The 6th Nagasaki Symposium

on Tropical and Emerging Infectious Diseases

The 11th Nagasaki-Singapore Medical Symposium

December 10th (Mon) - 12th (Wed), 2012
Ryojun Auditorium Nagasaki University Sakamoto Campus, Nagasaki, Japan

Supported by

Global COE Program
-Integrated Global Control Strategy for the Tropical and Emerging Infectious Diseases-

Program for Leading Graduate Schools
-Program for Nurturing Global Leaders in Tropical and Emerging Communicable Diseases-

JSPS Bilateral Joint Projects

Time	December. 10, Mon
8:00	Registration
9:10–9:20	Opening Remarks Shigeru Katamine (Nagasaki University)
9:20–9:30	Greetings from Singapore John Wong (National University of Singapore)
9:30–9:45	GCOE for Tropical and Emerging Infection Kenji Hirayama (NEKKEN, Nagasaki University)
9:45–10:30	William A Petri Jr. (University of Virginia, USA)
10:30–10:45	Tea Break at Sensai Hall
10:45–11:30	Kiyoshi Takeda (Osaka University)
11:30–12:00	Putting up Posters and Discussion
12:00–13:00	Lunch Time
13:00–13:20	Shinjiro Hamano (Nagasaki University)
13:20–13:40	Tetsu Yamashiro (Nagasaki University)
13:40–14:00	Ho Bow (National University of Singapore)
14:00–14:15	Tea Break at Sensai Hall
14:15–14:35	Osamu Nakagomi (Nagasaki University)
14:35–14:55	Sylvie Alonso (National University of Singapore)
14:55–15:15	Koya Ariyoshi (Nagasaki University)
15:15–15:35	Masaaki Kai (Nagasaki University)
15:35–15:50	Tea Break at Sensai Hall
15:50–16:10	Osamu Kaneko (Nagasaki University)
16:10–16:30	Kevin SW Tan (National University of Singapore)
16:30–16:50	Kenji Hirayama (Nagasaki University)
16:50–17:10	Katsuyuki Yui (Nagasaki University)
17:30–19:00	Poster Presentation at 1F Sensai Hall

Time	December. 11, Tue
8:30	Registration
9:00–9:45	Rogerio Amino (Pasteur Institute, France)
9:45–10:30	Alexander E Gorbalenya (Leiden University, Netherlands)
10:30–10:45	Tea Break at Sensai Hall
10:45–11:30	Matthias Frosch (University of Wurzburg, Germany)
11:30–12:15	Naoki Yamamoto (National University of Singapore)
12:15–13:15	Lunch Time
13:15–13:35	Volker Patzel (National University of Singapore)
13:35–13:55	Justin Jang Hann Chu (National University of Singapore)
13:55–14:15	Mary Mah Lee Ng (National University of Singapore)
14:15–14:30	Tea Break at Sensai Hall
14:30–14:50	Paul A Macary (National University of Singapore)
14:50–15:10	Yongliang Zhang (National University of Singapore)
15:10–15:30	Vincent TK Chow (National University of Singapore)
15:30–15:45	Tea Break at Sensai Hall
15:45–16:05	Paul A Tambyah (National University of Singapore)
16:05–16:25	Yee Joo Tan (National University of Singapore)
16:25–16:45	Gijsbert Marnix Grotenbreg (National University of Singapore)
16:45–17:05	Nobuhiro Yuki (National University of Singapore)
17:05–17:25	Daisuke Ishibashi (Nagasaki University)
19:00–21:00	Welcome Reception at Hotel New Nagasaki

Time	December. 12, Wed
8:30	Registration
9:00–9:45	Keith Arnold, MD FACP: (Retired from Roche Asian Research Foundation, Hong Kong) and Muoi M Arnold, MD FCAP:(Consultant Pathologist, Sutter Lakeside Hospital, USA) On behalf of Guoqiao Li (Guangzhou University)
9:45–10:30	Nicholas J White (Mahidol University, Thailand)
10:30–10:45	Tea Break at Sensai Hall
10:45–11:05	Richard Culleton (Nagasaki University)
11:05–11:25	Noboru Minakawa (Nagasaki University)
11:25–11:45	Ryuichiro Atarashi (Nagasaki University)
11:45–11:55	Conclusion Yoshiyuki Nagai (RIKEN Center of Research Network for Infectious Diseases)
11:55–12:00	Closing Remarks Tsutomu Takeuchi (Nagasaki University)

Day 1 : December 10th (MON)

Registration
08:00-09:10

Opening remarks
09:10-09:20 Shigeru Katamine (President, Nagasaki University)

Greetings from Singapore
09:20-09:30 John Wong (Vice Provost Academic Medicine, National University of Singapore)

Greetings from GCOE program leader
09:30-09:45 Kenji Hirayama (NEKKEN, Nagasaki University)
"The impacts of the GCOE program on Nagasaki University "

Keynote lecture 1.
(Chair: Shinjiro Hamano)
09:45-10:30 William A Petri Jr. (University of Virginia, USA)
"The effect of tropical enteropathy on vaccination"

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

Keynote lecture 2.
(Chair: Katsuyuki Yui)
10:45-11:30 Kiyoshi Takeda (Osaka University)
"Regulation of intestinal inflammation by innate immunity"

11:30-12:00 **Putting up posters and discussion at 1F Sensai Hall**

12:00-13:00 **Lunch Time**

Session 1.
(Chair: Toshiya Hirayama)
13:00-13:20 Shinjiro Hamano (NEKKEN, Nagasaki University)
"*Entamoeba moshkovskii* is associated with diarrhea in infants and causes diarrhea and colitis in mice."

13:20-13:40 Tetsu Yamashiro (NEKKEN, Nagasaki University)
"Integration of novel filamentous phages into the sites flanking the CTXΦ in the genome of *Vibrio cholerae*"

13:40-14:00 Ho Bow (National University of Singapore)
"Use of real-time phase contrast microscopy in the study of disruption of cell-cell junctions of gastric epithelial cells caused by *Helicobacter pylori*"

14:00-14:15 **Tea Break at 1F Sensai Hall**

Session 2.
(Chair: Hiroyuki Moriuchi)
14:15-14:35 Osamu Nakagomi (Nagasaki University)
"Molecular epidemiology of gastroenteritis viruses from the global perspective"

14:35-14:55 Sylvie Alonso (National University of Singapore)
"Development of a novel mouse model of EV71 infection"

14:55-15:15 Koya Ariyoshi (NEKKEN, Nagasaki University)
"What was accomplished in the Lampang HIV cohort in northern Thailand"

(Chair: Osamu Nakagomi)

15:15-15:35 Masaaki Kai (Nagasaki University)
"Inhibition of the epression of HIV-1 protease in CD4+ T cells owing to DNA aptamer-mediated specific delivery of siRNA"

15:35-15:50 **Tea Break at 1F Sensai Hall**

Session 3.

(Chair: Osamu Nakagomi)

15:50-16:10 Osamu Kaneko (NEKKEN, Nagasaki University)
"Deciphering the export signal of *Plasmodium falciparum* protein exported to the parasite-infected red blood cell"

16:10-16:30 Kevin SW Tan (National University of Singapore)
"Unicellular eukaryotic cell death: What *Plasmodium* and *Blastocystis* can teach us"

(Chair: Toshifumi Matsuyama)

16:30-16:50 Kenji Hirayama (NEKKEN, Nagasaki University)
"Novel malaria vaccine candidate PyTAM (*Plasmodium yoelii* GPI8p-transamidase related protein) confirmed by NANOBALL vaccine delivery system"

16:50-17:10 Katsuyuki Yui (Nagasaki University)
"Regulation of T cell responses during infection with *P. berghei* ANKA leading to the protective immunity and pathogenesis of cerebral malaria."

17:30-19:00 **Poster Presentation at 1F Sensai Hall**

Day 2 : December 11th (TUE)

Keynote lecture 3.
(Chair: Katsuyuki Yui)
09:00-09:45 Rogerio Amino (Pasteur Institute, France)
"In vivo imaging of *Plasmodium* life and death"

Keynote lecture 4.
(Chair: Koichi Morita)
09:45-10:30 Alexander E Gorbalenya (Leiden University, Netherlands)
"Why virus discovery effort should be expanded"

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

Keynote lecture 5.
(Chair: Toshifumi Matsuyama)
10:45-11:30 Matthias Frosch (University of Wurzburg, Germany)
"Deciphering evolution and virulence of the strictly human pathogen *Neisseria meningitides*"

Keynote lecture 6.
(Chair: John Wong)
11:30-12:15 Naoki Yamamoto (National University of Singapore)
"Unique association of high-risk human papilloma viruses (HPV) with breast carcinoma"

12:15-13:15 **Lunch Time**

Session 4.
(Chair: Vincent TK Chow)
13:15-13:35 Volker Patzel (National University of Singapore)
"Homologous SV40 RNA trans-splicing: a new mechanism for diversification of viral genotypes and phenotypes"

13:35-13:55 Justin Jang Hann Chu (National University of Singapore)
"Antiviral strategies to combat chikungunya virus infection."

13:55-14:15 Mary Mah Lee Ng (National University of Singapore)
"Non-structural functions of flavivirus capsid protein are important for virus replication."

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

Session 5.
(Chair: Shinjiro Hamano)
14:30-14:50 Paul A MacAry (National University of Singapore)
"The derivation and characterization of a therapeutic human antibody for dengue virus infection"

14:50-15:10 Yongliang Zhang (National University of Singapore)
"MAP kinase phosphatase 5 regulates immune response to influenza via IRF3."

15:10-15:30 Vincent TK Chow (National University of Singapore)
"Mice deficient in interferon regulatory factor-4 are more susceptible to infection with mouse-adapted influenza H3N2 virus than to PR8 H1N1 virus."

15:30-15:45 **Tea Break at 1F Sensai Hall**

Session 6.

(Chair: Shigeru Kohno)

15:45-16:05 Paul A Tambyah (National University of Singapore)
"Influenza epidemiology and control"

16:05-06:25 Yee Joo Tan (National University of Singapore)
"Targeting different viral proteins of the influenza A virus for the development of broad-spectrum antibody-based immunotherapy"

16:25-16:45 Gijsbert M Grotenbreg (National University of Singapore)
"Conditional ligands for Asian HLA products"

(Chair: Noriyuki Nishida)

16:45-17:05 Nobuhiro Yuki (National University of Singapore)
"Guillain–Barré syndrome and vaccination"

17:05-17:25 Daisuke Ishibashi (Nagasaki University)
"Protective role of host innate immune response against prion pathogen"

Reception

19:00-21:00 **Hotel New Nagasaki 3F Hoohkaku Room**
14-5, Daikoku-machi, Nagasaki-city,
Nagasaki 850-0057
TEL:095-826-8000

Day 3 : December 12th (WED)

Keynote lecture 7.
(Chair: Akira Kaneko)
09:00-09:45 Keith Arnold MD FACP: (Retired from Roche Asian Research Foundation, Hong Kong)
and Muoi Arnold MD FCAP:(Consultant Pathologist, Sutter Lakeside Hospital, USA)
On behalf of Guoqiao Li (Guangzhou University)
" Discovery of Artemisinin (qinghaosu) and Early Research by My Team"

Keynote lecture 8.
(Chair: Koya Ariyoshi)
09:45-10:30 Nicholas J White (Mahidol University, Thailand)
"Bridging laboratory science and the field in malaria research."

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

Session 7.
(Chair: Kenji Hirayama)
10:45-11:05 Richard Culleton (NEKKEN, Nagasaki University)
" Quantitative whole genome resequencing and genetic linkage analyses identify genes controlling medically important phenotypes of malaria parasites."

(Chair: Taro Yamamoto)
11:05-11:25 Noboru Minakawa (NEKKEN, Nagasaki University)
"Climate and malaria in western Kenya."

(Chair: Jiro Yasuda)
11:25-11:45 Ryuichiro Atarashi (Nagasaki University)
"Ultrasensitive human prion detection in cerebrospinal fluids using real-time quaking-induced conversion."

Conclusion
11:45-11:55 Yoshiyuki Nagai
(Director, RIKEN Center of Research Network for Infectious Diseases)

Closing Remarks
11:55-12:00 Tsutomu Takeuchi
(Dean, Institute of Tropical Medicine, NEKKEN, Nagasaki University)

Abstracts

Dec 10th (MON) 9:45-10:30

Keynote Lecture 1

The Effect of Tropical Enteropathy on Vaccination

Evan Newell, Rashidul Haque, Brian Kidd, Natalia Sigal, Mark M. Davis,
William A. Petri, Jr., M.D., Ph.D.

University of Virginia

Oral live virus vaccines are less effective in children in the developing world. Greater than 95% of children with wild poliovirus infection in India reported having received more than three doses, and 77% more than 7 doses of trivalent OPV (1,2). Similarly, oral rotavirus vaccine (RotaTeq) was only 58% effective at preventing severe rotavirus infection in Nicaragua, compared to > 98% in Finland (3); the vaccine was 49% effective in Malawi and 77% in South Africa (4). Hypotheses for this lower efficacy of oral vaccination in developing countries include tropical enteropathy, malnutrition and interference from maternal antibodies (5). Reasons found not to explain this include lack of a cold chain (resulting in damage to the live virus vaccine) or interference by infection with endogenous enteroviruses (6) or co-administered OPV and rotavirus vaccines.

Tropical enteropathy is common in children in the developing world (8). It is pathologically characterized by villous shortening with increased intraepithelial lymphocytes in the small intestine (9). Tropical enteropathy is commonly measured by increased lactulose absorption (due to increased intestinal mucosal permeability) and decreased mannitol absorption (due to decreased intestinal surface area). Intestinal inflammation is hypothesized to underlie tropical enteropathy, with observed increases in gut CD3+CD69+, CD3+HLADr+ and TNFa and IFNg producing cells (10). Another measure of the increased mucosal permeability is endotoxin in the circulation, or the presence of anti-endotoxin (endocab) antibodies resulting from that exposure (11). There is extremely limited data on the effectiveness of oral vaccines in children with tropical enteropathy. The oral poliovirus vaccine (OPV) is less effective if given during episodes of diarrhea, with the geometric mean antibody response only 50% of that of children without diarrhea (12).

We hypothesized that failure of orally administered vaccines such as OPV were due to tropical enteropathy and resultant inflammation from endotoxin exposure. We tested this hypothesis in a cohort of children followed from birth, starting in January 2008, in an urban slum of Dhaka Bangladesh. The majority of newborns were from poor families as indicated by the median household expenditure of <6000 BDT (< $100 USD) per month; in addition, 40% of the mothers had no education. The average family size was 5, ranging from 3 to 14. The vast majority of households had access to municipal water supplies and used safe food handling practices. Nineteen percent of the mothers were underweight with a BMI of <18.5. Poor nutritional status was common at birth and increased by one year of age (measured both by underweight [WAZ] and stunted [HAZ]). At birth 28.6% and 16.3% of the newborns had WAZ and HAZ scores of <-2 respectively, worsening at 12 months of age to 34.0% and 42.4% of the children underweight or stunted, respectively. The total number of diarrheal episodes suffered by children in the first year of life was 4.7 episodes per child per year. Protozoa, rotavirus and ETEC were the most common pathogens isolated from the diarrheal stool specimens.

Response to oral poliovirus vaccine was measured in the children who had received the Expanded Program on Immunization (EPI) recommended minimum of three doses of OPV by age 6 months. Vaccine response at 6 and 12 months of age was measured by the titer of serum neutralizing antibodies. Vaccine failure was defined as a titer of less than 1/8 ($\log_2$ 3).

OPV failure (titer < $\log_2$ 3) and diminished responses to OPV were associated with malnutrition (HAZ or WAZ ≤ -2), serum endocab antibodies, shorter breastfeeding duration, systemic inflammation and hypo-responsiveness of the immune system, but not vitamin A levels (as measured by retinol binding protein). Antibody titers to the systemically-administered vaccines measles, diptheria and tetanus were not associated with any of these factors.

