DENV IgM. Four cases were positive with both of DENV IgM and ZIKV IgM tests. Neutralizing antibody to ZIKV with the $PRNT_{50} \geq 40$ was detected in 8 out of 22 cases (36.4%) with $PRNT_{50}$ values ranging from 40-1280. Of these 8 cases, 6 exhibited cross-reactive neutralizing antibody to DENV ($PRNT_{50} \geq 40$): 6 (75%) to DENV-1 (6/8), 2 to (25%) DENV-2, 5 (62.5%) to DENV-3 and 1 (12.5%) to DENV-4 . This study demonstrated that residents in Central Vietnam had recent exposure to ZIKV infection. The presence of high levels of neutralizing antibody to ZIKV in all ($PRNT_{50}$ = 40 to 1280) confirmed that the residents have been exposed to ZIKV. The findings suggest that there has been a recent ZIKV outbreak in Central Vietnam. This serosurveillance study shows its usefulness in the determination of recent outbreaks as compared to molecular studies due to transient ZIKV viremia. This study also emphasize on the need to continue ZIKV surveillance in the region.

Presenter's profile: 1st year Ph.D. student, Department of Virology, Institute of Tropical Medicine and Graduate School of Biomedical Sciences, Nagasaki University

Seroepidemiological survey for Zika Virus antibodies in febrile patients, Central and North Vietnam, 2014-2015

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Zika virus (ZIKV) is a mosquito born flavivirus. The disease is first reported in humans approximately 60 years ago in 1953. Until recently, there have been limited data on the ZIKV epidemics. As of early 2017, more than 80 countries have reported transmission of ZIKV. In Vietnam, 23 confirmed ZIKV cases have been reported since April 2016. However, little is known on the extent of prior ZIKV epidemic in Vietnam. In this study, we aim to determine the extent of ZIKV outbreak in Vietnam during 2014 to 2015 by performing seroepidemiological cross sectional studies. A total of 599 serum samples collected from Hue, Vietnam and 180 serum samples collected from Hanoi, Vietnam were used in this study. Serum samples were tested for the presence of ZIKV IgM antibodies using an in-house kit. ZIKV IgM positive samples were further tested for anti-DENV IgM using a commercial kit and dengue NS1 antigen test. A total of 20 positive samples for ZIKV IgM antibodies were tested for the presence of ZIKV RNA using real-time PCR. Of the 599 Hue samples, 148 (24.7%) were positive for anti- ZIKV IgM antibody. Among 35 ZIKV positive samples, 16 (45.7%) were also positive for DENV IgM antibodies. Additionally, one sample (1/16, 6.25%) was positive for DENV NS1 antigen. Real-time PCR assay was also performed for 20 anti-ZIKV IgM antibody positive samples. Three samples (3/20, 15%) were positive for ZIKV RNA (Ct level = 36-37). Of the 180 samples obtained from Hanoi, 58 (58/180, 32.2%) were positive for anti-ZIKV IgM antibody. These results suggest the occurrence of ZIKV in Central and North Vietnam prior to 2016.

Presenter's profile: Visiting researcher, Department of Virology, Institute of Tropical Medicine, Nagasaki University

Rapid detection of all known ebolavirus species by reverse transcription-loop-mediated isothermal amplification (RT-LAMP)

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P15

Ebola virus disease (EVD), a highly virulent infectious disease caused by ebolaviruses, has a fatality rate of 25-90%. Without a licensed chemotherapeutic agent or vaccine for the treatment and prevention of EVD, control of outbreaks requires accurate and rapid diagnosis of cases. In this study, five sets of six oligonucleotide primers targeting the nucleoprotein gene were designed for specific identification of each of the five ebolavirus species using reverse transcription-loop mediated isothermal amplification (RT-LAMP) assay. The detection limits of the ebolavirus species-specific primer sets were evaluated using *in vitro* transcribed RNAs. The detection limit of species-specific RT-LAMP assays for *Zaire ebolavirus*, *Sudan ebolavirus*, *Taï Forest ebolavirus*, and *Bundibugyo ebolavirus* was 256 copies/reaction, while the detection limit for *Reston ebolavirus* was 64 copies/reaction, and the detection time for each of the RT-LAMP assays was 13.3 ± 3.0, 19.8 ± 4.6, 14.3 ± 0.6, 16.1 ± 4.7, and 19.8 ± 2.4 min (mean ± SD), respectively. The sensitivity of the species-specific RT-LAMP assays were similar to that of the established RT-PCR and quantitative RT-PCR assays for diagnosis of EVD and are suitable for field or point-of-care diagnosis. The RT-LAMP assays were specific for the detection of the respective species of ebolavirus with no cross reaction with other species of ebolavirus and other viral hemorrhagic fever viruses such as Marburg virus, Lassa fever virus, and Dengue virus.

The species-specific RT-LAMP assays developed in this study are rapid, sensitive, and specific and could be useful in case of an EVD outbreak.

Presenter's profile: 3rd year student of Program for Nurturing Global Leaders in Tropical and Emerging Communicable Diseases, Graduate School of Biomedical Sciences, Nagasaki University.

The therapeutic effect of antiserum on severe fever with thrombocytopenia syndrome virus infection in a mouse model

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Severe fever with thrombocytopenia syndrome (SFTS) is an emerging viral infection that is endemic in China, Korea and Japan. There are no effective vaccines or specific treatments for SFTS infection. In the present study, we used a mouse model to examine the effects of ribavirin, site-1 protease inhibitor, corticosteroids, and combination of minocycline and ciprofloxacin (MC) on SFTS infection. The antiserum from a patient who recovered from SFTSV infection was also examined for its effect on mice. Administration of antiserum completely protected mice against lethal infection (10^6 ffu/ml) with SFTSV. It could also protect mice from showing clinical signs of the disease due to non-lethal infection. Minocycline and ciprofloxacin treatment resulted in prolonged survival times during lethal infection. Although other agents had no significant protective effects, they did not provide detrimental effects that could lead to progression of the disease in mice.

Presenter's profile: 4th year PhD student and pediatrician, Department of Virology, Institute of Tropical Medicine and Graduate School of Biomedical Sciences, Nagasaki University

Difference in Clinical Presentation of Human Metapneumovirus Group-A Lineages Associated with Pediatric ARI Hospitalizations in Central Vietnam

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Background: Human Metapneumovirus (hMPV) is one of the primary viruses for acute respiratory infection (ARI). hMPV Group-A and B possess distinct Subgroups and Lineages based on genetic diversity of surface G and F glycoproteins. Clinical manifestation of hMPV is similar to RSV; however, the detailed clinical and molecular epidemiological information of hMPV is limited worldwide, particularly in developing countries.

Objective: We aim to investigate (i) seasonal circulation pattern of hMPV and (ii) demographic, clinical and molecular epidemiological characteristics of hMPV-related pediatric ARI hospitalizations in central Vietnam.

Methods: The study was conducted at Khanh Hoa Province, Vietnam where population-based ARI surveillance is on-going. We utilized the clinical-epidemiological data of hospitalized ARIs during 2007-2015. Multiplex PCRs were performed to screen 13 respiratory viruses using nucleic acids extracted from nasopharyngeal swabs. Phylogenetic analysis of *F* gene nucleotide sequence was performed to reveal the molecular epidemiological characteristics with Maximum Likelihood and Bayesian MCMC. Multivariable logistic regression was done to identify the demographic and clinical characteristics associated with hMPV Groups/Subgroups and Lineages.