We concluded that the block to mucosal OPV, but not systemic vaccination, was associated with indicators of tropical enteropathy, including malnutrition, serum markers of tight junction dysfunction, systemic inflammation and a hyporesponsive immune system. Encouragingly, prolonged exclusive breastfeeding was associated with a better response to OPV and may represent a positive means to intervene.

Dec 10 (MON) 10:45-11:30

Keynote Lecture 2

Regulation of intestinal inflammation by innate immunity

Kiyoshi Takeda

Department of Microbiology and Immunology, Graduate School of Medicine, Osaka University

Innate immunity has been shown to control antigen-specific adaptive immune responses. In addition, abnormal activation of innate immunity, due to the breakdown of negative regulatory mechanisms, causes development of several inflammatory disorders, including inflammatory bowel diseases. Therefore, activity of innate immunity is finely regulated at the intestinal mucosal surfaces. Intestinal mucosa is a unique site, where many numbers of symbiotic microbiota exist. In the intestinal mucosa, there are several unique subsets of innate immune cells, which orchestrate a peculiar immune response. For example, intestinal $CD103^{+}$ dendritic cells have been shown to instruct development of regulatory T cells in the mesenteric lymph nodes and the lamina propria. We previously identified $CD70^{+}$ dendritic cells that show microbiota-dependent induction of Th17 cells in the lamina propria. In addition, we recently identified a unique intestinal $CX3CR1^{high}$ $CD11b^{+}$ $CD11c^{+}$ cell subset, which we have named regulatory myeloid (M_{reg}) cells, that is responsible for prevention of intestinal inflammation through inhibition of T cell responses. These cells inhibit $CD4^{+}$ T cell proliferation in a cell contact-dependent manner, and prevent T cell-dependent colitis. The suppressive activity of M_{reg} cells is abrogated in the absence of the IL-10/Stat3 pathway. Thus, activity of several unique subsets of innate immune cells is responsible for maintenance of intestinal homeostasis.

Entamoeba moshkovskii is Associated with Diarrhea in Infants and Causes Diarrhea and Colitis in Mice

Shinjiro Hamano [1, 2]

[1]Department of Parasitology, Institute of Tropical Medicine (NEKKEN),
[2]Global COE program, Nagasaki University, Nagasaki 852-8523, Japan

Entamoeba moshkovskii is prevalent in developing countries and morphologically indistinguishable from pathogenic *Entamoeba histolytica* and non-pathogenic *Entamoeba dispar*. However, it is not known if *E. moshkovskii* is pathogenic. The purpose of the study was to elucidate the pathogenicity of *E. moshkovskii.*

Mice were intracecally challenged with the trophozoites of each *Entamoeba* spp. to test the ability to cause diarrhea, and infants in Bangladesh were prospectively observed to see if newly acquired *E. moshkovskii* infection was associated with diarrhea.

E. moshkovskii and *E. histolytica* caused diarrhea and weight loss in susceptible mice. *E. dispar* infected none of the mouse strains tested. In Mirpur, Dhaka, Bangladesh, *E. moshkovskii, E. histolytica* and *E. dispar* were identified in 42 (2.95%), 66 (4.63%) and 5 (0.35%) respectively out of 1426 diarrheal episodes in 385 children followed prospectively from birth to one year of age. Diarrhea occurred temporally with acquisition of a new *E. moshkovskii* infection: in the two months preceding *E. moshkvskii*-associated diarrhea, 86% (36/42) of monthly surveillance stool samples were negative for *E. moshkovskii.*

E. moshkovskii was found to be pathogenic in mice. In children, the acquisition of *E. moshkovskii* infection was associated with diarrhea. These data are consistent with *E. moshkovskii* causing disease, indicating that it is important to re-examine its pathogenicity.

Session 1-2

Dec 10 (MON) 13:20-13:40

Integration of novel filamentous phages into the sites flanking the CTXΦ in the genome of *Vibrio cholerae*

Nguyen Dong Tu[1], Ngo Tuan Cuong[1], Nguyen Binh Minh[1], Tetsu Yamashiro[2, 3, 4], and Masahiko Ehara[2]

1: Department of Bacteriology, National Institute of Hygiene and Epidemiology

2: Institute of Tropical Medicine, Nagasaki University, Vietnam Research Station Nagasaki University, J-GRID

3: Institute of Tropical Medicine, Nagasaki University

4: Global Center for Excellence, Nagasaki University

Vibrio cholerae, the causative agent of cholera, is a gram-negative bacterium that is able to colonize the human small intestine, where it secrets cholera toxin. Several filamentous phages specific for *V. cholerae* have been described previously, and the most well-studied of these phages is CTXϕ, which plays a crucial role in the pathogenicity of *V. cholerae*, since it carries the genes that encode cholera toxin (Waldor et al. Science 272: 1910-1914, 1996). The isolation of filamentous phage fs1 and fs2 from *V. cholerae* O139 epidemic strains were first reported in 1997 (Ehara et al. FEMS Microbiol. let. 154, 1997). They are long slender tubes that range from 0.8 to 2 μm in length and are about 6 nm in diameter. The particles each contain a single-stranded circular DNA genome that is converted to a double-stranded replicative form in infected cells. In a survey in Nam Dinh province (northern Vietnam) in 2010, a *V. cholerae* O139 strain was first isolated in Vietnam from an environmental water sample which tested positive for genes relevant to *V. cholerae*. This strain was in lysogenic status harboring a genome similar to that of the filamentous phage fs1. The phage, ND1-fs1, had a structure similar to those of fs1, VGJϕ, and VSK phages and carries *att*-like site which may function as a site for integration. The ND1-fs1 phage is estimated to integrate into the region between the core region of CTXϕ and *rtxA* region of the host *V. cholerae* O139 strain.

Use of Real-Time Phase Contrast Microscopy in the Study of Disruption of Cell-Cell Junctions of Gastric Epithelial Cells Caused by *Helicobacter pylori*

Alvin Soon Huat Wee[1,2] and Bow Ho[1,2]

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

[2]Mechanobiology Institute, National University of Singapore, Republic of Singapore

Helicobacter pylori has been reported to disrupt cell-cell junction of gastric epithelial cells. Among the various virulence factors of *H. pylori*, cytotoxin-associated gene A (CagA) has been regarded as the main player in the disruption process. However, the exact mechanism of tight junction disruption caused by the bacteria is still not well-established. In the study, *H. pylori* 26695 wildtype or Δ*cag*A were used to infect MKN28 cells which form functional tight junctions. The real-time event of disruption of tight junctions of the gastric cells caused by respective *H. pylori* strains was recorded over a period of 44 hours using Nikon Biostation IM-Q, a high resolution imaging system that is able to maintain perfect focus and optimal growth conditions for cells. The images were then analyzed using ImageJ software. Results show that the tight junctions in uninfected MKN28 cells remained intact up till the end of recording period. Interestingly, tight junction disruption as observed in wildtype-infected and Δ*cag*A-infected cells occurred at the similar rate, implying that CagA may not be the major virulence factor in the disruption of cell-cell junctions. The process of tight junction disruption as shown by the real-time microscopy observations was furhter supported by results obtained from barrier function test. Our findings show that real-time microscopy can provide a highly supportive role in illuminating the mechanistic events occurring during host-pathogen interactions. The implications of tight junction disruption on the physical environment and its relationship to biological events will be discussed.

Session 2-1

Dec 10 (MON) 14:15-14:35

Molecular epidemiology of gastroenteritis virus from the global perspective

Osamu Nakagomi

Department of Molecular Microbiology and Immunology,
Graduate School of Biomedical Sciences, Nagasaki University

While there are five established gastroenteritis viruses and many others are emerging as potential gastroenteritis viruses, rotaviruses and noroviruses have been recognized as the two most important pathogens, responsible for 40-50% and 15-20% of severe acute gastroenteritis cases occurring children less than 5 years of age worldwide. Recent advances of molecular techniques including next-generation sequencing and real-time reverse-transcription PCR together with the widespread use of rotavirus vaccines in both developing and developed countries have begun to make a great impact on our understanding of molecular epidemiology of these gastroenteritis viruses. Two most important issues are: (1) Will noroviruses fill the vacuum created after the introduction of rotavirus vaccine and become the most important pathogen causing severe acute gastroenteritis in children? (2) What impact will rotavirus vaccine make on the distribution of circulating strains of rotaviruses? To address these questions we set up surveillance activities in Hai Phong, Nha Trang and Thai Binh, Vietnam, Recife, Brazil, Kathmandu, Nepal, and Blantyre, Malawi with various collaborators. Most of our own observations were related to the use of the monovalent vaccine, and it became evident that this vaccine favoured the increase in the prevalence of wild-type strains heterotypic to the vaccine virus, yet more importantly that the vaccine substantially reduced the absolute number and proportion of rotaviruses that caused severe gastroenteritis in children. I will also discuss the remaining issues and challenges that we are facing from the view point of science as well as global public health.

Development of a Novel Mouse Model of EV71 Infection

Sylvie ALONSO

Department of Microbiology, Yong Loo Lin School of Medicine and Immunology Programme, Life Sciences Institute, National University of Singapore

Enterovirus 71 (EV71) is a neurotropic pathogen that has been consistently associated with the severe neurological forms of Hand, Foot and Mouth Disease. The lack of a relevant animal model has hampered our understanding of EV71 pathogenesis, in particular the route and mode of viral dissemination. It has also hindered the development of effective prophylactic and therapeutic approaches, making EV71 one of the most pressing public health concerns in Southeast Asia.

We have developed a novel mouse model of EV71 infection. We show that two-week-old and younger immunodeficient AG129 mice, which lack type I and II interferon receptors, are susceptible to the infection with a non mouse-adapted EV71 strain via both the intraperitoneal (ip.) and oral route of inoculation. The infected mice displayed progressive limb paralysis prior to death. Dissemination of the virus was dependent on the route of inoculation, but eventually resulted in virus accumulation in the central nervous system (CNS) from both animal groups, indicating a clear neurotropism of the virus. Histopathological examination revealed massive damage in the limb muscles, brainstem and anterior horn areas. We have extended our study with various EV71 strains from different genogroups and our results indicated that the ability to reach and accumulate in the CNS correlates with the limb paralysis. Thus our results support the hypothesis that limb paralysis is a consequence of EV71 neuroinvasion and not a direct viral cytopathic effect in the limb muscle. Together, our observations support that young AG129 mice display polio-like neuropathogenesis upon infection with non-mouse adapted EV71 strains, making this mouse model relevant for EV71 pathogenesis study and an attractive platform for EV71 vaccine and drug testing.

Session 2-3

Dec 10 (MON) 14:55-15:15

What was accomplished in the Lampang HIV cohort in northern Thailand

Koya Ariyoshi MD, DTMH, PhD

Department of Clinical Medicine, Institute of Tropical Medicine (NEKKEN), Nagasaki University

The Lampang HIV cohort was commenced at Lampang Hospital, northern Thailand, in July 2000 and the whole field activity was completed in December 2010. The objective of this talk is to introduce the scientific achievement and human-resource development accomplished from this cohort.

The Lampang HIV cohort initially began as a natural HIV history cohort with 756 HIV-infected patients, which investigated the natural course of HIV-infection determining death as the end-point. Since the National Access to Antiretroviral Program for People with HIV/AIDS (NAPHA) introduced generic anti-retroviral (ARV) drug therapy (combined tablet of NVP, 3TC, d4T called GPOvir®), the cohort was continued as GPOvir cohort with 979 patients on ARV therapy, which investigated the ARV treatment outcome of HIV-infected patients, whereas those who remain healthy and do not need to receive any ARV drug therapy, were followed up as CTL cohort, which investigate Cytotoxic T-Lymphocyte (CTL) responses among these HIV viral load well-controllers. Moreover, one of strengths of the cohort was that we recruited a large number of HIV-affected couples, including 185 HIV-serology discordant couples.

The Lampang HIV cohort has played an essential role in establishing International Research Collaboration Network with a number of prominent Japanese scientists, creating numerous research opportunities for both Thai and Japanese researchers and students, ranging from clinical and socio-behavioral studies, host genetic polymorphisms studies, to virology and immunology studies. Consequently it led to over 20 internationally recognized publications and 7 PhD degrees.

Inhibition of the Expression of HIV-1 Protease in CD4+ T cells owing to DNA aptamer-mediated specific delivery of siRNA

Takayuki Shibata[1], Qinchang Zhu[1], Tsutomu Kabashima[1], Masaaki Kai[1, 2]

[1] Faculty of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Nagasaki University, 1-14, Bunkyo-Machi, Nagasaki 852-8521, Japan

[2] Global Center of Excellence Program, Nagasaki University

RNA interference-based gene therapy is a promising treatment for a variety of diseases. However, delivery of small interfering RNA (siRNA) into specific tissues and cells is still a hurdle. Aptamer has been exploited as a quality vehicle for the delivery due to its highly specific binding to the target molecule. For siRNA delivery, DNA aptamer has not been used, although several RNA aptamers have been reported. Compared to RNA aptamer, DNA aptamer has some potential advantages, such as higher chemical stability, easier synthesis, and lower possibility to induce interferon response. In this study, we show that a DNA aptamer could be used to deliver siRNA into $CD4^+$ T cells specifically. The DNA aptamer was obtained from the conversion of a reported RNA aptamer that binds to CD4 protein on the surface of T cells. It was covalently conjugated to the sense strand of the siRNA targeting HIV-1 protease (HIV-PR).The cellular binding and uptake of this resulting DNA aptamer-siRNA chimera (DAS) was analyzed with fluorescence microscopy. The gene silencing effect of DAS was evaluated by real-time RT-PCR. And its stability in the serum was analyzed with polyacrylamide gel electrophoresis stained with SYBR Glod. Results showed that DAS could specifically enter into $CD4^+$ T cells and efficiently knock down the expression of exogenous HIV-PR gene. This study provides the first evidence that the DNA aptamer with intrinsic stability has a greater potential to be used for siRNA delivery.

Session 3-1

Dec 10 (MON) 15:50-16:10

Deciphering the export signal of *Plasmodium falciparum* protein exported to the parasite-infected red blood cell

Xiao-tong Zhu, Kazuhide Yahata, Jean SF Alexandre, Osamu Kaneko

Department of Protozoology, Institute of Tropical Medicine, Nagasaki University

Plasmodium falciparum SURFIN is a type one transmembrane (TM) protein encoded by a *surf* gene family. SURFINs show a sequence similarity with known parasite proteins that are exported to the parasite-infected red blood cell (iRBC) surface; the extracellular cysteine-rich domain (CRD) with *P. vivax* VIR protein and the intracellular tryptophan-rich domain (WRD) with *P. falciparum* PfEMP-1. One member, $SURFIN_{4.2}$ was shown to be located on the iRBC surface and Maurer's clefts (MCs), however, another member, $SURFIN_{4.1}$, was reported not to be exported to iRBC. In order to clarify what determine this difference, we re-evaluated the gene structure and the protein location of $SURFIN_{4.1}$ by using a transgenic *P. falciparum* parasite expressing a recombinant $SURFIN_{4.1}$. By comparing gDNA and cDNA, we detected an earlier internal stop codon, resulting $SURFIN_{4.1}$ in one line had only one WRD, and in 3 *P. falciparum* lines, $SURFIN_{4.1}$ had only 19 amino acids cytoplasmic tail (Cyt) after the TM region. Unexpectedly full length of 3D7 type recombinant $SURFIN_{4.1}$ fused with tag proteins was detected in the MCs. We found that TM region was essential for exportation and together with N-terminal 50 amino acids and Cyt, recombinant protien was targeted to MCs. Further analysis detected more than one export motifs within N-terminal, which were distinct from known "*Plasmodium* export element (PEXEL)" motif. Replacement of TM or Cyt by that of unrelated proteins dramatically reduced export efficiency. These results suggest that N-terminal, TM and Cyt regions contain critical information for the efficient export of $SURFIN_{4.1}$ to iRBC, which is independent from PEXEL motif.

S3

Unicellular Eukaryotic Cell Death: What *Plasmodium* and *Blastocystis* Can Teach Us

Kevin SW TAN,[1] Jun Hong CH'NG,[1] Amanda GOH,[1] Yan Quan LEE,[1] Su Peng YEO,[1] Wendy WU,[1] Bruce RUSSELL,[1] François NOSTEN,[2] Laurent RENIA[3]

[1]Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, [2]Shoklo Malaria Research Unit, Mahidol, Maesot, Thailand, [3]Singapore Immunology Network (SIgN), A*STAR, Singapore

Programmed cell death (PCD) in unicellular Eukaryotes is increasingly accepted as a *bona fide* process that occurs, in this group of microbes, during growth, differentiation and in response to undesirable stimuli. Our laboratory has observed both conserved and unique PCD features between mammalian systems and the unicellular protists *Blastocystis* and *Plasmodium*, causative agents of blastocystosis and malaria respectively. Specific inhibition of *Blastocystis* surface legumain triggers a rapid apoptosis-like death involving mitochondrial dysregulation, caspase-like activity and DNA degradation. Interestingly, this molecule may also play a role in *Blastocystis* pathogenesis. Malaria PCD pathways are also apoptosis-like and may be triggered by lysosomotropic drugs that breach the parasite digestive vacuole (DV) membrane. In our laboratory, the features of malaria parasite cell death have been exploited in a variety of ways. Firstly, the distinct PCD features of drug-induced parasites can be quantified and used to generate a Cell Death Index (CDI), a tool to classify antimalarial drug action and to derive useful drug combinations. Secondly, fluorescent probes that indicate DV stability have been used to screen compounds that specifically breach this organelle, resulting in PCD. Finally, the use of fluorescent lysosomotropic drug chloroquine has been developed into a novel, rapid screening tool for the identification of compounds that reverse drug resistance in malaria parasites.

References:

1. Ch'ng JH, Renia L, Nosten F, Tan KSW (2012) Can we teach an old drug new tricks? Trends Parasitol. 28:220-224.
2. Ch'ng JH, Liew K, Goh ASP, Sidhartha E, Tan KSW (2011) Drug-induced permeabilization of parasite's digestive vacuole is a key trigger of programmed cell death in *Plasmodium falciparum*. Cell Death Dis. 2 e216.
3. Ch'ng JH, Kotturi SR, Chong AGL, Lear MJ and Tan KSW (2010) A programmed cell death pathway in the malaria parasite *Plasmodium falciparum* has general features of apoptosis but is mediated by clan CA proteases. Cell Death Dis. 1 e26.
4. Wu BH, Yin J, Texier C, Roussel M and Tan KSW (2010) *Blastocystis* legumain is localized on the cell surface and specific inhibition of its activity implicates a pro-survival role for the enzyme. J. Biol. Chem. 285:1790-1798.

Acknowledgment: This work was supported by generous grants from the National Medical Research Council (NMRC/1071/2006, NMRC/1310/2011 and NMRC/EDG/1038/2011) and National Research Foundation (NRF). LYQ is an NGS Graduate Scholarship recipient.

Session 3-3

Dec 10 (MON) 16:30-16:50

Novel malaria vaccine candidate PyTAM (*Plasmodium yoelii* GPI8p-transamidase related protein) confirmed by NANOBALL vaccine delivery system

Hirayama K, Shuaibu MN, Cherif MS, Kurosaki T, Kodama K, Tsuboi T, Helegbe G, Kikuchi M, Yanagi T, Yui K. and Sasaki H.

Department of Immunogenetics and Global COE Program, Institute of Tropical Medicine (NEKKEN), Nagasaki University,
Department of Hospital Pharmacy, Nagasaki University Hospital,
Cell-Free Science and Technology Research Center, Ehime University
Animal Research Centre for Tropical Infections, NEKKEN,
Division of Immunology, Department of Molecular Microbiology and Immunology, Nagasaki University

Candidate gene screening by using web-based bioinformatics: 29 genes from the annotated Plasmodium yoelii genome sequence (www.PlasmoDB.org and www.tigr.org) were identified as encoding GPI-anchored proteins. Target genes were those with orthologues in P. falciparum.

Screening for protective DNA vaccine in mouse system: Focusing on the blood stage, we obtained PCR amplified cDNAs from mRNA of the infected RBCs, and eventually cloned nine of these into a DNA vaccine plasmid, pVAX 200-DEST. Immunization with one gene (PY03470) resulted in a significantly increased survival (50%) compared to other vaccinee mice although the antibody was marginal. Recombinant protein from this gene, GPI8p transamidase-related protein (PyTAM) emulsified with GERBU adjuvant was also used to immunize another set of C57BL/6 mice to increase its immunogenicity. Though much higher antibody response was obtained, all were dead with 2 days delay of onset of parasitaemia suggesting DNA vaccine had some advantage.

Application of a NANOBALL delivery system: Plasmodium yoelii MSP-1 C-terminal DNA vaccine was formulated with newly designed nanoparticle—an anionic ternary complex of polyethylene imine and γ-poly glutamic acid (pVAX-MSP-1/PEI/γPGA), and intraperitonealy vaccinated by 100μg, three times at 3-week interval. The mice were subsequent challenged with 10^5 P. yoelii-infected red blood cells revealed 100% survival in repeated experiments.

Confirmation of TAM as a strong candidate vaccine: NANOBALL-coated PyTAM-DNA vaccinated mice were able to resolve parasitaemia from challenge infection and showed 100% survival. Before challenge, the vaccinees' plasma levels of TAM specific lgG1 and lgG2b were significantly high. They produced IL-4, IFN-g and IL-12 in the plasma as well as in the supernatant after the antigen stimulation of spleen cells.

Using the mouse antiserum for PyTAM, TAM protein was revealed to be expressed in the schiszont stage parasite and located on the parasitophorus vacuoles.

Conclusions: We found the NANOBALL vaccine had a potential to strengthen the immunogenicity of already abandoned DNA vaccine. With NANOBALL we found a brand new candidate vaccine molecule TAM expressed in the blood stage.

References

1. Cherif MS, Shuaibu MN, Kurosaki T, Helegbe GK, Kikuchi M, Yanagi T, Tsuboi T, Sasaki H, Hirayama K. Immunogenicity of novel nanoparticle-coated MSP-1 C-terminus malaria DNA vaccine using different routes of administration. Vaccine. 2011 Nov 8;29(48):9038-50. Epub 2011 Sep 20.
2. Shuaibu MN, Kikuchi M, Cherif MS, Helegbe GK, Yanagi T, Hirayama K Selection and identification of malaria vaccine target molecule using bioinformatics and DNA vaccination. Vaccine. 2010 Oct 4;28(42):6868-75. Epub 2010 Aug 13.
3. Kurosaki T, Yamashita Y, Aki K, Harasawa H, Nakagawa H, Kodama Y, Higuchi N, Nakamura T, Kitahara T, Sasaki H. Secure and effective gene vector of polyamidoamine dendrimer pharmaceutically modified with anionic polymer. J Pharm Sci, 2011, 100 (4855-63)doi: 10. 1002/jps. 22701

Regulation of T cell responses during infection with *Plasmodium berghei* ANKA leading to the protective immunity and pathogenesis of cerebral malaria

Kimura Daisuke[1], Mana Miyakoda[1], Kazumi Kimura[1], Masao Yuda[3], Katsuyuki Yui[1, 2]

[1]Division of Immunology, Department of Molecular Microbiology and Immunology, Graduate School of Biomedical Sciences, [2]Global COE program, Nagasaki University, [3]Department of Medical Zoology, School of Medicine, Mie University

We generated *Plasmodium berghei* ANKA expressing OVA (PbA-OVA) and have been investigating the mechanisms underlying the modulation of specific T cell-responses during infection with malaria parasites. In the first part, we will report $CD8^+$ T cell responses during infection with PbA-OVA using T cell receptor transgenic mice, OT-I. We show that specific $CD8^+$ T cells are activated during infection with PbA-OVA by cross-presentation, and are involved in the pathogenesis of cerebral malaria. We also show that long-lived memory $CD8^+$ T cells can be generated after infection with PbA-OVA, but their responses are more profoundly perturbed than those of naive $CD8^+$ T cells during recall responses. In the second part, we will report our study on $CD4^+$ T cell responses. $CD4^+$ T cells from PbA-infected mice produce reduced levels of IL-2. Our study indicated that $CD4^+$ T cells from PbA-infected mice produce potent inhibitory molecule, which is EBI3-related cytokine. EBI3 forms IL-27 and IL-35, IL-12 family cytokines, by pairing with p19 and p35, respectively. $CD4^+$ T cells from PbA-infected EBI3 KO mice did not produce inhibitory cytokines, implying the involvement of EBI3 in the inhibition of IL-2 production. TCR β KO mice that were transferred with EBI3 KO $CD4^+$ T cells showed improved resistance to the PbA-infection when compared with their control. These results suggest that malaria-specific $CD4^+$ T cells produce an inhibitory $EBI3^+$ cytokine and regulate their protective immune responses during infection with PbA.

Dec 11th (TUE) 9:00-9:45

Keynote Lecture 3

In vivo imaging of *Plasmodium* life and death

Rogerio Amino

Unit of Malaria Biology and Genetics, Institut Pasteur - Paris

Malaria starts with the inoculation of *Plasmodium* sporozoites in the host skin by the bite of an infected mosquito. After invading skin blood vessels, sporozoites arrest in the liver, where they invade and develop, as a liver-stage, inside hepatocytes. The liver stage then generates and releases thousands of merozoites in to the blood circulation, where they repetitively invade erythrocytes causing the symptoms of the disease.

Using *in vivo* imaging techniques in a rodent malaria model, we are analyzing the host and parasite determinants implicated in *Plasmodium berghei* survival in the skin and liver of mice. Our goal is to define these determinants at cellular and molecular levels, focused on the requirements needed to hinder the progression and development of the parasite in these tissues. I will present and discuss the escape mechanisms used by the parasite to evade the host innate immunity in the liver sinusoids and the strategies that we are developing to eliminate the plasmodial liver-stage via the host acquired immunity.

Keynote Lecture 4

Why virus discovery effort should be expanded

Alexander E. Gorbalenya[1,2]

[1]Department of Medical Microbiology, Leiden University Medical Center, P.O. Box 9600, E4-P, 2300 RC Leiden, The Netherlands

[2]Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, 119899 Moscow, Russia

The causative link between viruses and infectious diseases in cellular life forms, primarily humans, has been driving research in virology from the time of discovery of the first viruses. Prompted by technology advancements, it has led recently to the launching of specialized programs, including metagenomics studies, that are concerned with the searching for pathogens causing diseases of unknown etiology as well as emerging infections. Thanks to this research effort, our knowledge about the natural virus diversity has been improved impressively, especially during the last decade. These studies have identified numerous viruses whose characterization is often limited to the genome sequence. The accumulation of genome sequences seems to outpace any other characterization in modern virology, the remarkable development that requires reflection. In this talk I will present results of comparative genomics of RNA viruses to show that genomes offer an unparalleled perspective on the function, structure and evolution of viruses, often challenging paradigm. It reveals that genome sequences, regardless of their origins, have considerable value for the original goals of the concerned programs and far beyond. Furthermore and somewhat paradoxically, it shows that the current sampling of the virus diversity remains rudimentary. Its expansion is expected to benefit greatly both fundamental research and diverse applications. Consequently, the virus discovery effort must be continued and broadened.

Dec 11th (TUE) 10:45-11:30

Keynote Lecture 5

Deciphering Evolution and Virulence of a Strictly Human Pathogen *Neisseria meningitidis*

Matthias Frosch

Institut für Hygiene und Mikrobiologie, der Universität Würzburg, Josef-Schneider-Straße 2, 97080 Würzburg

Neisseria meningitidis (the meningococcus) usually lives as a commensal bacterium in the upper airways exclusively of humans. Whereas most isolates from healthy carriers are considered as non-pathogenic, a small number of strains belonging to so called hyperinvasive lineages can cause life-threatening diseases such as acute bacterial meningitis or sepsis. Despite timely antibiotic treatment invasive meningococcal infections in particular represent a major childhood disease with a mortality rate of 10% and high morbidity in survivors. Apart from epidemic outbreaks, approximately 500,000 cases of meningococcal disease are estimated to occur every year on a worldwide basis posing a heavy burden on the public health systems especially in developing countries.

Due to the lack of a suitable animal model we use ex vivo infection models together with genome-wide approaches to experimentally investigate the genetic basis as well as the mechanisms of meningococcal pathogenicity and their evolution. By combining computational genome analyses with comparative genome hybridization using microarrays (mCGH) of a set of 29 meningococcal strains, we could recently detect a novel association of meningococcal virulence with a previously described canonical genomic island termed IHT-E and a differential distribution of genes encoding RTX toxin- and two-partner secretion systems among hyperinvasive and non-hyperinvasive lineages. In addition, we have demonstrated that recombination comprising lateral transfer of minimal mobile elements as well as prophages and homologous intragenic recombination in core genes has a profound impact on meningococcal population structure and genome composition. By further comparing the transcriptome of a serogroup B strain from a hyperinvasive lineage with the transcriptome of a related serogroup B carriage strain upon adhesion to human nasopharyngeal cells using microarrays, we have also shown that almost 10% of the 1731 genes that both strains have in common were differently expressed. These included in particular genes involved in inorganic ion as well as amino acid transport and metabolism, energy metabolism as well as stress response. In line with these transcriptomic differences, both strains also showed marked differences in their in vitro infectivity and in serum resistance. Our data thus support the concept of a polygenic nature of meningococcal virulence comprising differences in gene content as well as in the regulation of metabolic genes, and we suggest a prominent role for immune selection and genetic drift in shaping the meningococcal genome.

Keynote Lecture 6

Unique Association of High-Risk Human Papillomaviruses (HPV) with Breast Carcinoma

Naoki YAMAMOTO, Misako YAJIMA and Kenji OHBA

Translational ID Lab, Department of Microbiology,
Yong Loo Lin School of Medicine, National University of Singapore

Breast carcinoma (BC) is one of the major health threats for women in western countries occupying first place in their mortality, and its rapid increase in developing countries poses a big problem. It is well known that several hereditary as well as environmental risk factors affect the etiology of BC. Several studies have been conducted and data are accumulated as to BC and human papillomaviruses (HPV) from 1990s. But these studies have not provided consistent results which are quite varied and sometimes contradictory. Therefore, we investigated prevalence of HPV in BC with much larger number of samples including pre-malignancy, invasive BC as well as matched normal of different Asian ethnic groups. Samples were analyzed by a new DNA chip technology detecting viral L1 gene of 13 high-risk types of HPV simultaneously and sensitively. As the results, high prevalence of HPV infection was noted among BC patients. Most of the positive cases were infected with high–risk HPVs such as 16 and 18 types. When matched normal and tumor portions of BC samples were compared, HPV positivity in tumor was significantly lower than matched normal. Viral subtypes in both samples were closely related. We also noticed that all samples at various stages of BC tissues represented low viral copy numbers being far below than 1 copy/cell throughout the disease between pre-malignant and late stages. Statistical analysis also revealed that virus-positive BC patients progress faster than negatives when death was used as a parameter for disease progression. These data show that high-risk HPV is uniquely associated with BC.

Session 4-1

Dec 11 (TUE) 13:15-13:35

Homologous SV40 RNA Trans-Splicing: a new Mechanism for Diversification of Viral Genotypes and Phenotypes

Joachim EUL[1] and Volker PATZEL[2]

[1]Institute for Biochemistry and Molecular Biology, Freie Universitaet Berlin; Present address: Steinbeis Transfer Centre for Nucleic Acids Design, Germany (Stuttgart) & Singapore

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

Simian Virus 40 (SV40) is a polyomavirus found in both monkeys and humans which causes cancer in some animal models. In humans, SV40 is associated with cancers but causality has not been proven yet. The transforming activity of SV40 is mainly due to its 94 kD large T antigen which perturbs the function of the retinoblastoma (pRb) and p53 tumour suppressor proteins. Here we report a novel mechanism involving homologous RNA trans-splicing of SV40 early transcripts in transformed rodent cells. Enhanced trans-splice activity was observed in clones carrying a single point mutation in the large T antigen 5' donor splice site (ss). This mutation impaired cis-splicing in favour of an alternative trans-splice reaction via a cryptic 5'ss within a second cis-spliced SV40 pre-mRNA molecule and enabled detectable gene expression. The resulting trans-splice product encodes for a 100 kD super T antigen carrying a duplication of the pRB binding domain and was associated with unusual high cell transformation activity. Next to the cryptic 5'ss we identified additional trans-splice helper functions including putative dimerisation domains and a splice enhancer sequence. Our findings suggest (i) SV40 may spontaneously convert into a highly transforming virus, (ii) RNA trans-splicing as causative molecular event in the genesis of cancer and other diseases, and (iii) RNA trans-splicing as SV40-intrinsic mechanism that supports the diversification of viral genotypes and phenotypes.