Results: During the study period, a total of 6167 pediatric ARI cases were enrolled, and 206 (3.3%) hMPV positive cases were detected. Yearly hMPV incidence was 127 (per100,000) among children under five before 2009, which increased to 216 in post-A/H1N1pdm09 period (2010-2015). Group-A and B were co-circulating, yet Group-A was dominant in most of the seasons. Time-scaled Bayesian phylogenetic tree presented that recently dominant A2c Lineage diverged from A2b around 2004 (95%HPD:2002-2006) and became dominant in post-A/H1N1pdm09. With respect to the clinical manifestation of Group-A, multivariable logistic regression presented that A2b ARI cases were clinically more severe than A2c: Adjusted odds ratios were 6.30 (95%CI:1.65-24.09) among wheeze and 6.93 (95%CI:2.34-20.50) among lower respiratory tract infections.

Conclusion: These results highlight the clinically important role of hMPV among pediatric ARI hospitalizations in post-A/H1N1pdm09 era. Furthermore, the difference in clinical presentation of hMPV Group-A Lineages will add important information for the future clinical management and vaccine development.

Presenter's profile: 4th year Ph.D. (candidate) in Leading program, Graduate School of Biomedical Sciences

Respiratory Viruses Increase the Risk of Subsequent Hospitalization for Acute Respiratory Illnesses in Young Children

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Rationale: Respiratory viruses cause acute respiratory illness (ARI) in early childhood, but their effect on subsequent ARI admissions is not fully understood.

Objectives: This study aimed to determine the association between initial ARI admission due to viruses including human rhinovirus (HRV), respiratory syncytial virus (RSV), human adenovirus (HAdV), and human metapneumovirus (hMPV) and the risk of ARI readmission in children.

Methods: Clinical information and nasopharyngeal swab samples were collected from children <2 years old at their initial ARI admission in Nha Trang, Vietnam. The incidence of ARI readmission during the follow-up period (initial admission to 5 years of age) was compared between children with and without one of 13 respiratory viruses at initial admission.

Measurements and Main Results: A total of 1,941 children were enrolled in the study. Viruses were detected in 1,254 (64.6%) children at enrollment; HRV, RSV, HAdV, and hMPV were detected in 499 (25.7%), 439 (22.6%), 156 (8.0%), and 47 (2.4%) children, respectively. During the follow-up period (4,572.7 person-years), 277 children were readmitted with ARI. Virus-related ARI admission was associated with an increased risk of ARI readmission for children who were initially admitted before 6 months of age (adjusted rate ratio, 1.6; 95%CI, 1.1-2.5). HAdV (4.6; 1.8-11.9), hMPV (20.4; 6.2-66.9), and HRV (1.6; 1.0-2.4) were independently associated with the outcome. These associations were not observed for children whose initial admission occurred after 6 months of age.

Conclusions: HAdV-, hMPV-, and HRV-related initial ARI admissions increased the risk of subsequent ARI admission when occurring during early infancy.

Presenter's profile: Assistant professor of Department of Pediatric Infectious Diseases in Institute of Tropical Medicine, Nagasaki University

Erythrocyte binding-like protein is essential for the erythrocyte invasion by the rodent malaria parasite *Plasmodium yoelii*

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Malaria is one of major global infectious diseases caused by *Plasmodium* species parasites. In the mammalian host, malaria parasites multiply by repeating erythrocyte invasion and schizogony. The erythrocyte invasion process consists of multistep steps involving sequential interaction between parasite ligands and host receptors and is a target of vaccine and drug development. Erythrocyte binding-like protein (EBL) was suggested to be essential in the rodent malaria parasite *Plasmodium yoelii*, because of unsuccessful trials to establish a gene deletion mutant for this gene locus. However, the direct evidence is lacking and in which step *ebl* deletion would affect is unknown. To answer these points, we established a transgenic *P. yoelii* 17XL-background line for which the *ebl* gene locus will be silenced with anhydrotetracycline (ATc). Parasitemia was significantly reduced 12 hours after ATc administration and almost all parasites disappeared 24 hours after administration. Time-lapse imaging of the erythrocyte invasion by *ebl*-knockdown parasites revealed that most knockdown parasites failed to complete the invasion process. In conclusion, we have succeeded to knockdown *ebl* gene in *P. yoelii* for the first time and proved that this gene was essential during the erythrocyte invasion.

Presenter's profile: 4th year PhD student of leading program

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EBL secretion dynamics of a malaria parasite during erythrocyte invasion

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Malaria remains as a heavy burden on human societies in the world. Despite long-term efforts to develop effective malaria vaccine, none is still available. This is due to, in part, the poor understanding of the dynamics of parasite antigens during host-parasite interaction. In mammalian hosts, malaria parasites proliferate by repeated invasion of and multiplication within erythrocytes. During erythrocyte invasion, the invasive merozoite stage of the malaria parasite sequentially secrete various molecules from organelles, which participates at different steps in the invasion process. However, the molecular background of this tightly regulated protein secretion mechanism is not well understood. In order to understand this system, we developed a novel method to evaluate the timing of the protein secretion using a rodent malaria parasite, *Plasmodium yoelii*, focusing on the unique feature of this species that the secretion from an organelle called microneme can be seen at the merozoite stage. We purified merozoites from fully matured schizonts and performed immune fluorescent assay at various time points. We were successfully able to observe the one microneme protein Apical membrane protein 1 (AMA1) was secreted earlier than the other microneme protein erythrocyte binding like protein (EBL) in 17XNL line. This suggests that signals to trigger the secretion of these proteins are different, although both are secreted from "micronemes". We also found that EBL in 17XL line was not secreted up to 80 min in our assay. One amino acid mutation found in 17XL line EBL is known to mislocate this protein from microneme to the other organelle "dense granule". Thus our result raises a possibility that EBL plays no direct role in the host cell recognition during erythrocyte invasion process in 17XL line.

Presenter's profile: 2nd year student of Graduated School of Biomedical Sciences, Nagasaki University, Japan

Asian G6PD-Mahidol reticulocytes sustain normal *Plasmodium vivax* development

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymatic disorder in humans and appears to be protective against falciparum severe malaria. Controversially, it is also thought that Plasmodium vivax, the most common cause of malaria in Asia, has driven the recent selection of G6PD alleles in this region. We use an experimental approach to determine whether G6PD-MahidolG487A variant, a widespread cause of severe G6PD deficiency in Southeast Asia—, provides a barrier against vivax malaria. Our results show that the immature reticulocytes (CD71+) targeted by *P. vivax* invasion are enzymatically normal even in hemizygous G6PD-Mahidol G487A mutants; thus allowing the normal growth, development and high parasite density in severely deficient samples. Our findings challenge preconceptions that human red cell mutations are protective against all haemoparasites.

P21

Tether-like structures connect Sinton-Mulligan's clefts and erythrocyte membrane in *Plasmodium knowlesi*-infected erythrocyte

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P22

Brain autopsy of a fatal case of *Plasmodium knowlesi*, a recent emerged zoonotic malaria parasite in Southeast Asia has revealed its capacity to cause cerebral malaria. However, *P. knowlesi* is significantly different from *Plasmodium falciparum* at both genome and structural levels. Cerebral malaria, one cardinal feature of severe falciparum malaria is associated with a major virulence protein erythrocyte membrane protein 1 (PfEMP1), for which *P. knowlesi* lacks its homolog. *P. falciparum* restructures the inert infected erythrocyte by forming a membranous structure called Maurer's cleft (MC) for protein sorting and trafficking to the erythrocyte surface. The MCs are connected to the erythrocytes membrane via a tether structure with membrane-associated histidine rich protein 2 (MAHRP2) as its marker. We have previously reported skeleton binding protein 1 (SBP1) homolog in *P. knowlesi*, a resident protein at MC in the *P. falciparum*-infected erythrocyte. PkSBP1 localizes in both unilateral and circular clefts in *P. knowlesi* infected erythrocytes at the electron microscopic level and Sinton and Mulligan's Giemsa-stained "clefts" (SMC). The SMC's role in protein trafficking in *P. knowlesi* is not known. Our objective was to further characterize the structural platform for protein trafficking in the erythrocyte infected with *P. knowlesi*. We have combined gene identification, immunofluorescence and electron microscopic techniques to show that SMCs connect each other and with erythrocyte membrane through a tether-like structure similar to the *P. falciparum* MC. We have also identified a novel marker (We refer to as PkMAHRP2) that localizes at this structure. The marker (PkMAHRP2) is conserved in seven other *Plasmodium* species. In conclusion, we showed the existence of tether-like structures that connect different membranous structures in *P. knowlesi*-infected erythrocytes, which may form a platform for protein trafficking in *P. knowlesi*. Although the structural features among *Plasmodium* species are different, these membranous structures involved in the protein trafficking machinery appear to be conserved among malaria parasites species.