Antiviral Strategies to Combat Chikungunya Virus Infection

Justin Jang Hann CHU

Laboratory of Molecular RNA Virology and Antiviral Strategies, Department of Microbiology,
Yong Loo Lin School of Medicine, National University of Singapore
Email: miccjh@nus.edu.sg

Chikungunya virus (CHIKV) is a re-emerging arthropod-borne virus responsible for recent epidemics in the Asian Pacific regions. This medically important alphavirus, Chikungunya, is known to cause CHIKV fever and disease symptoms such as maculopapular rash and persistent arthralgia in human. The current lacks of effective anti-viral or vaccine against this virus leave the human populations around the world at high risk of infection by this mosquito-borne viral pathogen.

To address this urgent need for treatment options, cell-based high-throughput screening platforms were developed in an attempt to identify potential anti-viral compounds from a diverse collection of highly purified small molecule natural product libraries against this viral pathogen. The high throughput screening of the natural product libraries using viral specific immunofluorescence assay platform have yielded an interesting primary hit list which include alkaloids, ionophores, cardiac glycosides and flavones that inhibit the different replication processes of the CHIKV (including the entry, viral RNA replication as well as assembly of virus particles). For example, a cephalotaxine alkaloid, harringtonine was selected for detailed analysis due to its strong inhibitory profile against CHIKV infection with minimal cytotoxicity. A dose-dependent study revealed harringtonine to have an IC_{50} of 0.45μM. Time-of-addition studies on harringtonine-treated and CHIKV-infected cells suggested that harringtonine may be involved in inhibiting the post-entry stages of viral replication during CHIKV infection. Quantitative RT-PCR studies showed drastic reduction in viral positive and negative strand RNA levels upon treatment with harringtonine. Proteomic and ultrastructural analyses further revealed that the antiviral mechanism of harringtonine as likely to be associated with the specific disruption of viral protein synthesis.
Furthermore, due to the rapid replicating nature and high genetic adaptability of the CHIKV to new strains of mosquito vector, a highly effective antiviral strategy at the viral genomic level is required. Gene silencing technologies including small hairpin RNA and morpholino oligomers targeting specific region of the CHIKV 5' UTR, 3' UTR, Capsid, E1, nSP1 and nSP4 genes conserved among different CHIKV phylogenic strains, were designed and evaluated. The effectiveness of these gene silencing technologies as novel antiviral approaches against CHIKV will be discussed. These studies have provided the basis for the development of novel antiviral approaches that could be further validated for as viable therapeutic option against CHIKV infection.

Session 4-3

Dec 11 (TUE) 13:55-14:15

Non-Structural Functions of Flavivirus Capsid Protein are Important for Virus Replication

Mun-Keat CHONG, Krupakar PARTHASARATHY, Bhuvanakantham RAGHAVAN,
Yuen Kuen CHEONG and Mah-Lee NG

Flavivirology Laboratory, Department of Microbiology, MD4, #01-04, 5 Science Drive 2,
Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597

Dengue virus (DENV) is a re-emerging arthropod-borne pathogen that accounts for hundred millions cases in tropical and subtropical countries annually. To date, antiviral drugs and vaccines are yet to be available in the market. This is mainly due to the incomplete understanding of the viral and host factors in the pathogenesis of DENV. Studying virus-host protein interactions will unravel the underlying molecular mechanism of the virus assembly and infection, which will be useful for novel antiviral strategies.

Capsid protein was found to have several non-structural functions during West Nile (WN) and dengue virus infections. This protein was transported into the cell nucleus to ensure that infection was established. Phosphorylated capsid protein which interacted with importin protein facilitated the entry of this protein into the nucleus. To further understand the function of capsid protein in virus replication cycle, high through-put protoarray was performed with purified biotinylated-full length capsid protein. The results unravelled several novel host interacting proteins which could be involved in the non-structural roles of DENV C protein during infection.

The interacting host proteins were found both in the nucleus as well as the cytoplasm modulating ribosomal RNA processing, cell cycle control, apoptosis and transportation pathways. This confirmed that capsid protein has multiple functions both in the nuclear and cytoplasmic phase during the replication process. One such example is the specific interaction of capsid proteins and human Sec3 protein (hSec3p). hSec3p was found to be a host anti-viral response against the infecting virus but the capsid protein was found to be able to abolish this response. Work is in progress to decipher the roles of other interacting host partners.

The Derivation and Characterization of a Therapeutic Human Antibody for Dengue Virus Infection

Paul A MACARY

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

Dengue virus is the major mosquito-borne viral pathogen that afflicts human populations. The virus is transmitted through the bite of an infected Aedes aegypti mosquito and infection can be asymptomatic; cause a self-limiting fever; or result in potentially fatal plasma leakage and hemorrhage. There are no approved vaccines or anti-viral therapies for dengue and current treatment is restricted to fluid replacement. Thus, there is an urgent need for new preventative and/or therapeutic options for this disease. Dengue virus consists of four related but distinct serotypes and infection is proposed to elicit life-long immunity to the infecting serotype but only short-term immunity against the others in those patients that recover. Immunity is mediated by serotype-specific antibodies but little is known about their specificity or mode of action. In this report, we characterize a neutralizing human monoclonal antibody engendered by natural dengue infection. This antibody is specific for dengue virus serotype one and shows little or no binding or neutralizing activity for serotypes two, three or four. We show that the antibody binds across two adjacent viral envelope proteins and identify the contributing amino-acids that comprise the binding site. The anti-viral activity of this antibody is linked principally to a blockade of virus binding to target host cells and this results in a strong anti-viral effect in a mouse model. This human antibody represents a new therapeutic candidate for dengue serotype 1 infection. These findings also provide a structural and molecular context for understanding the nature of durable, serotype-specific immunity to dengue infection and thus have implications for the design and evaluation of dengue vaccines.

Session 5-2

Dec 11 (TUE) 14:50-15:10

MAP Kinase Phosphatase 5 Regulates Iimmune Response to Influenza via IRF3

Hong Ying TEH,[1] Sharmy Jennifer JAMES,[1] Vincent T. K. CHOW,[1] Richard A. FLAVELL,[2] Chen DONG[3] and Yongliang ZHANG[1]

[1]Department of Microbiology, National University of Singapore, Singapore 117597

[2]Department of Immunology, Yale University, New Haven, CT06520, USA

3Department of Immunology, MD Anderson Cancer Center, Houston, TX77030, USA

MAP kinase phosphatases (MKP), also known as dual specificity phosphatases, are major negative regulators of MAP kinases and play important roles in immunity. Previously, we have shown that MAP kinase phosphatase 5 (MKP5), one member of MKP protein family, plays critical roles in both innate and adaptive immune responses. However, the role of this protein in anti-viral immune responses has not been well studied. To investigate the role of MKP5 in innate immune response against influenza, wild-type (WT) and MKP5 knockout (KO) mice were infected with A/Putero Rico/8/34 (PR8, H1N1) viruses. We found that viral titers in the lung of MKP5 KO mice are significantly lower on day 3 and day 5 post-infection than those in the lung of WT mice, which is associated with increased expression of IFN-beta in the lung and IFN-beta concentrations in the BAL from KO mice compared with those from WT mice. Overexpression of MKP5 in macrophages inhibits IFN-beta promoter activity. MKP5 KO macrophages produced significantly higher amount of IFN-alpha and IFN-beta in response to PR8 virus infection than WT cells which is associated with enhanced IRF3 activation in MKP5 KO cells. The regulation of IRF3 and type I interferon by MKP5 was further investigated. Our study demonstrated that MKP5-regulated signaling plays an important role in regulating IRF3 activation and type I interferon expression in innate immune responses against virus infection.

Mice Deficient in Interferon Regulatory Factor-4 are more Susceptible to Infection with Mouse-Adapted Influenza H3N2 Virus than to PR8 H1N1 Virus

W.P. POH[a], F. SETIAWAN[a], K.S. TAN[a], J.P. HSU[a], M.C. PHOON[a], T. NARASARAJU[a], K.B. TAN[a], S. WANG[a], K. HONMA[b], T. MATSUYAMA[b], D.R. KOH[a], Vincent T. CHOW[a]

[a] Yong Loo Lin School of Medicine, National University of Singapore, Kent Ridge, Singapore.[b] Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

Interferon regulatory factor-4 (IRF4) plays critical roles in both innate and adaptive immunity. IRF4 is thus likely to modulate antiviral innate immunity by regulating interferon-mediated responses. The objective of this study is to elucidate the involvement of IRF4 during influenza viral pneumonitis using an IRF4 knockout mouse model infected with highly virulent mouse-adapted influenza A/Aichi/2/68 H3N2 virus compared with A/PR/8/34 H1N1 virus. Mice of different IRF4 genotypes, i.e. wild-type (WT or +/+), heterozygous (HZ or +/-), and homozygous-knockout (KO or -/-) were infected via the intra-tracheal route with lethal challenge doses of H3N2 or H1N1 strains. Various parameters were assessed, i.e. weight loss, survival, lung histopathology, virus titers, cytokine/chemokine and immune cell profiles, etc.

Following H3N2 viral challenge, IRF4 KO mice exhibited higher mortality rate, as well as more severe lung damage. Viral quantification by plaque assay and real-time RT-PCR revealed higher viral burden in the lungs of IRF4 KO compared to IRF4 WT mice. Influenza virus-specific neutralizing antibodies were notably absent in infected IRF4 KO mice. Moreover, heterozygous IRF4 HZ mice consistently exhibited intermediate responses, thus implying a gene dosage effect. Levels of cytokines and chemokines were also altered in infected IRF4-deficient mice, including IL-1a, IL-2, IL-3, IL-6, IL-13, IL-17, MIP-1b, GM-CSF and TNF-alpha. Flow cytometric analyses of these mice also revealed changes in the population of immune cell subsets including CD3, CD4, CD8, CD19, and NK cells. Interestingly however, when IRF4 KO mice were challenged with H1N1 virus, there were no significant differences in mortality and morbidity compared with wild-type mice. Cytokine analysis of lung homogenates of H1N1-infected IRF4 KO mice revealed that levels of IL-1b, IL-2, IL-9, IL-10, IL-12, IL-13, IFN-gamma and KC were suppressed, whereas IL-17, RANTES and TNF-alpha were augmented. Noteworthy observations were that several IRF4-deficient mice spontaneously developed large lymphomatous lesions involving lymph nodes, while others succumbed to severe *Pneumocystis carinii* pneumonia in the absence of influenza infection. Our findings indicate an important role of IRF4 in regulating innate and adaptive immune responses, and in viral clearance during virulent influenza viral pneumonitis. Deficiency in IRF4 culminates in differential outcomes following challenge with different influenza strains, and is also associated with opportunistic infections and lymphomas.

Session 6-1

Dec 11 (TUE) 15:45-16:05

Influenza Epidemiology and Control

Paul A TAMBYAH

Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Influenza has been with us for centuries and in recent years, there has been an increased interest in influenza particularly as the last major subtype change occurred in 1968. In 2009, the World Health Organisation declared a pandemic of influenza with a novel strain of an existing subtype. This was the first time this has happened and led to the activation of influenza pandemic plans globally. We have learned a great deal about influenza in the years since. In particular, in the field of epidemiology, the transmission of influenza in hospitals and in semi-closed communities is better understood. The efficacy of antiviral therapy for influenza is also better known and there are numerous efforts at vaccine development, the most exciting of which is the possibility of a universal influenza vaccine. Southeast Asia and East Asia are uniquely positioned to collaborate in controlling and preventing the spread of influenza and its complications

Targeting Different Viral Proteins of the Influenza A Virus for the Development of Broad-Spectrum Antibody-Based Immunotherapy

Tze Minn MAK[1], Kedar Diwakar MANDAKHALIKAR[2] and Yee-Joo TAN[1,2]

[1]NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore,
[2]Department of Microbiology, Yong Loo Lin School of Medicine, National University Health System (NUHS), National University of Singapore

Current strategies to control influenza A virus include vaccination and antiviral drug treatment. However, predicting the major strain that may cause the next pandemic is the main obstacle in current vaccine development. Moreover, some viruses have acquired resistance to either the M2 ion-channel inhibitors or the neuraminidase inhibitors. Passive immunotherapy is now increasingly being used to treat human infectious diseases and there is a demand for the development of neutralizing monoclonal antibodies (mAbs) for passive immunotherapy in the event of a highly pathogenic flu pandemic.

As the hemagglutinin (HA) protein mediates viral entry, it has been the main target for the preclinical studies on antibody-based immunotherapy and these studies suggest that it may be a viable option to administer neutralizing HA mAbs as a form of passive immunotherapy. We have previously generated a mouse monoclonal antibody, 9F4, with the ability to neutralize different clades of H5N1. Here, we evaluate the ability of 9F4 to cross-neutralize other avian subtypes, namely H7N7 and H9N2, and characterize the binding epitope in details. As murine mAb will elicit a non-self response from the human immune system, we also constructed a chimeric 9F4 antibody where the murine constant regions were replaced by human sequences. The ability of the chimeric 9F4 antibody to prevent viral entry was found to be similar to the murine 9F4 antibody, thus suggesting that it may possible to humanize this antibody for therapeutic use.

Combination therapy, where multiple steps in the virus life cycle are inhibited simultaneously, is highly recommended to minimize the development of escape viruses. With the promising results obtained with anti-HA mAbs, there is now an urgent need to obtain an extensive collection of mAbs that target and inhibit different viral proteins. We have generated several monoclonal antibodies (mAbs) targeting the non-structural protein 1 (NS1) of influenza A virus and construct single-chain fragment (scFv) for intracellular expression. Interestingly, the overexpression of the scFv prevented the nuclear localization of DsRed-tagged NS1 protein. The intracellular localization of NS1 in infected cells has been shown to be predominantly nuclear and this is important for numerous functions of NS1. By preventing the transport of NS1 to the nucleus, it is probable that the scFv can inhibit the functions of NS1 during viral infection. Indeed, MDCK cells stably expressing the scFv showed reduction in viral replication. Current work is focused on understanding the mechanism of inhibition by NS1 mAbs.