Presenter: 4th year student of Graduate School of Biomedical Sciences, Nagasaki University

Identification and Characterization of Plasmodium falciparum Digestive Vacuole Disruption and Programmed Cell Death through High Throughput Screening using the MMV Compound Libraries – Malaria and Pathogen Box

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Malaria is a mosquito-borne infectious disease caused by parasitic protozoans of the genus *Plasmodium*. To date, *P. falciparum* infections have been established to be the deadliest in terms of clinical manifestations and the severity of the disease. In 2016, the World Health Organization (WHO) reported an estimated 429 000 malaria deaths over 200 million disease-ridden cases.

Since the 1950s, chloroquine (CQ) has long been used in the treatment or prevention of malaria infection. In contrast to the canonical mechanism of action in inhibiting hemozoin formation, it was recently found that upon treatment with micromolar levels of CQ, the parasite's digestive vacuole membrane was permeabilized, leading to Ca^{2+}efflux. Further downstream events also displayed hallmark features of mammalian programmed cell death (PCD) such as mitochondrial outer-membrane permeabilization and DNA degradation. In the search for novel antimalarials against rampant CQ-resistance and rising artemisinin-resistance, this biological pathway can be leveraged upon to elucidate the PCD-like characteristics in the parasite for maximal killing activity. Through private-public partnerships with St. Jude Children's Research Hospital, GlaxoSmithKline and Novartis, Medicines for Malaria Venture (MMV) had assembled the Malaria Box (2013) and the Pathogen Box (2015) respectively consisting of 396 and 400 diverse compounds against malaria and a wide range of neglected tropical diseases. High throughput screening was performed to select for compounds which disrupt the digestive vacuole membrane as measured by the leakage of intravacuolar Ca^{2+} using a calcium probe. Mitochondrial membrane depolarization and DNA fragmentation were assayed to validate the PCD-like features. From this panel of 32 hits, hemozoin biocrystallization inhibition assays and dose-response IC_{50} assays across CQ-resistant and sensitive strains were quantified to investigate the possible molecular mechanism of action, cross-resistance and potency at the nanomolar range. The timing of action of the non-cross resistant hits was also defined over a growth period of 72 hours to identify the rapidly parasiticidal hits from the moderate or slow-acting ones; while the cross-resistant hits were further assayed for improved *in vitro* efficacy against the resistant strains using chemoreversal agents. From the drug development perspective, the work presented can offer a basis for subsequent hit-to-lead generation and optimization through structure-activity relationship analysis. Follow-up studies with non cross-resistant and fast-acting antimalarial analogues which target the digestive vacuole can provide a positive and alternative outlook in the pursuit towards novel therapeutic intervention.

P23

Modulation of the metabolic status enhances protective immune responses against infection with malaria parasites

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Antigen stimulation modulates metabolic status of T cells via several signal transduction pathways such as mTORC1 and AMP-activated protein kinase (AMPK). Type II diabetes drug, metformin, was reported to be a AMPK activator, and can inhibit exhaustion and promote memory differentiation of effector $CD8^+$T cells. Therefore, we investigated the effects of metformin on the immune responses against infection with malaria parasites. C57BL/6 mice were infected with *Plasmodium yoelii* 17XNL and were treated with metformin in drinking water. The levels of parasitemia in the treated mice began to reduce ~18 days after infection, earlier than untreated mice. We examined spleen cells on day 18 after infection using flow cytometry. Activated $CD4^+$ and $CD8^+$ T cells slightly increased, while γδT and germinal center B cells dramatically increased. Immunohistolochemical analysis indicated that the expansion of γδT cells occurred in red pulp of spleen in the treated mice. We are currently analyzing the effects of metformin treatment on the signal transduction and metabolism of immune cells during malaria infection.

Presenter's profile: Senior assistant professor of Graduate School of Biomedical Sciences, Nagasaki University

Hepatic spheroids as an *in vitro* model to study malaria relapses

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Plasmodium vivax is responsible for up to 50 million cases of malaria worldwide each year. Unlike many other causes of malaria, *P. v*ivax forms dormant liver stages (hypnozoites), which cause 'relapses' of infection weeks after the initial infection. The search for alternative hypnozoite- active drugs is hampered due to the lack of suitable *in vitro* hepatic models to better understand the biology of the elusive *P.vivax* hypnozoites. For the reactivation of the hypnozoite to develop into schizont, viable and functional primary hepatocytes are required for long term culture so as to capture the entire liver stage life cycle of the parasite. However, when primary hepatocytes are cultured *in vitro* as a conventional 2D monolayer, they de-differentiate and rapidly lose their hepatocyte-specific functions. Thus, the use of such 2D primary hepatic monolayer cultures for drug-screening assays of hypnozoite-active drugs is not ideal. Primary hepatic 3D spheroids are widely used as better alternative to conventional hepatic monolayer for the study liver biology and functions, drug induced hepatotoxicity and liver diseases as it closely mimics the *in vivo* liver.

Here, we have adapted primary simian hepatic 3D spheroids as a potential platform for screening hypnozoite-active drugs for *Plasmodium cynomolgi* (established surrogate model for *Plasmodium vivax*) where infected hepatic 3D spheroids were cultured long term and were able to sustain the parasite development so to accurately capture the relapses from the hypnozoites.

P25

Kinetics of antigen-specific CD8$^+$ T cells response in the spleen infected with *Plasmodium berhgei*

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Spleen is the main organ of the immune responses during blood-stage infection with malaria parasites. However, little is known regarding the dynamics of the immune responses in the spleen during malaria infection. We previously reported that antigen-specific CD8$^+$ T cells expand in the spleen during malaria infection using a combination of *Plasmodium berghei* ANKA expressing model antigen OVA (PbA-OVA) and CD8$^+$ T cells from OVA-specific T-cell receptor transgenic mice (OT-I). In this study, we examined dynamic of OT-I cells in the spleen during infection with PbA-OVA, and compared it with that during infection with *Listeria monocytogenes* expressing OVA (LM-OVA). OVA-specific CD8$^+$ T cells were mainly activated in the white pulp of the spleen during malaria infection, as similarly observed during Listeria infection. However, further development of these activated CD8$^+$ T cells was distinct. During infection with malaria parasites, activated CD8$^+$ T cells accumulated in the red pulp and/or marginal zone, where they expressed multiple inhibitory receptors and their cytokine production reduced, suggesting that these cells became exhausted. In mice infected with LM-OVA, OVA-specific CD8$^+$ T cells only transiently expressed inhibitory receptors in the white pulp and maintained their ability to produce cytokines becoming memory cells. These results suggest that activation and exhaustion of specific CD8$^+$ T cells occurr in distinct spleen compartments during infection with malaria parasites.