Session 6-3

Dec 11 (TUE) 16:25-16:45

Conditional Ligands for Asian HLA Products

Cynthia Xin Lei CHANG,[1,4,5] Anthony T TAN,[6] Ming Yan OR,[1,4] Kai Yee TOH,[1,4] Pei Yiing LIM,[1,4] Adeline Siew Eng CHIA,[6] Thomas Moerch FROESIG,[9] Karen D NADUA,[6] Hsueh-Ling Janice OH,[7] Hoe Nam LEONG,[8] Sine Reker HADRUP,[9] Adam J GEHRING,[6] Yee-Joo TAN,[1,7] Antonio BERTOLETTI,[3,6,10] and Gijsbert M GROTENBREG[1,2,4,5]

[1]Department of Microbiology, [2]Department of Biological Sciences, [3]Department of Medicine, [4]Immunology Programme, Yong Loo Lin School of Medicine, and [5]NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore; [6]Infection and Immunity Programme, Singapore Institute for Clinical Sciences, and [7]Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore; [8]Singapore General Hospital, Singapore; [9]Center for Cancer Immune Therapy, Department of Hematology, University Hospital Herlev, Herlev, Denmark; [10]Program of Emerging Viral Diseases, Duke-NUS, Singapore

Antigen-specific T cells typically confer protection by cytolytic clearance of infected cells and produce cytokines to engage additional immune effectors. The enumeration of lymphocyte subsets that respond to discrete MHC-restricted epitopes remains technically challenging which hampers the determination of immunodominance of T cell subsets, their exact function and functional plasticity following infection. Class I MHC tetramers allow direct phenotypic identification of $CD8^+$ T cell populations but the production of these reagents is laborious and time-consuming. A peptide exchange strategy, which employs class I MHC molecules loaded with conditional ligands, provides a fast and straightforward method to obtain arrays of class I MHC tetramers of diverse specificity, and facilitates $CD8^+$ T cell epitope discovery. The application and refinement of this technology, frequently in combination with cell surface marker staining or intracellular cytokine staining followed by multidimensional flow cytometry analysis, allows for the accurate dissection of the T cell response following pathological challenge. Proof-of-concept studies with MHC tetramer libraries in several mouse models of infectious disease, such as *Chlamydia trachomatis*, *Toxoplasma gondii*, and MHV-68, will be highlighted. We will further present the recent implementation of conditional ligands for a large number of HLA products typically associated with Asian ancestry, and how the peptide exchange strategy can be employed for the determination of HLA restriction and mapping epitope fine-specificity. Well-defined T cell epitopes for viral infections such as Dengue, SARS and HBV are scarce for Asian HLA variants, and from our expanding portfolio a picture is gradually emerging where HLA-B and HLA-C restricted epitopes are readily identified. This can profoundly influence our understanding of human antiviral T cell responses and has specific relevance to vaccine design.

Acknowledgment: This work was supported by the NRF Research Fellowship (NRF2007 NRF-RF001-226) and an A*STAR BMRC grant (10/1/21/19/652). CXLC is an NGS Graduate Scholarship recipient.

Guillain–Barré Syndrome and Vaccination

Nobuhiro YUKI, MD, PhD

Department of Medicine, National University of Singapore, Singapore

Guillain–Barré syndrome is currently the most frequent cause of acute flaccid paralysis world wide, and constitutes one of the serious emergencies in neurology. Two-thirds of cases are preceded by symptoms of upper respiratory tract infection or diarrhea. The most frequently identified cause of infection is *Campylobacter jejuni*, and in one meta-analysis, 30% of infections were attributed to *C. jejuni*, whereas cytomegalovirus was identified in up to 10%. We have proved that molecular mimicry between GM1 ganglioside and *C. jejuni* is a cause of GBS.

During a 1976 mass immunization against A/NJ/1976/H1N1 “swine flu” in the US, vaccinees had an increased risk of developing GBS. No other conventional vaccines have been associated with a significant risk of developing GBS except for brain-derived rabies vaccines One report demonstrated that the 1976 swine flu and seasonal flu vaccines induced anti-GM1 antibodies in mice, thus suggesting molecular mimicry in post-vaccination GBS but this was not replicated in mice and men. In contrast, a sheep brain–derived rabies vaccine that is contaminated with gangliosides may trigger GBS associated with IgG anti-GM1 antibodies.

References:

1. Yuki N, Hartung HP. Guillain–Barré syndrome N Engl J Med 2012.
2. Laouini D, Kennou MF, Khoufi S, Dellagi K. Antibodies to human myelin proteins and gangliosides in patients with acute neuroparalytic accidents induced by brain-derived rabies vaccine. J Neuroimmunol 1998;91:63-72.
3. Sakai H, Harun FM, Yamamoto N, Yuki N. Contamination with gangliosides in brain-derived rabies vaccine may trigger Guillain-Barré syndrome. J Neurology, Neurosurg Psychiatry 2012;83:467-9.

Session 6-5

Dec 11 (TUE) 17:05-17:25

Protective role of host innate immune response against prion pathogen

Daisuke Ishibashi

Division of Cellular and Molecular Biology, Department of Molecular Microbiology and Immunology, Nagasaki Univ. Graduate School of Biomedical Sciences

Host defenses mechanism against prion infection was seldom focused until now. The detail of host innate immune systems had to be determined in prion infection. We elucidated that the transcription factor IRF3 locating in downstream of innate immune signal pathways including TLR signaling had a protective role in prion infection and reduced PrP^{Sc} production. This work suggests that innate immune responses have a key role for invasion of prion pathogen.

Keynote Lecture 7

Discovery of Artemisinin (qinghaosu) and Early Research by My Team

Guoqiao Li[1)], Keith Arnold[2)], Muoi Arnold[3)]

1. Guangzhou University of Chinese Medicine, China
2. Retired from Roche Asian Research Foundation, Hong Kong
3. Consultant Pathologist, Sutter Lakeside Hospital, USA

Artemisinin (also known as qinghaosu) and its derivatives are now standard treatment worldwide for *P. falciparum* malaria. Qinghaosu was developed by group of Chinese scientists in 1970's, as the results of collective efforts by collaborative units in 523 Project. In May 23rd, 1967, the General Logistics Department of the Chinese People's Liberation Army and the State Science and Technology Commission of China organized a National Malaria Control & Research Cooperative Workshop. The outcome was "Project 523"; an urgent program of anti-malaria drug research endorsed by Zhou En Lai and Mao Ze Dong.

During 1970-1974, collectively, the researchers from four units found qinghaosu has a rapid onset of action with high efficacy against malaria. In 1975-1978, the collaborative teams, organized by the National 523 office, successfully developed qinghaosu, around 60 institutions and over 500 scientists became involved.

In any wild mosquito existing endemic site, integrated malaria control methods should mainly focus on transmission source control. The integrated measures include mass drug administration (MDA) with ACT (artemisinin combined therapy) + low dose primaquine to fast eliminate the infectious reservoirs; early diagnosis and early treatment to block transmission; medication in mobile population to stop imported transmission. Such as the pilot project in Comoros.

Keynote Lecture 8

Bridging laboratory science and the field in malaria research

NJ White

Faculty of Tropical Medicine, Mahidol University,
420/6 Rajvithi Rd, Bangkok 10400, Thailand
E-mail: nickw@tropmedres.ac

Although there have been substantial advances in malaria control in recent years, with an estimated reduction in global mortality by one third, malaria still kills approximately 2000 people each day. We do not have a malaria vaccine, although a first generation vaccine providing partial protection may become available in 2015. Malaria control depends therefore on effective insecticides and effective treatment, and will continue to do so for the foreseeable future. Most laboratory research on malaria takes place in temperate countries which have already eliminated the disease. Much of this has been on basic science and animal models, and very little has translated into improvements in the prevention and treatment of malaria.
Global malaria control, and plans for malaria elimination, are now threatened by the emergence of artemisinin resistance in falciparum malaria and pyrethroid resistance in some anopheline vectors. Artemisinin resistance has emerged in Western Cambodia. Chloroquine resistance arose in exactly the same place 50 years ago, spread to Africa, and killed millions of children. Characterising and containing artemisinin resistance is now considered of the highest priority. Clinical and pharmacological research has provided methods of assessment. This is now an opportunity for laboratory science to demonstrate its value to global health by identifying the molecular basis for resistance, and providing a molecular marker that can be used to map spread and so support the containment effort.

Quantitative whole genome resequencing and genetic linkage analyses identify genes controlling medically important phenotypes of malaria parasites.

Richard Culleton

The Malaria Unit, Institute of Tropical Medicine (NEKKEN), Nagasaki University

Efforts are currently underway to produce a malaria vaccine, although the reality of a safe, affordable, and above all highly efficient vaccine appears to be some way off. Vaccines may be based on attenuated whole organism approaches or on sub-unit vaccines. Although attenuated sporozoite vaccines are currently being considered for development, most work to date has focused on sub-unit vaccines, as they are potentially easier to produce and distribute. The first, and potentially most crucial, step the in the development of a sub-unit vaccine is the identification of *protective* parasite antigens that may form their basis. Here, we describe an approach to identify such antigens that fuses classical genetic linkage analysis with next generation whole genome sequencing.

Using the rodent malaria parasite *Plasmodium yoelii*, we first identified parasite strains that elicit immunity in mice that is completely protective against the immunising strain, but not against a heterologous strain. Genetic crosses were then performed with these strains, and the resulting progeny grown in mice made immune to one or other of the parental strains. Whole genome sequencing at greater than 200 times coverage was then performed on both parental strains, and on the progeny pre- and post-selection, and genomic regions targeted by immune selection were identified. Three major loci under immune-selection were identified, one of which contains the major vaccine candidate gene *msp1*.

Using the same approach, we also show that major differences in growth-rate phenotypes between two *P. yoelii* strains is controlled exclusively by a gene on chromosome 13, the most likely candidate being *Pyebl*.

Session 7-2

Dec 12 (WED) 11:05-11:25

Climate and malaria in western Kenya

Noboru Minakawa[1], Masahiro Hashizume[1] and Luis Chaves[2]

[1] Institute of Tropical Medicine, Nagasaki University

[2] Graduate School of Environmental Earth Science, Hokkaido University

Global warming was considered as a cause of malaria epidemics in the highland of East Africa during the 1990's. However, the association of global warning with the malaria epidemics is unclear. Since a malaria epidemic occurred following the El-Niño-Southern Oscillation (El Niño) event in 1997, climate variability drew scientists' attention. While El Niño is a global event, we were interested in the influence of a local oceanic event, the Indian Ocean Dipole (IOD), on the highland malaria. We found that IOD had a greater association with highland malaria than El Niño. The association was stronger in the highland than the lowland in the Lake Victoria basin. Further, we found that climate variability affects the water level in Lake Victoria, and the fluctuation of lake water influences malaria transmission in villages on the lakeshore. Our findings suggest that a climate based early-warning system is useful for reducing disease burden.

Ultrasensitive human prion detection in cerebrospinal fluids using real-time quaking-induced conversion

Ryuichiro Atarashi

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

Current diagnostic investigations to evaluate suspected human prion disease, although of proven utility, rely on non-specific bio-markers, such as detection of 14-3-3 proteins in the cerebrospinal fluid (CSF). The development of *in vitro* protein amplification technologies has generated the potential for a novel diagnostic assay that directly detects the abnormal, protease-resistant conformers of the prion protein (PrP^{Sc}). Hitherto, attempts at ultrasensitive PrP^{Sc} detection in CSF have not been successful in human prion diseases. Previously, we developed a new *in vitro* PrP^{Sc} amplification assay designated quaking-induced conversion (QUIC), which involves intermittent, automated shaking of the substrate, soluble recombinant PrP (rPrP-sen). To further improve the rapidity and practicality of this method, we combined QUIC technology with thioflavin T (ThT) fluorescence to monitor amyloid fibril formation. This assay, termed "real-time QUIC (RT-QUIC)", allows within 48h, the detection of ≥1 fg of PrP^{Sc} in diluted sporadic Creutzfeldt-Jakob disease (sCJD) brain homogenate. Moreover, we assessed the technique first in a series of Japanese patients, and then in a blind study of 30 CSF specimens from Australia, which achieved greater than 80% sensitivity and 100% specificity. These findings indicate the promising enhanced diagnostic capacity of RT-QUIC in the ante-mortem evaluation of suspected sCJD. More recent studies have shown that the RT-QUIC is able to rapidly determine the relative prion concentration when used in combination with end-point dilution of the specimens. Furthermore, we recently examined CSF specimens from human genetic disease patients in Japan using RT-QUIC and compared the sensitivity with that of biochemical markers such as 14-3-3 protein and total tau protein.

Poster Presentation
December 10th（MON）
at 1F Sensai Hall

No.	Name	University
P01	Bruce Russell	National University of Singapore
P02	Lucky Amuza	Nagasaki University
P03	Xiaotong Zhu	Nagasaki University
P04	Jun Hong Ch'ng	National University of Singapore
P05	Xangsayarath Phonepadith	Nagasaki University
P06	Yan Quan Lee	National University of Singapore
P07	Miako Sakaguchi	Nagasaki University
P08	Jephtha Nmor	Nagasaki University
P09	Hussein M. Abkallo	Nagasaki University
P10	Lam Quoc Bao	Nagasaki University
P11	Tamotsu Niikura	Kyorin University
P12	Daisuke Kimura	Nagasaki University
P13	Kazuhide Yahata	Nagasaki University
P14	Rika Kamei	Nagasaki University
P15	Masahito Asada	Obihiro University
P16	Md Moshiur Rahman	Kanazawa University
P17	Fumika Miichi	Saga University
P18	Chikako Shimokawa	Nagasaki University
P19	Mudyawati Kamaruddin	Kanazawa University
P20	Masoud Akbari	Nagasaki University
P21	Adrian P. Ybanez	Obihiro University
P22	Sock Yue Thong	National University of Singapore
P23	Keishi Adachi	Nagasaki University
P24	Joanna Ai Ling Choo	National University of Singapore
P25	Evaristus C. Mbanefo	Nagasaki University
P26	Sachiyo Nagi	Nagasaki University
P27	Michiko Toizumi	Nagasaki University
P28	Yen Hai Doan	Nagasaki University
P29	Mitsuru Toda	Nagasaki University
P30	T. N. Hoa Tran	Nagasaki University
P31	Masayuki Nakano	Nagasaki University
P32	Shakh M. A. Rouf	Nagasaki University
P33	Toshiya Hirayama	Nagasaki University
P34	Sayuri Shigematsu	Nagasaki University

P1

Field Based Flow Cytometry for the Ex Vivo Characterization of *Plasmodium vivax*, *Plasmodium falciparum* and Antimalarial Sensitivity: a Comparison with the Gold Standard Microscopic Assay

RUSSELL Bruce,[1,2*] MALLERET Benoit,[3*] SUWANARUSK Rossarin,[3*] ANTHONY Claudia,[2] TAN Kevin S.W,[1] SRIPRAWAT Kanlaya,[4] NOSTEN Francois,[4] RÉNIA Laurent[1,2]

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

[2] Department of Parasitology, Faculty of Medicine, University of Malaya, KL, Malaysia

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

[4] Mahidol–Oxford University Tropical Medicine Research Programme, Shoklo Malaria Research Unit, Mae Sot, Thailand

Microscopic examination of *ex vivo* matured malaria parasites, remains the gold standard method used to determine the intrinsic sensitivity of *Plasmodium vivax* and *Plasmodium falciparum* to antimalarials. However, the microscopic examination of Giemsa stained thick films central to this method is tedious, time-consuming and requires skilled microscopists. Moreover, large inter-observer variations of parasite staging are frequently recorded. We have developed a precise, accurate, fast and simple flow cytometery (FC) method to conduct *ex vivo* drug sensitivity assays of *P. vivax* and *P. falciparum* under field conditions using only 2 colours.

The results for FC matched those of the traditional microscopy very closely. In the one case where there was a significant difference between the IC50 analysis of FC and Microscopy the actual mean difference in AS IC50 for *P. vivax* was less than 0.1 ng/ml which is unlikely to be of biological significance. This 0.1 ng/ml disparity should also be put in the context of inter reader variability between SMRU microscopists, where the difference in the AS IC50 for *P. vivax* can be as high as 1.54 ng/ml for one isolate. It should also be noted that the time to accrue data from the FC method is only ~2mins per drug (8 wells) compared to 18 mins by microscopy. Additionally, the use of a novel fluorescent-linked chloroquine hybrid reporter molecule was also investigated for diagnostic applications.