Presenter's profile: Research assistant in Graduate School of Biomedical Sciences

Modulation of pathogenic T-cell responses after infection with *Plasmodium berghei* ANKA by prior infection with *P. chabaud*

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The protective immunity against *Plasmodium* species is thought to be species-specific. However, the consequences of the infection with one species to another is not clearly understood. To determine the effect of previous infection with *Plasmodium* parasites, C57BL/6 mice were infected with *Plasmodium chabaudi chabaudi* (Pcc) and treated with anti-malarial drug at chronic phase of the infection. $CD4^+$ T cells from these mice produced IFN-□ in response to soluble lysate of Pcc or *P. berghei* ANKA (PbA), suggesting cross reactivity of $CD4^+$ T cells. Two weeks after the treatment, mice were infected with Pcc or PbA. Mice re-infected with Pcc exhibited showed strong protective immune responses. When these mice were infected with PbA, the levels of parasitemia was initially lower than controls, but continuously increased. These mice, however, did not develop cerebral malaria, while all control mice developed cerebral malaria and died within 10 days after infection.

$CD4^+$ T cell from Pcc-infected mice produced IL-10 at high levels in response to PbA antigen. We are studying the possible regulatory mechanisms that inhibited the development of cerebral malaria in Pcc-infected mice.

P27

Presenter's profile: 4th year student of Graduate School of Biomedical Sciences

IRF4^{+} dendritic cells play pivotal roles for the induction of protective immunity against infection with *Plasmodium chabaudi*

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Dendritic cells (DCs) play indispensable roles for the induction of T cell immune responses during infection with malaria parasites. Transcription factors IRF4 and IRF8 play critical roles for the differentiation of CD4^{+} and CD8^{+} DCs, respectively. Previous studies revealed that CD8^{+} DCs are essential for cross-presentation of malaria antigens and activation of CD8^{+} T cells during blood-stage malaria infection. However, the DC subtype critical for CD4^{+} T cell immune responses, which are essential for the protective immunity, has not been determined.

In this study, we used IRF4$^{fl/fl}$CD11c-Cre (CKO) mice, that lack IRF4 in DCs, to determine the role of IRF4^{+} DCs in the generation of protective immunity against infection with *Plasmodium chabaudi*. CKO mice showed severer and prolonged parasitemia when compared with IRF4$^{fl/fl}$ mice, and the majority succumbed to death, while all control mice survived. CKO mice exhibited lower levels of activated T cells and lower levels of plasmablasts during infection. Furthermore, IFN-γ production by CD4^{+} T cells from CKO mice in response to TCR-stimulation was severely impaired, suggesting that IRF4^{+} DCs play pivotal roles for the induction of CD4^{+} and B cell immune responses during blood-stage infection with *P. chabaudi*.

P28

Presenter's profile: 4th year student of Graduate School of Biomedical Sciences, Nagasaki University

High throughput screening and combinatorial chemistry for the discovery of novel anti-malarial (s)

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Malaria is a major global health concern. Rapid spread of drug resistant malaria parasites and limited available drugs – increase the urgency to develop new anti-malarial compounds. In previous study, core 9600 compounds assigned with various structural diversity from >200,000 compounds in chemical library of University of Tokyo were screened. Among 9600, 224 compounds were found after an initial *in vitro* anti-hemozoin high throughput screening (HTS). Next, an *in vitro* erythrocytic anti-malarial assay was performed to screen positive hit compounds at 10 μM using the Chloroquine/Mefloquine sensitive *Plasmodium falciparum (Pf)* strain, 3D7. Subsequently, dose-response assay was done at ten different dilutions ranged between 0.5 nM and 10 μM to measure the 50% inhibitory concentration (IC_{50}). Each experiment was conducted twice. Eventually, an *in vitro* anti-malarial assay followed by dose-response assay were performed using Chloroquine/Mefloquine resistant *Plasmodium falciparum (Pf)* strain, Dd2, at similar condition as mentioned above (with 3D7 strain). SYBR Green-l was used for the staining and detection of parasite. The cytotoxicity was measured using human liver carcinoma (HepG2) and adult mouse brain (AMB) cells at 20 μM concentration. A total of 22 compounds were found to exhibit anti-malarial activity against 3D7 strain with $IC_{50} \leq 10$ μM. Out of 22, only six compounds possessed $IC_{50} < 7$ μM for both 3D7 and Dd2 strains. However, five compounds demonstrated anti-malarial activity at $IC_{50} < 7$ μM for both *Pf* strains where 3 showed $IC_{50} < 0.8$ μM and $CC_{50} > 20$ μM. In conclusion, 5 compounds were selected as potential "Hit compounds". Based on the chemical similarity scores using 'Tanimoto similarity measure' of the five hit compounds, we received the available analogues (>1500) from University of Tokyo for further analysis.

Presenter's profile: 4th year student of Department of Immunogenetics, Institute of Tropical Medicine (NEKKEN), Graduate School of Biomedical Sciences, Nagasaki University

Kampo Compounds and Extracts as a Promising Japanese Traditional Medicine Based Antimalarial Drug

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Abstract

Introduction: Malaria is critical global health issue especially tropical and subtropical countries. The emergence of resistance to the available antimalarial drug requires the urgent development of new medicine for new mechanisms of action. Herbal medicine is still the attractive source of new antimalarial drug since the isolation of artemisinin. Kampo is known Japanese traditional medicine, and the aim of the study is to evaluate the *In Vitro* antimalarial activity of Kampo compounds and extracts.

Methods and Material: The antimalarial activity of 96 compounds and 120 crude drug extracts were evaluated under *In Vitro* antimalarial assay against *Plasmodium falciparum* in erythrocytic cycle. After the drug treatment, the red blood cell (RBC) were stained with CYBR Green to detect the parasite. In addition to this, the cytotoxicity assay was also examined.

Result: From compounds 12 of them had antimalarial activity, but 3 of them were toxic. One compound show high activity (IC_{50} 1.1 μM) and its SI = 37.8. Forty-seven extracts showed antimalarial effect against *Plasmodium falciparum*. One extracts which showed high activity against plasmodium falciparum (IC_{50} 2.5 μg/mL) and its SI > 200.

Conclusion: Some Kampo compounds and extracts showed antimalarial activity. These results lead us to examine Kampo formula as candidate of antimalarial drug. This study will be an input for advance development of antimalarial drug from herbal medicine.

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Presenter's profile:Awet Teklemichael is second year student of Masters of Science of Global Health and Medicine and he will be graduated on September 2017. His background is pharmacist and worked as Industrial pharmacy researcher in Ethiopia until he joined Nagasaki University in School of Tropical Medicine and Global Health. Now he belongs to Immunogenetics department in Institute of Tropical Medicine (NEKKEN). E- mail: awetekle@gmail.com

Pre-existing *Schistosoma mansoni* infection dampened sporozoite-induced malaria liver burden

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Plasmodium and *Schistosoma* species often cause a coinfection in the endemic areas. The interactions of *Schistosoma* with *Plasmodium* had been investigated in animal models using experimental infection with *Plasmodium*-parasitized erythrocytes; however, the host immune responses against pre-erythrocytic malaria in the liver should play an important role to initiate immunity against blood-stage malaria. We investigated the impact of pre-existing *S. mansoni* infection on malaria liver stage using experimental sporozoite inoculation. Malaria parasite burden in the liver was measured 42 hours post SPZ challenge in *S. mansoni coinfected* mice along with *Schistosoma*-non-infected controls. *P. yoelii* parasite load in the liver was reduced by 90 % in the presence of pre-existing *S. mansoni* infection. Although the reduction of malaria parasite density in the liver caused delay in development of the blood-stage malaria, pre-existing *S. mansoni* infection did not affect the peak parasitaemia and mortality in SPZ-induced *P. yoelii* infection. The diminishment of *Plasmodium* parasite was took place after SPZ reaching in the liver. Applying the intraportal infusion of *S. mansoni* eggs, we were able to identify that the main factor of the reduction of malaria liver burden was not caused by fibrotic or granulomatous liver damage but immune environment altered by *S. mansoni* infection. Our results contribute to further understanding of interactions between *Schistosoma* and *Plasmodium*.

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Presenter's profile: PhD student of Leading Program, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

T-cell responses to *Plasmodium falciparum* and *Schistosoma mansoni* infections in children living in the endemic region of Mbita, western Kenya

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The balance between pro-inflammatory and anti-inflammatory immune responses during malaria infection determines the outcome of the infection. In areas that are co-endemic for malaria and schistosomiasis co-infections are common and this may influence immune response to either infection. To study the immune-response modulations that occur during the single and co-infections, we collected blood, urine and stool samples from school going children aged 5-16 years in Mbita area, Kenya. Children were stratified by infection state. PBMC were subjected to flow cytometric analysis or cultured with anti-CD3/CD28 beads and/or specific antigens. Cytokine levels (IL-2, IFN□ and IL-10) were assayed from culture supernatants and T-cell ($CD4^+$ and $CD8^+$) proliferative responses to antigen stimulation tracked by flow cytometry. The percentage of $CD3^+CD4^+$/$CD3^+CD8^+$ cells and their corresponding subsets did not differ significantly amongst the uninfected, single infected and co-infected groups. In response to malaria antigen stimulation, the proportion of proliferating T cells was similar between uninfected and infected groups, showing the heterogeneity of T cell responses. In response to polyclonal stimulation, T cells produced cytokines at levels higher in uninfected compared to malaria-infected group. These findings suggest that T cell immune responses are generally suppressed in asymptomatic children actively infected with malaria parasites.

Presenter's profile: Fourth year doctoral student of Graduate School of Biomedical Sciences, Program for Nurturing Global Leaders in Tropical and Emerging Infectious Diseases, Nagasaki University.

P32

Identification of *Babesia bovis* proteins expressed on the surface of infected erythrocytes

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Babesia bovis causes the most pathogenic form of babesiosis in cattle, which results up to 90% mortality in naive adults. *B. bovis* invades host erythrocytes where they multiply and cause clinical symptoms. The terminally differentiated erythrocyte lacks much of the cellular machinery, presenting a unique challenge for the parasites to modify it suitable for their survival and simultaneously to be able to avoid the removal from circulation by spleen. To overcome these challenges, *B. bovis* needs to export numerous proteins, called "exportome", into erythrocyte cytosol and membrane to change permeability and cytoadherence. Although the exportome, especially proteins exposed on the infected erythrocytes, play a crucial role in *B. bovis* virulence and pathogenesis, such proteins are still not comprehensively characterized. In order to exclusively identify proteins expressed on the erythrocyte surface infected with this pathogen, proteome analysis following the surface biotinylation of infected erythrocytes was performed. Biotinylated proteins were extracted, purified and subjected to LC-MS/MS. As a result of three times MS, in addition to the known surface exposed protein, Variant Erythrocyte Surface Antigen (VESA), we were able to find several uncharacterized proteins, which will be further examined for their importance in the pathogenesis. This is a fundamental and initial step for characterizing the *B. bovis* proteins on the surface of infected erythrocytes which will open up new and exciting possibilities for the development of new therapeutic strategies as well as novel vaccine candidates to combat with bovine babesiosis.

P33

Presenter's profile: JSPS Postdoctoral Fellow

Sharing the experiences of soil-transmitted helminth elimination in Japan to contribute to developing elimination strategies

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In Japan, the prevalence of the soil-transmitted helminth (STH) was high until the 1960s. Due to various control measures, the national prevalence drastically dropped to finally mark 0.01% in 1998. Since then, the population-level prevalence of STH in Japan has been unknown. On the other hand, 1.5 billion people are infected with STH in tropical and subtropical areas in the world. The Natural History Museum of London launched a research project called DeWorm3 in 2015 to test the feasibility of integrated approaches to the STH elimination. As a part of this project, we started collecting stool samples in Japan to identify and quantify current STH prevalence, obtaining information on the trend of prevalence, and reviewing literature on the control measures taken in the past in Japan. The trend of prevalence and literature review revealed that the mass screening and the selective drug administration were effective. Sharing such experiences of Japan may contribute to developing STH elimination strategies for the current endemic areas.

Presenter's profile: 2nd year student of Graduate School of Biomedical Sciences, Nagasaki University

P34

Spatial Distribution and Risk Factors of *Schistosoma haematobium* and Hookworm Infections among Schoolchildren in Kwale, Kenya

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A key epidemiological feature of schistosomiasis is its small-scale heterogeneity. Locally profiling disease dynamics including risk factors associated with its transmission is essential for designing appropriate control programs. We examined 368 schoolchildren from six primary schools. Soil-transmitted helminths and *Schistosoma mansoni* eggs in stool were evaluated by the Kato-Katz method. We measured the intensity of *Schistosoma haematobium* infection by urine filtration. The geometrical mean intensity of *S. haematobium* was 3.1 eggs/10 ml urine (school range, 1.4–9.2). The hookworm geometric mean intensity was 3.2 eggs/g feces (school range, 0–17.4). Heterogeneity in the intensity of *S. haematobium* and hookworm infections was observed in our study. To identify factors associated with the intensity of helminth infections, we utilized negative binomial generalized linear mixed models. The intensity of *S. haematobium* infection was associated with religion and socioeconomic status, while that of hookworm infection was related to sex and time since last anthelmintic treatment.

Our findings link religion and socioeconomic status to the intensity of *S. haematobium* infection. Religious practices may influence behavior of contact with infested water and participation in schistosomiasis control initiatives. In addition, Muslims in Kwale may have genetic traits that increase their susceptibility to infection. Profiling heavily infected individuals could be important for better understanding of the disease epidemiology.

Presenter's profile: 3rd year student Leading Program, Graduate School of Biomedical Sciences, Nagasaki University.

P35

IL-17A may help to persist of *Entamoeba histolytica* by keeping antagonistic relationship with Th1 response in animal model of intestinal amebiasis

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Entamoeba histolytica is the third leading parasitic cause of mortality in the world. Interestingly all individuals are not equally susceptible to amebic infections. *In vitro* studies have suggested that particular cell-mediated immune responses may play some critical role to control or deteriorate this disease conditions. It has already been reported that Th1 responses, for example IFNɣ, play a protective role by clearing ameba from the site of infection, while Th2 responses showed the opposite phenomena. Up-regulation of IL-17 has also been reported during intestinal amebiasis but its role was not clearly defined. The aim of this study was to investigate the role of IL-17 during intestinal amebiasis. Unexpectedly, the number of ameba was lower in IL-17 KO mice than wild type mice. The ceca of wild type infected mice were white and shrink, while those from infected IL-17 KO mice were unchanged. In the absence of IL-17, it has also been observed that Th1 response especially IFNγ was upregulated, which is beneficial for reducing ameba burden. In conclusion, IL-17 helps balance Th1 and Th2 responses that influence disease burden in the mouse model.

4th grade PhD students of the program for nurturing global leaders in tropical and emerging communicable diseases

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Type 2 innate lymphoid cells exacerbate severe amebic liver abscess in mice

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Amebic liver abscess (ALA) is caused by a protozoan parasite, *Entamoeba histolytica* (Eh). After direct injection of Eh into hepatic parenchyma, NKT cells and IFN-γ are known to suppress ALA development. Large amount of Th2 cytokines are also produced at early after Eh infection, but the source and function of this initial Th2 cytokines are unknown. Type 2 innate lymphoid cell (ILC2) can produce abundant IL-5 and IL-13. Therefore, we examined whether ILC2 was involved in this early Th2 cytokine production and the pathogenesis of ALA. In newly established ALA model by inoculating Eh via portal vein, C57BL/6 IFN-γ KO mice showed severer ALA with higher number of ameba than wild-type (WT) mice, reiterating the importance of IFN-γ for host defense to ameba even after their translocation via natural route. In the setting, the number of ILC2 and those producing IL-5 and IL-13 were significantly increased, suggesting the negative association between ILC2 and IFN-γ. To examine the impact of ILC2 on ALA formation, we depleted ILC2 in T/B cells deficient Rag2 KO mice using anti-CD25 mAb. In ILC2-depleted Rag2 KO mice, both the number of ameba and ALA development were better controlled than non-depleted mice with significance. These results indicate that ILC2 exacerbates the pathogenesis of ALA and IFN-γ regulates the number and function of ILC2 in liver.