Equal Contribution*

Trafficking and assembly of malarial exported proteins in the *Plasmodium falciparum*-infected red blood cell

Amuza Byaruhanga Lucky [1, 2], Kazuhide Yahata[1], Nobuhisa Iwata[2], Osamu Kaneko[1]

[1]Department of Protozoology, Institute of Tropical Medicine (NEKKEN) and the Global COE Program, Nagasaki University, [2]Division of Molecular Biology and Biotechnology, Department of Molecular Medicinal Sciences, School of Pharmaceutical Sciences, Nagasaki University.
Email address: amuza7@yahoo.com

An important part of virulence for *Plasmodium falciparum* malaria infection, results from rosetting, cytoadherence and immune evasion strategies by the parasite. These phenotypes are mediated by exported proteins to the parasite-infected red blood cell (RBC), where the parasite newly generates the parasitophorous vacuolar membrane (PVM) and the Maurer's clefts and modifies the host RBC membrane. Currently, proteins exported using N-terminally located pentameric motif termed *Plasmodial export element* (PEXEL) and those not depending on the PEXEL motif are known. PEXEL-positive proteins were proposed to use a PVM-located translocon termed *P. falciparum translocon of exported proteins* (PTEX), however the export mechanism of PEXEL-independent proteins is not well elucidated. Because the parasite proteins exported to the RBC cytosol are mobile at early ring stage when Maurer's clefts are not yet established, we focused on this stage to evaluate if PEXEL-positive and -negative proteins are co-exported or not. To achieve this, we generated transgenic *P. falciparum* parasites expressing PEXEL-positive PfMC2TM and PEXEL-negative PfSBP1 proteins fused with fluorescent tags. Live imaging and immunofluorescence assays showed detectable signals beyond the PVM by 12 hours after RBC invasion. We found that these two types of proteins located differently at early ring stage and eventually merging together in later trophozoite stage, suggesting a difference in route of export of these PEXEL-positive and PEXEL-negative proteins from the PVM to Maurer's clefts.

P3

The N-terminal segment of *Plasmodium falciparum* SURFIN$_{4.1}$ is required for its trafficking to the red blood cell cytosol through the endoplasmic reticulum

Xiaotong Zhu[1], Kazuhide Yahata[1], Jean Semé Fils Alexandre[1,2], Takafumi Tsuboi[3], Osamu Kaneko[1,*]

[1] Department of Protozoology, Institute of Tropical Medicine (NEKKEN) and the Global COE Program, Nagasaki University, Sakamoto, Nagasaki 852-8523, Japan

[2] Centro Nacional de Control de Enfermedades Tropicales, Santo Domingo, República Dominicana

[3] Cell-Free Science and Technology Research Center, Ehime University, Matsuyama, Ehime 790-8577, Japan

* Corresponding author: Osamu Kaneko; address, Department of Protozoology, Institute of Tropical Medicine, Nagasaki University, 1-4-12 Sakamoto, Nagasaki 852-8523, Japan; Tel.: (+81) 95 819 7838; fax: (+81) 95 819 7805; E-mail address: okaneko@nagasaki-u.ac.jp

Plasmodium falciparum SURFIN is a type I transmembrane protein that shares domains with molecules expressed on the surface of the red blood cells (RBCs) infected with a variety of malaria parasite species, such as *P. falciparum* PfEMP1, *P. vivax* VIR proteins, and *P. knowlesi* SICA*var*. Thus, understanding the export mechanism of SURFIN to the RBC may provide fundamental insights into how malaria parasites export their proteins to RBC cytosol in general. We re-evaluate SURFIN$_{4.1}$ for its exon-intron boundaries, location, and the function of each region by expressing recombinant SURFIN$_{4.1}$ in *P. falciparum*. We found that, in two 3D7 lines and one Thai isolate, SURFIN$_{4.1}$ possesses only 19 amino acids after the predicted transmembrane region, whereas in the FCR3 line, it possesses two tryptophan-rich domains in its intracellular region. rSURFIN$_{4.1}$ based on the 3D7 sequence was detected in the Maurer's clefts of infected RBCs, suggesting that endogenous SURFIN$_{4.1}$ is also exported to Maurer's clefts. Brefeldin A-sensitive export of SURFIN$_{4.1}$ indicates that its export is endoplasmic reticulum (ER)/Golgi-dependent. By sequential deletion and replacement with unrelated protein sequences, we find that the SURFIN$_{4.1}$ TM region is essential for the initial recruitment of the protein to the ER, and the following sorting step to the parasitophorous vacuole is determined by two independent signals located in the N-terminus 50 amino acids. TM region with the adjacent cytoplasmic region also contain information for the efficient recruitment to the ER and/or for the efficient translocation across the PV membrane. We also found that SURFIN$_{4.1}$ might form a homomeric complex during the trafficking using cysteine rich domain and/or variable region.

Keywords: *Plasmodium falciparum*, red blood cell, trafficking

Target-Based Whole Cell Pathway Screen for Chemosensitizing Compounds using a Fluorophore-Tagged Antimalarial

P4

CH'NG Jun Hong and Kevin SW TAN

Laboratory of Molecular and Cellular Parasitology, Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore

The early promise of malaria eradication was dealt a major setback with the rise and spread of malaria parasites resistant to the antimalarial chloroquine (CQ). Notably, the recent discovery of artemisinin delayed-clearance strains has signaled an urgent need for novel strategies to treat this disease. While the development of novel antimalarials remains one of the key approaches, the possibility of redeploying outdated drugs in combination with chemosensitizing compounds that interfere with the resistance-conferring mechanisms is a potentially faster and cheaper alternative.

Using a coumarin-tagged chloroquine moiety (CM-CQ) and assaying for the extent of uptake, we are able to readily discriminate between the sensitive and resistant parasites as the latter possess drug-efflux transporters and accumulate less of the fluorescent compound. It is also possible for us to use this approach to assay for compounds which interfere with the resistance mechanism in a high throughput fashion.

While several chemosensitizing compounds like verapamil and desipramine have been previously elucidated, poor *in vivo* efficacy has limited the use of these compounds. The increased throughput has allowed us to expand the repertoire of known chemosensitizers in an effort to identify compounds that will be clinically useful. With a preliminary screen of 1240 compounds from the LOPAC library (Library of Pharmacologically Active Compounds, Sigma-Aldrich), 13 hits were shown to significantly increase CM-CQ uptake. Of these, 12 were shown to significantly reduce the IC_{50} of K1 to CQ at 10 μM and 2 remained effective even at 50 nM, well below their reported C_{max}.

While pharmacological and in vivo experiments have yet to be performed, it is hoped that these compounds will be suitable for co-administration with CQ or chemically coupled to CQ to increase its potency against drug resistant parasites.

Positive diversifying selection on the *Plasmodium falciparum* $surf_{4.1}$ gene in Thailand

P5

Xangsayarath Phonepadith

Department of Protozoology
Institute of Tropical Medicine (NEKKEN), Nagasaki University
Sakamoto 1-12-4, Nagasaki 852-8523, Japan
Phone: 095-819-7838
Email: xangsayalathdith@yahoo.com

Plasmodium falciparum $SURFIN_{4.1}$ is a type I transmembrane protein proposed to locate on the merozoite surface and responsible for a reversible adherence to the erythrocyte before invasion. In this study, we evaluated $surf_{4.1}$ gene segment encoding extracellular region for polymorphism, the signature of positive selection, the degree of linkage disequilibrium, and temporal change in allele frequency distribution in *P. falciparum* isolates from Thailand in 1988-89, 2003, and 2005. We found $SURFIN_{4.1}$ is highly polymorphic, particularly at the C-terminal side of the variable region located just before a predicted transmembrane region. A signature of positive diversifying selection on the variable region were detected by multiple tests and, to a lesser extent, on conserved N-terminally located cysteine-rich domain by Tajima's *D* test. Linkage disequilibrium between sites over a long distance (> 1.5 kb) was detected and multiple $SURFIN_{4.1}$ allele sequences detected in 1988/89 still circulated in 2003. Almost all of the single amino acid polymorphism allele frequency distribution were not significantly different between 1988/89 and 2003 groups, suggesting that frequency distribution of $SURFIN_{4.1}$ extracellular region were stable over 14 years.

A High-Content Screen for Drug Inducers of Digestive Vacuole Permeabilization in *Plasmodium falciparum* as a Novel Antimalarial Strategy

P6

YQ LEE,[1,2] Amanda SP GOH,[1] JH CH'NG,[1] Kevin SW TAN[1,2]

[1]Laboratory of Molecular and Cellular Parasitology, Yong Loo Lin School of Medicine, National University of Singapore

[2]NUS Graduate School of Integrative Sciences and Engineering

Background: Malaria is a global health concern and the cause of 655,000 deaths in 2010. *Plasmodium falciparum* is the major etiological agent of malaria. Recent work in our laboratory established that programmed cell death (PCD) in *P. falciparum* can be induced by chloroquine (CQ), associated with early digestive vacuole (DV) permeabilization. Lysosomal membrane destabilization has previously been implicated in mammalian PCD and could perhaps be exploited as a novel antimalarial strategy. However, CQ-resistant strain K1 was resistant to DV destabilization. We therefore developed an image-based high-content screening assay to identify novel compounds with DV destabilizing effects. **Methods:** *P. falciparum* 3D7 was treated with 5 known lysosome-destabilizing and 25 commercially-available compounds, most of which were reported to exhibit antimalarial properties, but with unknown modes of action. Cells were stained with Hoechst and Fluo-4-AM and assayed with ImageStream, a combined fluorescence microscope and flow cytometer. Fluo-4-AM is a calcium indicator which allows for visualization of the DV, a calcium store. Cells were then assessed for increase in mean area of Fluo-4 fluorescence as a measure of DV destabilization and verified by confocal microscopy. Hits were tested on 2 other strains, K1 and 7G8, both multidrug resistant. Mitochondrial dysfunction and DNA fragmentation were assayed with JC-1 and Hoechst staining respectively. **Results:** The 5 known lysosomotrophic agents resulted in significant Fluo-4 redistribution as assessed by confocal microscopy. ImageStream analysis had similar results except for 1 compound. Screening of the panel of 25 candidates resulted in 2 hits: quinacrine (QC) and dichlorobenzamil (DCB). Fluo-4 redistribution after treatment of the 3 strains with QC and DCB was confirmed by confocal microscopy. JC-1 assays of QC- and DCB-treated cultures showed mitochondrial disruption for all 3 strains. DCB also increased the proportion of sub-G1 cells in these 3 strains. However, QC resulted in increased DNA fragmentation in 3D7 but not in 7G8 and K1. **Conclusions:** A proof-of-concept is presented for the use of an image-based high-content platform to identify DV-destabilizing agents. Two compounds have been identified as promising antimalarials through a previously-unexploited PCD pathway.

Role of the C-terminal region of *Plasmodium falciparum* antigen 332 on the location in the parasite-infected red blood cell

Ning Jiang[1,2], Miako Sakaguchi[1], Jean Alexandre[1], Kazuhide Yahata[1],
Takafumi Tsuboi[3], Qijun Chen[2], Osamu Kaneko[1]

P7

[1] Department of Protozoology, Institute of Tropical Medicine, Nagasaki University, Japan. [2] Laboratory of Zoonosis, Jilin University, China. [3] Cell-Free Science and Technology Research Center, Ehime University, Japan.

Plasmodium falciparum dramatically remodels the membrane structure of the infected red blood cell (iRBC), giving a cytoadherence property responsible for the malaria severity. Pf332 is one of responsible proteins exported to the membranous structure Maurer's clefts (MCs) and the disruption of Pf332 gene locus altered the iRBC rigidity and decreased the cytoadhesion efficacy. Pf332 is a large type I transmembrane protein, consisting of an N-terminal Duffy-binding-like (DBL) domain followed by a transmembrane (TM) region, Glu-repeats, and a C-terminal tryptophan-rich (WR) region. As there are controversial reports for the location of Pf332 DBL region, one proposed this region was exposed on the iRBC surface and the other not, we generated transgenic *P. falciparum* lines expressing DBL-TM-WR (a mini-Pf332) and DBL-TM fused with GFP at their C-terminus. We found DBL-TM localized to the MC, whereas DBL-TM-WR showed diffused signal throughout the RBC membrane in addition to the MC. Both anti-DBL or anti-GFP antibody gave no signal on the iRBC surface, whereas both detected signals after permeabilization with saponin, suggesting that both regions were not exposed on the iRBC surface. After streptolysin O treatment, we detected only GFP signal as multiple dots in the iRBC cytoplasm for DBL-TM or as signal located beneath the iRBC membrane for DBL-TM-WR. These data suggest that Pf332 is integrated into the MC membrane with the C-terminal region exposed to the iRBC cytoplasm, whereas the N-terminal is localized inside of the MC. Our results also suggest that Pf332 C-terminal region is responsible for the translocation of Pf332 from MC to RBC membrane or the interaction between MC and RBC membrane.

Topographic indices: plausible proxies to model breeding sites of malaria vectors.

Nmor JC[1,2], T Sunahara[1] K Goto[3], K Futami[1], G Dida[4], G Sonye[5] and N Minakawa[1]

[1]Department of Vector Ecology and Environment, Institute of Tropical Medicine Nagasaki University, Japan
[2]Department of Animal and Environmental Biology, Delta State University, Abraka, Nigeria.
[3]Department of Eco-epidemiology, Institute of Tropical Medicine Nagasaki University, Japan.
[4]School of Public Health, Maseno University, Maseno, Kenya
[5]Spring of Hopes, Mbita, Kenya

P8

Abstract

Background: Topography is often one of the major controls on the spatial pattern of saturated areas, which in turn is a key to understanding much of the variability in hydrological processes across landscapes. Malaria vector breeding sites depends on surface water availability. Given that topography and surface water are closely linked, we thus used topographic indices as a proxy to model the possible locations of *Anopheles* larva habitats.

Methods: Using GIS technique, topographic variables were extracted from digital elevation model (DEM) captured by Shuttle Radar Topography Mission (SRTM, 90m resolution). We used the data of breeding sites from an extensive field survey conducted on an island of western Kenya in 2006. The topographic variables were extracted for 826 breeding sites, and 4520 negative point that were randomly assigned. A logistic regression model was applied to characterize topographic features of the malaria vector breeding sites and predict their locations. Model accuracy was evaluated using the area under Receiver Operating Characteristics curve (AUC).

Results: All topographic derivatives from DEM showed significant correlation with breeding habitats except for aspect. The multivariate model showed higher accuracy in the independent test site (AUC 0.829) compared to the training site (AUC 0.758). There was variation in the extent of predictability of different breeding habitats types. Overall, drainages, swamps and foot prints habitat types showed higher predictability while rock pool and river bed habitats types revealed least predictability. The visualised model coincided and had good correlations with the breeding sites locations.

Conclusions: We have demonstrated that SRTM DEM is useful in modelling likely breeding habitats of malaria vector and topographic indices offer great potential in locating breeding sites of malaria vectors. We expect that this model will help enhance surveillance or targeted control activities in regions where they are most needed.

The consequences of within-host competition in mixed strain malaria infections

Hussein Abkallo, Julie-Anne Tangena, Megumi Inoue, Richard Culleton

Malaria Unit, Institute of Tropical Medicine, Nagasaki University, Japan

P9

In endemic areas with high transmission intensities, malaria infections are very often composed of multiple genetically distinct strains of malaria parasites. It has been hypothesised that such a situation leads to intra-host competition, in which parasite strains compete for resources such as space and nutrients. Such competition may have repercussions for the host, the parasite, and the vector in terms of disease severity, vector fitness, and parasite transmission potential and fitness. It has been argued that within-host competition could lead to selection for more virulent parasites, and, as a consequence of this, vaccines that reduce disease severity may themselves select for parasites with increased virulence. Laboratory evidence for these hypotheses comes almost exclusively from experiments performed with the rodent malaria parasite *Plasmodium chabaudi*. Here we use the rodent malaria parasite *Plasmodium yoelii* to assess the consequences of mixed strain infections on disease severity and parasite fitness (including transmission potential). Three isogenic strains with dramatically different growth rates (and hence virulence) were maintained in mice in single infections or in mixed strain infections with genetically distinct strain. We compared the virulence of mixed strain infections (defined as harm to the mammalian host) with that of single infections, and assessed whether competition impacted on parasite fitness, including transmission potential. We found that mixed infections were associated with a higher degree of disease severity and a prolonged infection time. In the mixed infections, the strain with the slower growth rate was responsible for the competitive exclusion of the faster growing strain, presumably through host immune-mediated mechanisms. Importantly, and in contrast to previous work conducted with *P. chabaudi*, we found no correlation between parasite virulence and transmission potential to mosquitoes, suggesting that within-host competition would not drive the evolution of parasite virulence in *P. yoelii*.