Presenter's profile: Assistant Professor, Department of Parasitology, NEKKEN, Nagasaki Univ.

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DUSP10 knockout mice harbor altered gut bacterial populations, reduced susceptibility to DSS-induced colonic inflammation and enhanced colonic tumour development

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Dual specificity phosphatase 10 (DUSP10) is a negative regulator of mitogen-activated protein kinases (MAPKs) that are involved in various cellular signaling pathways. Dysregulation of MAPKs is often found in gastrointestinal disorders such as intestinal inflammation and colorectal cancer (CRC). In addition, altered gut microbiota may also contribute to disease pathogenesis together with other factors such as host genetics and immune response. In DSS-induced colitis model, we found that DUSP10 knockout (KO) mice developed less colonic inflammation with reduced colitis-associated cytokine gene expression and increase intestinal epithelial cell proliferation. Further analysis showed that DUSP10 KO mice developed increased colonic tumours in both inflammation-associated and hereditary models of CRC. Interestingly, apart from the effect on intestinal epithelial cell function, loss of DUSP10 resulted in altered gut bacterial population. While total fecal bacteria abundance based on bacterial 16S RNA gene quantification was similar between wildtype and DUSP10 KO untreated mice, total Bacteriodetes and Bifidobacteria were increased in DUSP10 KO mice. Ratio of Firmicutes:Bacteroidetes was also higher in DUSP10 KO mice. Detail characterisation of bacterial populations will be required to understand possible complex host-gut bacteria interaction during disease development. This could provide insights for the development of novel biomarkers based on changes in gut microbiome for the detection of early stage disease, particularly for CRC before the precancerous stage.

Malnutrition and survival in patients admitted to the Tuberculosis (TB) ward at San Lazaro Hospital, Manila, the Philippines

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Abstract:

Under-nutrition is a risk factor and complication of active TB disease. Appropriate nutritional management of malnourished TB patients might improve treatment outcomes. However, the prevalence of under-nutrition in TB in the Philippines and its association with outcomes is not known.

This is a prospective cohort study of patients admitted with clinically suspected tuberculosis (*n*=418), to determine association between clinical wasting (body-mass-index [BMI]<17.5kg/m^2) and in-hospital mortality after the acute admission period (48 hours). Secondary objectives include determining the prevalence of co-morbid diabetes and HIV, change in nutritional status during admission and prevalence of TB using GeneXpert test and microscopy.

In preliminary results (n=268), mean BMI on admission was 17.1kg/m^2 (IQR=15.1-19.9, n=227), with 45% BMI<17.5kg/m^2. Microscopy results (n=214) indicate 32.2% as TB positive, whilst GeneXpert tests (n=228) detected *Mycobacterium TB* in 46.9%, with 19.6% indicated as multi-drug-resistant. HgbA1C>6.5%, indicating diabetes (n=266) was 13.9%. There were 64 deaths (23.9% mortality), 53 occurred after 48hr for whom BMI was: unavailable, n=23 (59.0%); BMI<17.5kg/m^2, n=18 (16.4%); & BMI>17.5kg/m^2, n=12 (11.9%).

Clinical wasting is common in patients admitted with suspected TB, although assessment of BMI is difficult to achieve in this setting. We are investigating alternative assessments of clinical wasting. Data collection is ongoing.

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Presenter's profile: Young researcher/Assistant Professor at School of Tropical Medicine and Global Health

Characteristics of treatments and outcomes of adult empyemas at tertiary hospitals in JAPAN.

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No guideline has been established for empyema management which usually requires combination therapy with antibiotics, drainage, and/or surgery. The present study examines the characteristics of treatments and outcomes of empyemas. A retrospectively observational study from 2009 to 2016 was carried out at tertiary hospitals at Nagasaki, Japan. Adult patients hospitalized with empyema were identified by screening the electronic 'Clinical Data Repository'. Empyema patients (n=59) were predominantly male (74.5%); median age= 68 years, and smokers (61.0%). The 30-day mortality was 3.4% (n=2). The median length of antimicrobial therapy and hospital stay were, 33 days and 31 days, respectively. Most empyemas were community acquired (71.2%), and right-sided (59.3%). Microbiological diagnosis was obtained in 23 (39.0%) patients, included streptococci (16.9%), staphylococci (8.5%), and anaerobes (8.5%); 10.2% were polymicrobial. As the primary therapy, 76.3% (n=45) used antibiotics with drainage; 11.8% (n=7) underwent surgery, and 11.8% (n=7) only with antibiotics. Carbapenems (44.1%) were the most frequently used, and the predictors of extending antimicrobial therapy were: insufficient leukocyte decrease (p=0.045), diagnosis delay (p=0.002), metastatic malignancy (p=0.035), and prior antibiotic use (p=0.030), beta-lactamase combined penicillin (p=0.040). Predictors of extending hospital stay included: female (p=0.014), anti-MRSA agents (p=0.049), low body weight (p=0.014), and long period of drainage (p=0.028). The mortality of empyema was low even without surgery. Length of hospital stay and antibiotic use were affected by multiple factors, need to be clarified by further studies.

Presenter's profile: 1st year student of Graduate School of Biomedical Sciences.

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Cholera outbreak caused by drug resistant *Vibrio cholerae* strains in Kenya

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Cholera is a major cause of mortality and morbidity in sub-Saharan countries in Africa including Kenya. In Kenya, the largest outbreak was occurred during 2007-2010 resulting 1,362 deaths among 26,901 cases. The Recent outbreak started from January 2015 and continued to moving in different counties of Kenya, which causes 178 deaths among 11,033 positive cases. The main objective of our study was to investigate the occurrence of *Vibrio cholerae* and their drug resistance pattern. A total of 464 diarrheal stool samples were received at the Institute of Tropical Medicine, Nagasaki University-Kenya Medical Research Institute, Nairobi, Kenya (NUITM-KEMRI) and microbiologically processed for the isolation of enteric pathogens using standard bacteriological methods (CDC, 1999). Of these, 252 samples were found cholera positive. Alkaline peptone water and thiosulphate citrate bile salt sucrose agar (TCBS) were used to isolate the *V. cholerae*. The isolates were identified with the help of colony morphology, Gram's staining, conventional biochemical testing, serotyping and biotyping. Antimicrobial susceptibility testing was performed by determining the minimum inhibitory concentration (MIC) using E-test strip. All isolated *V. cholerae* was confirmed to be *V. cholerae* serogroup O1. Maximum isolates were resistant to nalidixic acid (86.8%), and trimethoprim sulfamethoxazole (SXT) was found resistant (89.5%) until 2010 while current outbreak samples were found SXT sensitive. According to the antimicrobial susceptibility pattern of *V. cholerae* in our study, we recommend to use antibiotics among ceftriaxone, ciprofloxacin, doxycycline, gentamicin, and chloramphenicol for the preliminary treatment of cholera in Kenya.

Presenter's profile: Assistant Professor, Centre for Infectious Disease Research in Asia and Africa, Nagasaki University Institute of Tropical Medicine, Nagasaki, Japan

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Descriptive analysis of Post caesarean infection at Mibilizi Hospital, 2014

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[1,2,3] Mibilizi Hospital,

Objective: To describe the situation of post caesarean section surgical site infections at Mibilizi Hospital in Rwanda.

A retrospective cases series study was conducted at Mibilizi Hospital to describe the situation of post cesarean section surgical site infection during a period of 6 months (from January to June 2014).

A total of 1250 deliveries have been recorded and among them 599 deliveries (47.9%) were by caesarian sections and 651 deliveries (52.08%) were eutocic. Most of operated pregnant women were ranging from age of 18 – 42years old (mean age = 27 years old). 95.2% of operated and got infected were within the category of low socio economic status. The majority (61.9%) of infected women were primiparas. Among the 599 cesareans, 21 cases (3.5%) have had post caesarian surgical site infections. 90.5% of 21 cases got infection involving the superficial incised skin, deep fascial and muscle layers while 9.5% of 21 cases were superficial infection. The mean period of infection occurrence was on day 6 of their hospitalization. All infected women have to stay 25 days in hospital for follow-up and at discharging time, 1 patient (4.8%) had non improved post cesarean infected and was transferred to tertiary Hospital for further management. There was some associated conditions to the infection, but the most important was that most of infected wound was attributed to poor hygiene, and with emergency cesarean section (90.5%).

Conclusion: the analysis revealed that the number of performed caesarian section at Mibilizi hospital is higher than what WHO recommends (~15% of all deliveries), and the outcome was not satisfactory. Therefore, further studies are to be conducted to make further analysis and determine the risk factors associated.

Presenter's profile:: The presenter is a 1st Ph.D. Student under Leading Program at Nagasaki University, Institute of Tropical Medicine, in the department of International Health. But during the time of analysis he was the Director General of Mibilizi Hospital located in Rwanda (www.mibilizihospital.rw).

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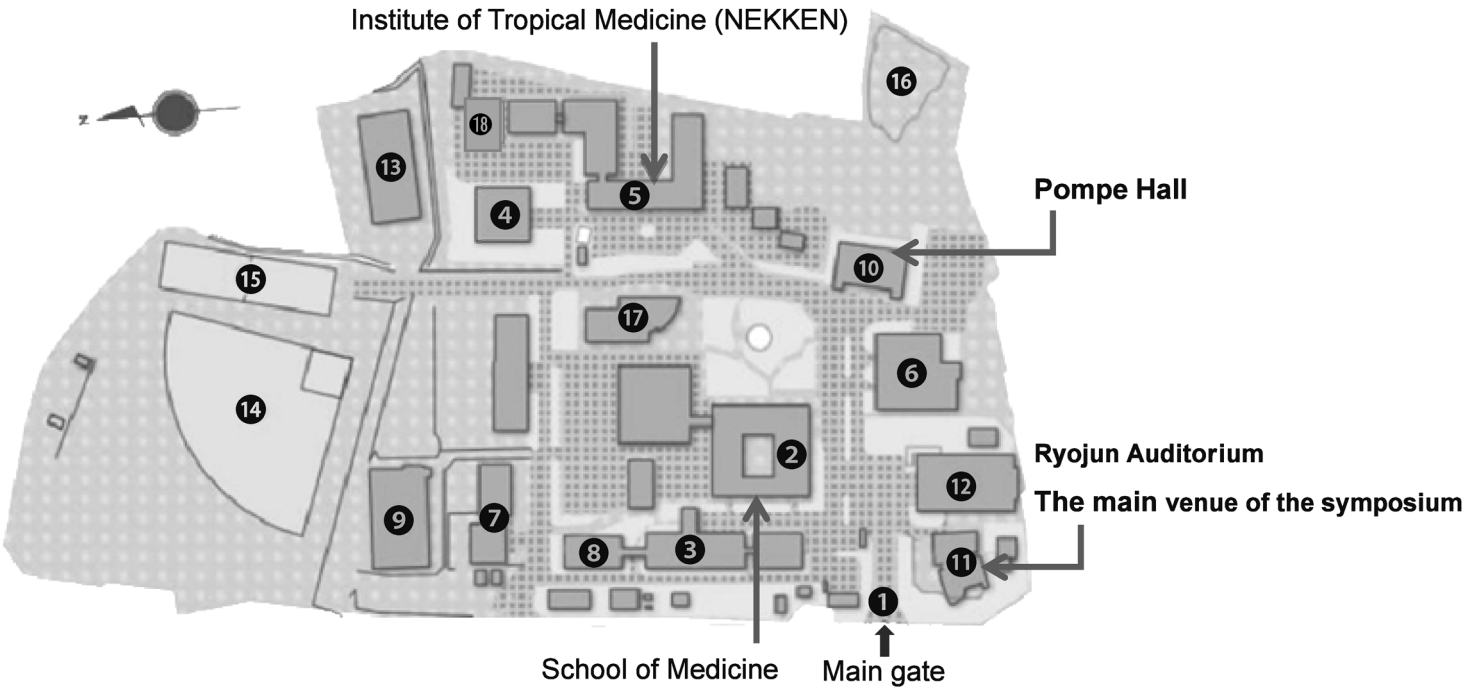
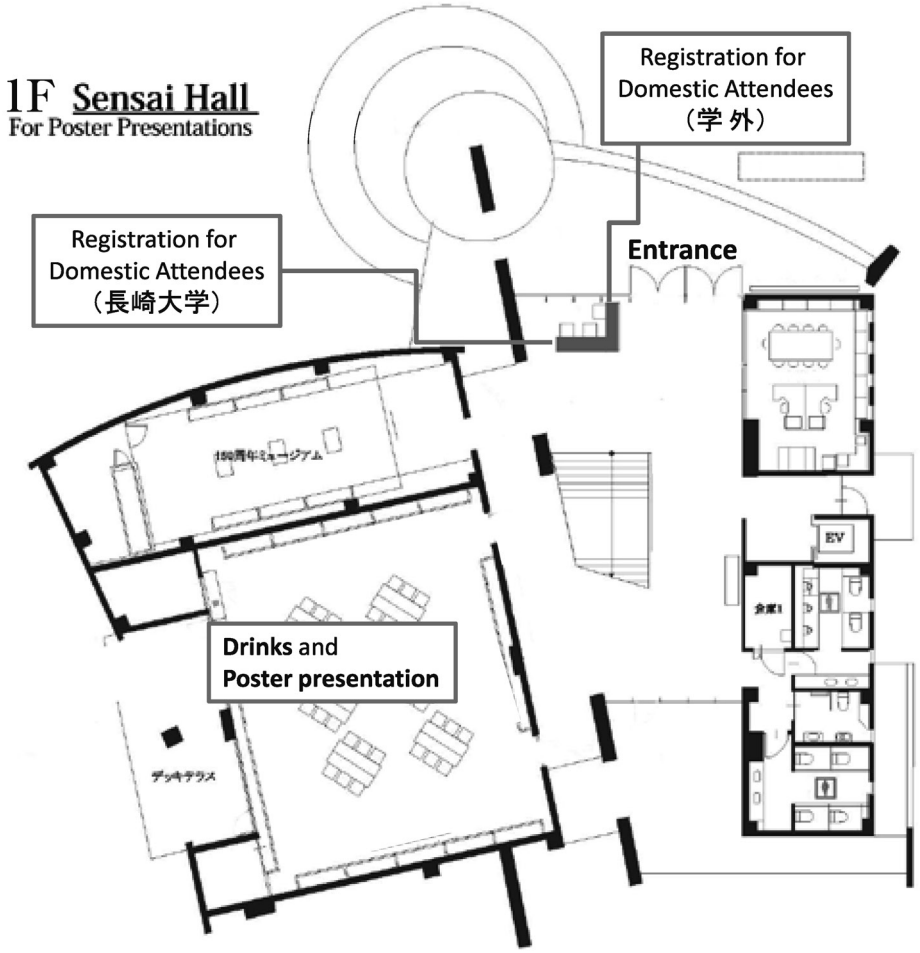
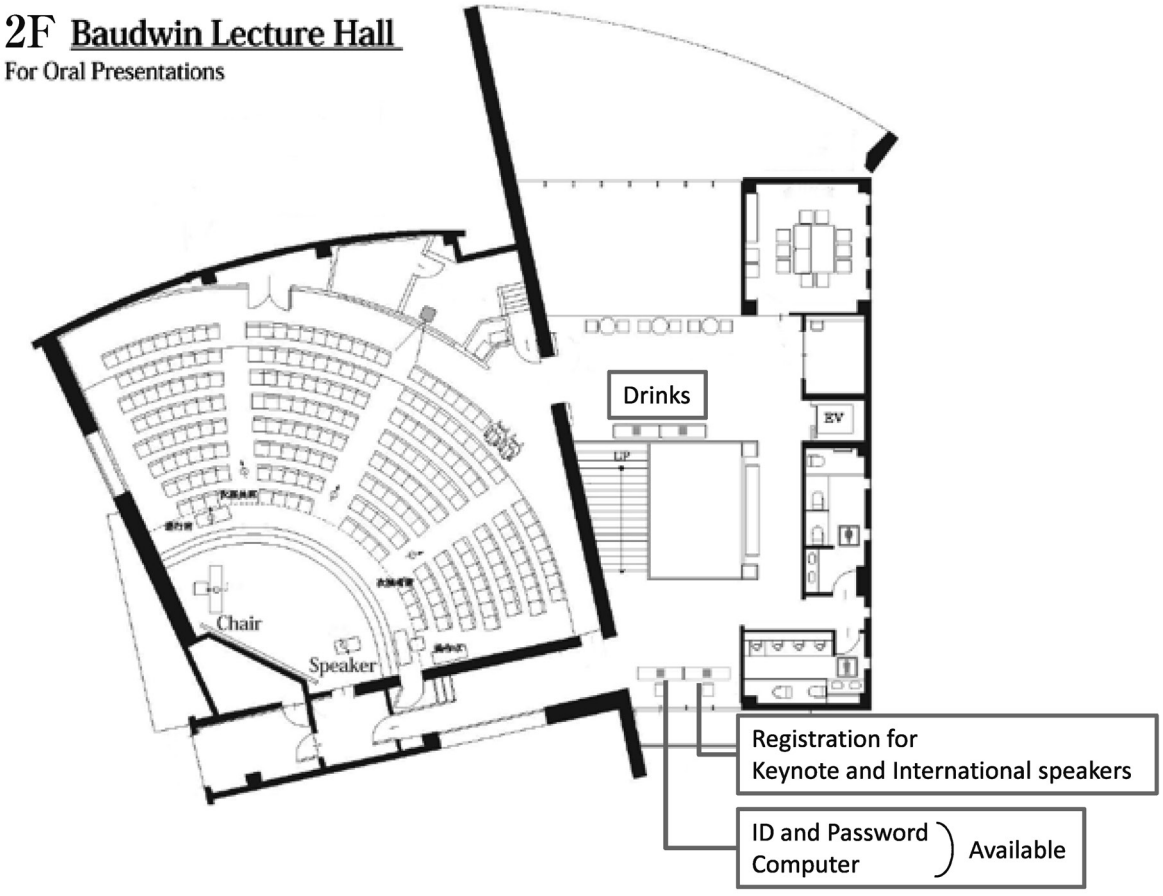
■Campus Map