CD19(+) B cells Confer Protection Against Experimental Cerebral Malaria in Semi-immune Rodent Model

Lam Quoc Bao[1], Nguyen Tien Huy[1], Mihoko Kikuchi[2], Tetsuo Yanagi[3], Masachika Senba[4], Mohammed Nasir Shuaibu[1,6], Kiri Honma[5], Katsuyuki Yui[5,6], Kenji Hirayama[1,6]

[1]Department of Immunogenetics, Institute of Tropical Medicine (NEKKEN)

[2]Center for International Collaborative Research, Nagasaki University (CICORN),

[3]Animal Research Center for Tropical Infections, NEKKEN, [4]Department of Pathology, NEKKEN, [5]Department of Molecular Microbiology and Immunology, Graduate School of Biomedical Sciences, Nagasaki University, [6] Global COE program, Nagasaki University

P10

ABSTRACT

In African endemic area, adults are less vulnerable to cerebral malaria (CM) than children probably because of acquired partial immunity or semi-immune status.

Here, we developed a CM model for semi-immune mice in which C57BL/6 (B6) mice underwent one, two and three cycles of infection and radical treatment (1-cure, 2-cure and 3-cure, respectively) before finally challenged with 10^4 Plasmodium berghei ANKA without treatment. Our results showed that 100% of naïve (0-cure), 67% of 1-cure, 37% of 2-cure and none of 3-cure mice died within 10 days post challenge infection. In the protected 3-cure mice, significantly higher levels of plasma IL-10 and lower levels of INF-γ than the others on day 7 post challenge infection were observed. Major increased lymphocyte subset of IL-10 positive cells in 3-cure mice was CD5(-)CD19(+) B cells. Passive transfer of splenic CD19(+) cells from 3-cure mice protected naïve mice from CM. Additionally, aged 3-cure mice were also protected from CM 12 and 20 months after the last challenge infection. In conclusion, mice became completely resistant to CM after 3 times of malaria exposure. CD19(+) B cells are determinants in protective mechanism of semi-immune mice against CM probably via modulatory IL-10 for pathogenic INF-γ production.

Development of severe pathology in immunized pregnant mice challenged with lethal malaria parasites

Mamoru Niikura[1], Mineo Shoichirou[1,2], Shin-ichi Inoue[1], Masahiko Kuroda[2], Fumie Kobayashi[1]

[1] Department of Infectious Diseases, Kyorin University School of Medicine, Tokyo 181-8611, Japan

[2] Department of Molecular Pathology, Tokyo Medical University, Tokyo 160-8402, Japan

P11

Pregnant women are highly susceptible to malaria infection because of their low immunity and at increased risk of maternal illness or death, in addition to the occurrence of spontaneous abortion, stillbirth, premature delivery and low birth weight. However, the detailed pathogenesis of maternal malaria remains unclear. In this study, we tried to establish a mouse model reproducing severe pathological features of pregnant women during *Plasmodium falciparum* infection and investigate the pathogenesis of maternal malaria using the mouse model. Pregnant mice immunized by infection of nonlethal *P. berghei* (*Pb*) XAT were more susceptible to lethal *Pb* NK65 challenged infection than non-pregnant mice and showed high levels of parasitemia and poor pregnancy outcome associated with placental pathology, such as accumulation of parasitized RBCs and decreasing number of vascular branches, in late phase of pregnancy. Notably, the pregnant immunized mice infected with *Pb* NK65 developed liver injury associated with microvesicular fatty infiltration in late phase of pregnancy. The pathological features are similar to acute fatty liver of pregnancy. Higher levels of IFN-γ and NO were observed in plasma from pregnant immunized mice infected with *Pb* NK65 compared with those in non-pregnant immunized mice infected with *Pb* NK65. These findings suggest that development of liver injury and placental pathology in pregnant immunized mice infected with *Pb* NK65 are associated with enhanced production of pro-inflammatory cytokines.

CD4 T cells produce EBI-3^{+} cytokine inhibiting their own protective immune responses during infection with malaria parasites.

D. Kimura[1], M. Miyakoda[1], K. Honma[1], K. Kimura[1], H. Hara[2], H. Yoshida[2], K. Yui[1]

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

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

P12

We previously reported that CD4 T cells from mice infected with *Plasmodium berghei* ANKA (PbA) have severe defects in IL-2 production in response to T cell receptor (TCR)-stimulation resulting in their poor proliferative responses, while they produced high levels of IFN-γ, IL-4 and IL-10. When CD4 T cells from uninfected mice were co-cultured with those from PbA-infected mice, their IL-2 production in response to anti-TCR mAb was severely impaired, suggesting that the reduction in IL-2 production was due to the inhibitory CD4 T cells. Furthermore, culture supernatant of CD4 T cell from the infected mice was inhibitory, indicating that the inhibition was mediated by soluble mediator(s). CD4 T cell subset that produced this inhibitory molecule was foxp3^{-}CD11a^{hi}CD49d^{hi}, and not CD11a^{lo}CD49d^{lo} or foxp3^{+} cells, implying that malaria-specific foxp3^{-}CD4 T cells produced these mediators. Monoclonal Abs specific for IL-10, TGF-β, IL-4 or IFN-γ were unable to neutralize the inhibitory effect of the culture supernatant. Thus, we examined a possible involvement of IL-27 and IL-35, IL-12 family heterodimeric cytokines containing EBI3. CD4 T cells from PbA-infected EBI3 KO mice showed normal level of IL-2 production, and their culture supernatant did not inhibit IL-2 production of other CD4 T cells, suggesting the involvement of EBI3 in the inhibition of IL-2 production. Furthermore, TCRβ KO mice that were transferred with CD4 T cells from EBI3 KO mice showed improved resistance to the PbA-infection when compared with control TCRβ KO mice that received wild-type CD4 T cells. These results suggest that malaria-specific CD4 T cells produce an inhibitory EBI3^{+} cytokine and regulate their protective immune responses during infection with PbA.

Time-lapse imaging of red blood cell invasion by rodent malaria parasites

Kazuhide Yahata, Osamu Kaneko

Department of Protozoology, Institute of Tropical Medicine (NEKKEN) and the Global COE Program, Nagasaki University, Sakamoto, Nagasaki 852-8523, Japan
Email to: kyahata@nagasaki-u.ac.jp

P13

In order to propagate within the mammalian host, malaria parasites must invade red blood cells (RBCs). This process offers a window of opportunity in which to target the parasite with drugs or vaccines. However, most of the studies relating to RBC invasion have analyzed the molecular interactions of parasite proteins with host cells under static conditions, and the dynamics of these interactions remain largely unstudied. Time-lapse imaging of RBC invasion is a powerful technique to investigate cell invasion and has been reported for *Plasmodium knowlesi* and *Plasmodium falciparum.* However, experimental modification of genetic loci is laborious and time consuming for these species. We have established a system of time-lapse imaging for the rodent malaria parasite *Plasmodium yoelii*, for which modification of genetic loci is quicker and simpler. We compared the kinetics of RBC invasion by *P. yoelii* with that of *P. falciparum* and found that the overall kinetics during invasion were similar, with some exceptions. The most striking of these differences is that, following egress from the RBC, the shape of *P. yoelii* merozoites gradually changes from flat elongated ovals to spherical bodies, a process taking about 60 sec. During this period merozoites were able to attach to and deform the RBC membrane, but were not able to reorient and invade. We propose that this morphological change of *P. yoelii* merozoites may be related to the secretion or activation of invasion-related proteins. Thus the *P. yoelii* merozoite appears to be an excellent model to analyze the molecular dynamics of RBC invasion, particularly during the morphological transition phase, which could serve as an expanded window that cannot be observed in *P. falciparum*.

Accumulation of MHC class II^+ $CD11c^-$ non-lymphoid cells in the spleen during infection with *Plasmodium yoelii* is lymphocyte-dependent

Rika Kamei[1], Mana Miyakoda[1], Takahiko Tamura[1, 2], Daisuke Kimura[1],
Kiri Honma[1], Kazumi Kimura[1], Katsuyuki Yui[1, 2]

[1]Division of Immunology, Department of Molecular Microbiology and Immunology, Graduate School of Biomedical Sciences, [2]Global COE program, Nagasaki University,

P14

The spleen is the main organ for immune defense during infection with *Plasmodium* parasites. Splenomegaly is one of the major symptoms developed after *Plasmodium* infection. Using a rodent model of *Plasmodium yoelii* infection, we characterized MHC class II^+CD11c^- non-T, non-B cells in the spleen. Although the proportion of conventional dendritic cells was reduced, that of MHC II^+CD11c^- non-T, non-B cells increased during the course of infection. The increased prevalence of this subpopulation was dependent on the presence of lymphocytes. Experiments using Rag-$2^{-/-}$ mice with adoptively transferred normal spleen cells indicated that these cells were non-lymphoid cells, but their accumulation in the spleen during infection with *P. yoelii* depended on lymphocytes. These cells were detected not only spleen but also bone marrow and blood during infection. Functionally, these MHC II^+CD11c^- non-T, non-B cells were able to produce the proinflammatory cytokines TNF-α and IL-6 in response to the infected red blood cells, but had only a limited ability to activate antigen-specific $CD4^+$ T cells. This study revealed a novel interaction between MHC II^+CD11c^- non-lymphoid cells and lymphoid cells in the accumulation of these non-lymphoid cells in the spleen during infection with *P. yoelii*.

In vitro imaging of gliding motility on *Babesia bovis* merozoites

[1]Masahito Asada, [1,2]Yasuyuki Goto, [1]Junya Yamagishi, [3]Kazuhide Yahata,
[1]Naoaki Yokoyama, [1]Noboru Inoue, [3]Osamu Kaneko, and [1]Shin-ichiro Kawazu

[1]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, [2]Laboratory of Molecular Immunology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, [3]Department of Protozoology, Institute of Tropical Medicine (NEKKEN) and the Center of Excellence Program, Nagasaki University

P15

Babesiosis is a zoonosis caused by tick-transmitted intraerythrocytic protozoa of the Phylum Apicomplexa. Some specific stages of apicomplexan parasites, such as sporozoites of *Plasmodium falciparum* and tachyzoites of *Toxoplasma gondii*, invade their target host cells using a unique, active process known as gliding motility. However, it is not thoroughly understood how the merozoites of *Babesia* parasites target and invade the host red blood cells (RBCs), and the gliding motility has so far not been observed in the parasite. In this study, we revealed the gliding motility of *B. bovis* merozoites by time-lapse video microscopy. The recorded images delineated that the processes included egress of the merozoites from the infected RBC, gliding motility, and succeeding invasion into new RBCs. Based on these images, the gliding motility of *B. bovis* merozoites was similar to the helical gliding of *Toxoplasma* tachyzoites. The trails left by the merozoites were detected by indirect immunofluorescence assay using antiserum against *B. bovis* merozoite surface antigen 1. Furthermore, inhibition of gliding motility by actin filament polymerizer or depolymerizer indicated that this movement was driven by actomyosin-dependent process. This first report of gliding motility in *B. bovis* is notable and significant for the apicomplexan parasites since merozoites of *Plasmodium* parasites do not glide on the substrate. Recent studies in *Plasmodium* have highlighted the essential role of the thrombospondin related anonymous/adhesive protein (TRAP) family in the gliding and cell invasion of the parasites. Currently, we are in the process of investigating the role of the TRAP-family in the gliding motility of *Babesia* merozoites.

(253/250Words)

First report of T5 genotypes of *Acanthamoeba* spp. from clinical isolates of human keratitis cases

Md Moshiur Rahman[1], Akira Kobayashi[2], Yosaburo Oikawa[3], Amjad I.A. Hussein[4], Takahiro Matsumura[1], Masaharu Tokoro[1]

[1]Department of Parasitology, Graduate School of Medical Science, Kanazawa University, Japan
[2]Department of Ophthalmology, Graduate School of Medical Science, Kanazawa University, Japan
[3]Department of Medical Zoology, Kanazawa Medical University, Japan
[4]Department of Medical Laboratory Sciences, An-Najah National University, Nablus, West-Bank, Palestine

P16

Keywords: *Acanthamoeba,* keratitis, genotype, 18S rRNA, 16S rRNA

Introduction: *Acanthamoeba* is well-recognized as a causal agent of keratitis. To assess the genetic characterization of *Acanthamoeba* spp., we have analyzed clinical isolates from keratitis cases using a nuclear small subunit ribosomal RNA (18S rRNA) and a mitochondrial small subunit ribosomal RNA (16S rRNA) gene loci.

Methods: Altogether, twenty-seven corneal scraping and preserving solution samples were collected from patients who complained vision and corneal problems. Firstly we investigated the isolates through cultivation by ameba saline, supplemented with *Escherichia coli.* To evaluate the genotype distribution we analyzed partial sequences of approximately 550bp of the 18SrRNA gene and 1540bp of the 16SrRNA gene.

Results: The phylogenetic analysis using partial sequences of the 18S rRNA gene locus indicated that most of the *Acanthamoeba* isolates are clustered into T4 genotype (23 isolates), and others were belonging to T3 (3 isolates) and T5 (single isolate). In this study, we found for the first time T5 genotype can involve the human keratitis cases. Moreover, there are some other available previous reports of isolation of the T5 genotype from the environmental isolates other than human cases. To reconfirm the findings and to reexamine the results, all isolates were subsequently analyzed by using the alternative gene locus (16SrRNA). The result confirmed again 26 isolates had unique sequences as the same genotypes with significant bootstrap values of T3, T4 and T5 as observed in 18sRNA analysis.

Conclusions: After evaluation and compare the multi gene analysis, we would like to confirm, this is the first time isolation of T5 genotype from a keratitis case.

Sulfate activation in mitosomes plays an important role in *Entamoeba histolytica*

Fumika Miichi

Division of Molecular and Cellular Immunoscience, Department of Biomolecular Sciences,
Faculty of Medicine, Saga University

P17

Hydrogenosomes and mitosomes are mitochondrion-related organelles in anaerobic/microaerophilic eukaryotes with highly reduced and divergent functions. *Entamoeba histolytica*, which causes intestinal and extraintestinal amebiasis in humans, possesses mitosomes. Previously, we demonstrated that sulfate activation is compartmentalized in *E. histolytica* mitosomes, and that activated sulfate is predominantly incorporated into seven sulfur-containing lipids. Furthermore, parasites in which genes involved in sulfate activation pathway were suppressed by gene silencing showed marked growth retardation. These results indicate that sulfur-containing lipids are important for parasites growth. However, the physiological role of sulfate activation in *E. histolytica* remains largely unknown. One of the important issues to solve this question is to characterize these sulfo-containing lipids. Currently, we are trying to establish the purification procedure of these sulfur-containing lipids and analyzing their structures. In this study, we report the progress of these trials.

IFN-γ is necessary for clearance of pathogenic amoebae from the intestine of mice

Chikako Shimokawa, Shinjiro Hamano

Institute of Tropical Medicine Nagasaki University

We revealed that not only *Entamoeba histolytica* (Eh) but also its relative species *E. moshkovskii* (Em) were pathogenic in mice.

Eh caused persistent infection for more than 4 weeks without severe symptoms. In contrast, Em-infected mice suffered from weight loss with severe diarrhea, and expelled amoeba within 2 weeks, suggesting that expulsion of Em is associated with vigorous inflammations accompanied by harmful responses to the host. To clarify the role of immune responses in protection and also in pathology, we first examined the kinetics of lymphocytes in several tissues. IFN-γ-producing CD8 T cells were found to increase in Peyer's patches (PPs) in Em-infected mice but not in Eh-infected mice. Then, neutralization of IFN-γ in mice infected with Em resulted in prolonged infection with attenuated symptoms. Taken together, it was suggested that IFN-γwas responsible for protection and pathology in mice infected with Em.

We propose that the clearance of Em from the intestine correlates with IFN-γproduction and currently we are trying to figure out how IFN-γexerts its function in the expulsion of Em.