❶ Main Gate	❷ School of Medicine	❸ Atomic Bomb Disease Institute
❹ Second Building of the Atomic Bomb Disease Institute	❺ Institute of Tropical Medicine (NEKKEN)	❻ Medical Library
❼ Center for Frontier Life Sciences (Radioisotope Research Center)	❽ Center for Frontier Life Sciences (Gene Research Center)	❾ Center for Frontier Life Sciences (Biomedical Research Center)
❿ Pompe Hall	⓫ Ryojun Auditorium	⓬ Commemoration Hall
⓭ Gymnasium	⓮ Athletic Ground	⓯ Tennis Court
⓰ Monument to the Atomic Bomb Victims Gubiroga Hill	⓱ Welfare Facilities (Cafeteria)	⓲ Global Health General Research Building

Ryojun Auditorium



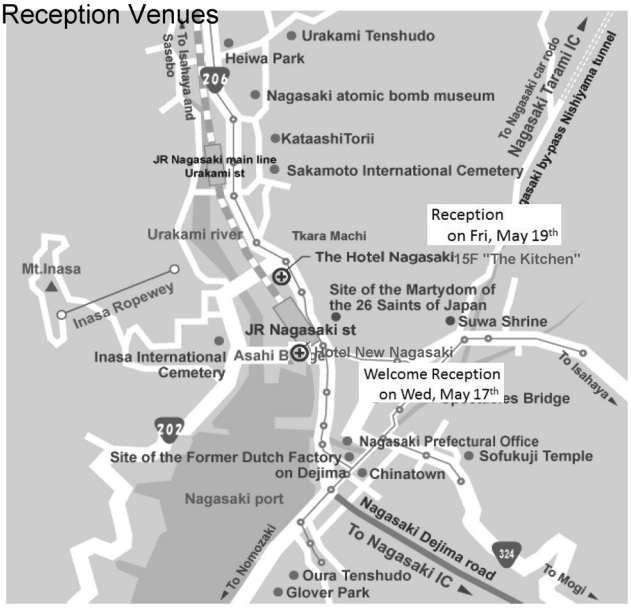


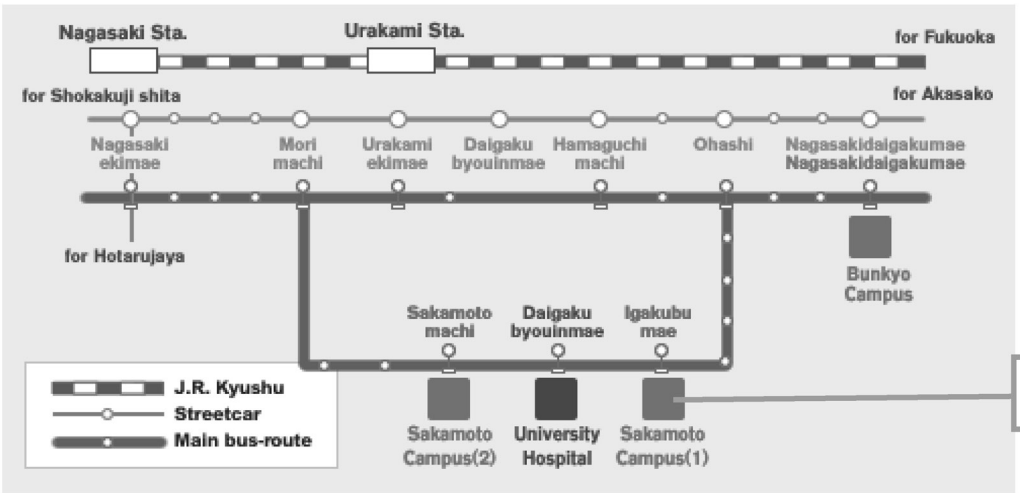
Traffic Access to Sakamoto Campus

From Hotel

From Hamaguchi machi

Reception Venues





1) BUS: Take a bus heading towards **SHIMO-OHASHI (No. 8)** and get off at **IGAKUBU MAE** (150yen).

2) RAM: From **NAGASAKI EKI MAE**, take a tram heading towards **AKASAKO** and get off at **HAMAGUCHI MACHI**(120yen). Then walk for 10 minutes.