Opportunistic Intestinal Parasitic Infection in HIV Positive Patients in Bali Island, Indonesia

Mudyawati Kamaruddin[1,2], Ei Kawahara[2], Din Syafruddin[3], Masaharu Tokoro[1]

[1]Department of Parasitology, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan; [2]Department of Laboratory Sciences, School of Health Sciences, Faculty of Medicine, Kanazawa University, Kanazawa, Japan; [3]Eijkman Molecular Biology Institute, Department of Parasitology, Jakarta, Indonesia.

P19

Human immunodeficiency virus (HIV)-infected individuals have greater susceptibility to infections by intestinal protozoan parasites, which can cause significant morbidity and mortality to the host compared to immunocompromised individuals. Prevalence of opportunistic infections (OIs) caused by intestinal protozoan parasites also vary according to the geographical area and the endemic levels in each location. Reports indicate that diarrhoea occurs in 30-60% of AIDS patients in developed countries, whereas it reaches up to 90% in developing countries.

To assess the status of opportunistic protozoan infection in developing areas, we conducted an intestinal protozoan molecular screening for HIV/AIDS patients at Bali Island, Indonesia. The 83 patients maintained by highly active anti-retroviral therapy (HAART) were recruited to this study. From the fecal samples, genomic DNA was extracted, and subjected to the PCR/DNA sequencing analyses for intestinal protozoan parasites.

Within those 83 samples, 7 (8.4%) *Cryptosporidium* spp. (2 *C. hominis*, 1 *C. parvum* and 4 *C. meleagridis*), one of AIDS related OIs, were detected. While, 27 (32.5%) *Giardia intestinalis*, and amebic infections such as 2 (2.4%) *Entamoeba dispar*, 3 (3.6%) *E. coli* , 5 (6.0%) *E. hartmanni* were also detected. These data showed that non-pathogenic amebic infection can be harmful as opportunistic infection in immunocompromised patients.

The high prevalence of certain opportunistic parasites among HIV positives is well known. Such co-infections present with more severe clinical symptoms compared to parasite infections of otherwise healthy people, and are more difficult to treat. Parasite-HIV co-infections are one of the neglected areas in HIV research although HIV generally has become a major public health concern and research topic in Indonesia. Even since the concerns regarding opportunistic parasite infections among HIV positives have been widely recognized, there is still lack of available data among HIV-infected individuals have been reported in Indonesia.

Key words: Opportunistic infections; Protozoan parasite; HIV/AIDS; immunocompromised

The role of IRF4 expressed in DCs during protective immune responses against *Leishmania major*

Masoud Akbari[1] ,Kiri Honma[1], Daisuke Kimura[1], Mana Miyakoda[1], Kazumi Kimura[1],
Toshifumi Matsuyama[2], Katsuyuki Yui[1]

[1]Division of Immunology, [2]Division of Cytokine signaling, Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Science

IFN regulatory factor-4 (IRF-4) is a member of IRF family, and are expressed in immune cells including lymphocytes, macrophages and dendritic cells (DCs). Our laboratory previously showed that IRF4 KO mice exhibited reduced footpad swelling 2-5 wks after infection with *Leishmania major (L. major)*, but the cell type responsible for this phenomenon was not identified. To examine the role of IRF4 expressed in DCs, we used conditional knock-out mice that lack IRF4 in DCs (CD11c-cre IRF4fl/fl mice, IRF4-/-(DC)).

IRF4-/-(DC) mice showed reduced footpad swelling when compared to the control IRF4fl/fl mice and to the mice that lack IRF4 in macrophages (LysM-cre IRF4fl/fl mice), suggesting that IRF4 expressed in DCs play critical roles for this phenomenon. IRF4-/-(DC) mice showed reduced parasite burden, and their $CD4^+$ T cells produced higher levels of IFN-γ in response to *L. major* antigen, indicating that the reduced footpad swelling was due to the enhancement of the Th1 type protective immune responses. In the draining lymph nodes (dLNs), the proportion of activated $CD4^+$ T cells in IRF4-/-(DC) mice was similar to the control, but that of IFN-γ-producing cells was increased, suggesting Th1 bias of the immune responses. The number of migrating Langerhans cells and regulatory T cells in the dLNs was reduced, but DCs produced higher levels of IL-12 in IRF4-/-(DC) mice. These results imply that IRF4 expressed in DCs promotes their migratory behavior while inhibiting their IL-12 production, and thus regulate $CD4^+$ T cell responses against local infection.

P20

Novel method using heat-shock operon (*groESL*) gene for the specific detection and phylogenetic analyses of *Anaplasma* species*

Adrian P. Ybanez

Obihiro University of Agriculture and Veterinary Medicine

Anaplasma spp. are important infectious vector-borne pathogens of humans and animals worldwide. The 16S rRNA gene, which is less divergent and prone to genetic recombination, is commonly utilized in PCRs that screens for these pathogens, while other genes are preferred for the specific detection of individual species. The present study aimed to develop a more convenient and novel diagnostic method that can detect all *Anaplasma* spp. and amplify a specific gene fragment that is sufficient to allow species delineation and robust phylogenetic analyses. A PCR assay based on the multiple sequence alignment of the heat-shock operon (*groESL*) gene of all registered *Anaplasma* spp. was developed. The method targeted 921-923 bp and 443-447 bp fragments for the 1st and 2nd round PCR, respectively. DNA samples from different *Anaplasma* spp., including *A. marginale, A. centrale, A. bovis, A. ovis, A. phagocytophilum,* and the potentially novel *Anaplasma* sp. of Japan were used. To evaluate for its specificity, *Ehrlichia*, *Babesia*, *Theileria* and *Trypanosoma* spp. were also tested. Results revealed that the new method was shown to be highly sensitive and specific, detecting dual infections of *A. centrale* and the potentially novel *Anaplasma* sp. of Japan from sika deer, and *A. ovis* from cattle in Mongolia. Moreover, phylogenetic analysis showed that *A. centrale* Aomori strain of Japan was divergent from other *A. centrale* sequences. The present study successfully developed a novel reliable method for the specific detection of *Anaplasma* spp., and clarified the phylogenetic relationships of closely related *Anaplasma* spp.

Keywords: *Anaplasma*; *groESL;* heat-shock operon

P21

Exploiting Multimeric T Cell (Co-)Receptors as Probes for Antigen Presentation

Sock Yue THONG,[1,2] Gladys YEO,[1,2] and Gijsbert GROTENBREG[1,2,3]

[1]Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[2]Immunology Programme, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[3]Department of Biological Sciences, Faculty of Science, National University of Singapore, Singapore

The interaction of peptide-MHC (pMHC) with a corresponding T cell receptor (TCR) governs the detection and subsequent activation of T cells. Intrinsically, the affinity of the TCR:pMHC interaction is weak (Kd ~1–100 μM). The co-receptors CD8 or CD4 that interact with class I MHC or class II MHC, respectively, on antigen presenting cells play an indispensible role. The association of CD8 with the class I MHC complex, for example, has two overlapping consequences; CD8 participates in signaling and it stabilizes the overall TCR:pMHC complex. In most cases, the sensitivity and strength of TCRs for their respective class I pMHC partners are enhanced by their CD8 co-receptors. Unlike pMHC tetramers, which are frequently applied in a wide range of immunological studies, including staining and activation of T cells, TCR tetramers are not as commonly used to probe antigen presentation on cells. While TCRs can be engineered to higher affinities for the pMHC partners, these non-native TCRs may lose their specificity. Hence, the introduction of co-receptors to TCR multimers would enable enhanced avidity and/or affinity for pMHC. We will present the detailed characterization of these interactions that occur during the association of pMHC and CD8+ T-cell surfaces through the use of fluorescent-labeled T cell (co-) receptor multimers in recently developed flow cytometry bead assays. These multimeric T-cell (co-) receptors are envisaged to facilitate the detection of antigen presentation, particularly those of infectious diseases and/or their accompanying opportunistic pathogen infections, in which early detection is important.

Acknowledgment: This work was supported by the Singapore National Research Foundation Research Fellowship (NRF2007NRF-RF001-226).

P22

Analysis of the unique hepatic T cell population induced during *Schistosoma mansoni* infection in mice

Keishi Adachi[1,2], Shinjiro Hamano[1,2]

[1]Department of Parasitology, Institute of Tropical Medicine (NEKKEN),
[2]Global COE program, Nagasaki University, Nagasaki 852-8523, Japan

BACKGROUNDS: In schistosomiasis, serious hepatic disorders are induced in the host. A liver possesses unique immune systems composed of the specialized cells different from those of other immune competent organs or tissues. Although a large amount of systemic as well as enteric blood-borne antigens constitutively enter liver, immune responses look suppressed in a homeostatic state, and hepatic lymphocytes show 'activated yet resting' phenotypes.

After cercarial infection, the host shows Th1-related responses in the early phase. As the parasites mature, mate and begin to produce the eggs, Th2 reactions are evoked. In the patients not showing serious symptoms, appropriate phase transitions are observed. Because it is believed that Th1 and Th2 inhibit their generation each other, we hypothesized that the unknown mechanisms bridging their generations exist in the liver between the Th1 and Th2 phases (transition phase).

P23

Last year, we reported several characteristic immune responses in the *Schistosoma mansoni* (*S. mansoni*)-infected mice during the transition phase; 1) elevation of serum levels of IL-18, 2) more vigorous cytokine productions by hepatic T cells than those by splenocytes upon TCR ligation, 3) the induction of the "unique T cell populations" in the liver which were doubly positive for IFN-γ and IL-13 and for IFN-γ and IL-4. Here we report more precise natures of the "unique hepatic T cells".

RESULTS and DISCUSSION: By flowcytometric analysis, we demonstrate that "the unique hepatic T cells"; 1) are consisted of DX5-positve and –negative subpopulations, 2) are γδ TCR-negative, 3) produce little IL-5, suggesting the selectivity of Th2 cytokine production, 4) include the population triply positive for IFN-γ, IL-13 and IL-4. Our findings indicate that liver-intrinsic immune environments and reactions are intimately involved in the hepatic disorders induced by *S. mansoni* infection.

We are now investigating the roles of IL-18 and the "unique T cell populations" upon *S. mansoni*-induced hepatic pathology.

The Right Chemistry to Initiate a Break-Up of Peptide-MHC

Joanna Ai Ling CHOO,[1, 2] Sock Yue THONG,[1, 2] Jiawei YAP,[1, 2] Pei Yiing LIM,[1, 2] Ton SCHUMACHER,[3] Steven H L VERHELST[4] and Gijsbert M GROTENBREG[1, 2, 5]

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

[2] Immunology Programme, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

[3] Division of Immunology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

[4] Center for Integrated Protein Science Munich, Lehrstuhl für Chemie der Biopolymere, Technische Universität München, Germany

[5] Department of Biological Sciences, Faculty of Science, National University of Singapore, Singapore

The development and application of fluorochrome-conjugated peptide-major histocompatibility complex (pMHC) multimers in conjunction with flow cytometry has made the direct visualization, quantification and phenotypic characterization of T cells possible. The utility of this technology has been extensively demonstrated in studies on $CD8^+$ T cell responses for a number of pathogenic challenges such as *Toxoplasma gondii*, *Chlamydia trachomatis*, Epstein-Barr virus and human immunodeficiency virus. A recently developed strategy employing photolabile peptides transiently loaded onto class I MHC molecules allows the generation of arrays of novel pMHC molecules with defined specificity from a common precursor upon UV irradiation. Nevertheless, this high-throughput method is unsuitable for several applications due to the generation of free radical species and the induction of heat during UV irradiation, as well as the lack of UV penetration within cell culture systems. Chemolabile pMHC molecules have been developed as an alternative. Although conceptually successful, the strategy in its current form is not practical as it suffers both from incomplete peptide exchange and epitope modification due to harsh cleavage conditions that abolishes T cell recognition. We present the development of class I pMHC tetramers loaded with an alternative chemically cleavable ligand that enables the generation of functional pMHC libraries under conditions that pose no deleterious effects on cell viability and leave the newly presented epitope intact. This advance represents an important milestone that permits broader application of pMHC tetramer library technology for the characterization of $CD8^+$ T cell responses against a variety of infectious agents.

Acknowledgement: This work was supported by the Singapore National Research Foundation Research Fellowship (NRF2007NRF-RF001-226). JALC is recipient of an NUS School of Medicine graduate scholarship.

P24

Molecular characterization of a Novel Protein-Coding Gene Family with SEA-like Domain in *Schistosoma japonicum*

Evaristus C. Mbanefo[1], Yu Chuanxin[2], Mihoko Kikuchi[1], Mohammed N. Shuaibu[1], Daniel Boamah[1], Masashi Kirinoki[3], Naoko Hayashi[3], Yuichi Chigusa[3], Yoshio Osada[4], and Kenji Hirayama[1*]

[1]Departments of Immunogenetics, and [5]Parasitology, Institute of Tropical Medicine (NEKKEN), Nagasaki University, [2]Jiangsu Institute of Parasitic Diseases, Wuxi, PR of China, [3]Tropical Medicine and Parasitology, Dokkyo Medical University, [4]Immunology and Parasitology,
University of Occupational and Environmental Health

Schistosoma japonicum infection remains a major public health problem. With its non-human reservoir hosts complicating control efforts; there is urgent need for protective and/or anti-pathology vaccines. Secreted antigens and parasite tegument proteins are potential vaccine candidates being located at the host-parasite interface. While isolating secreted and membrane binding antigens, we had identified a novel protein family with similar promoter region and signal sequence. Here we performed molecular and immunological characterization of some of the candidate proteins. No ancestral homologue of this protein family was found in the genome of any other organism. We therefore utilized three-dimensional structure modeling to show striking similarity between the molecular structure of the candidates and SEA domain (sea urchin proteins, enterokinase and agrin), a modules common among glycoproteins of diverse functions. We recorded differential stage specific mRNA expression, both among developmental stages and between characterized candidates. Using the expressed recombinant proteins, we confirmed expression of the candidate antigens in the adult worm and egg antigen preparations; and found that the molecules exist as oligomers in the native state, with the tetramer as the most stable state. Immunolocalization showed localization on adult worm teguments and epithelial linings of internal organs. The candidates induced significant levels of IgG and IgG subtypes in immunized mice. A robust cellular immune response was observed, with significant CD4+ T-cell proliferation. Cytokine analyses showed disproportionately Th1 cellular immune response. The characterized candidates are located at the host-parasite interface and highly immunogenic; eliciting a Th1 dominant immune response. Th1 polarized response, especially if sustained through the onset of egg production, is required for protective immunity. Thus, these candidates with SEA-like domain hold potential for application in protective or anti-pathology prophylaxis, in diagnostics, among other applications.

P25

Morbidity and risk factors of *Schistosoma mansoni* among primary school children in western Kenya

Sachiyo Nagi[1], Evans Chadeka[1], Faith Mutungi[2], Yombo K. Dan Justin[1], Makoto Ito[3], Satoshi Kaneko[1],Yoshio Ichinose[1], Sohkichi Mastumoto[4], Sammy M. Njenga[2], Toshihiko Sunahara[1], Masahiro Hashizume[1], Masaaki Shimada[1] and Shinjiro Hamano[1],

[1]Dept. of Parasitol, Institute of Tropical medicine, Nagasaki University, [2]Eastern & Southern Africa Centre of International Parasite Control (ESACIPAC), Kenya Medical Research Institute (KEMRI), [3] Dep. of Parasitology, Aichi Medical University,

[4] Dep. of bacteriology, Osaka City University

The transmission of schistosomiasis is closely related to socio-demographic and behavioral factors; however, limited intervention efforts based on definitive potential risk factors using morbidity as an indicator. To elucidate morbidity and risk factors related to helminthic infection, a cross-sectional study was conducted on 310 primary school children in Mbita district, a rural area of western Kenya. Biological samples and socio-demographic and behavioral information were collected to examine schistosomes, and soil-transmitted helminths.

We found that 238 (76.8%) students were infected with *Schistosoma mansoni* while 7 (2.3%) were infected with *S. haematobium*. The prevalence of hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* were 19 (6.1%), 16 (5.2%) and 7 (2.3%), respectively. Chi-square test and binomial logistic regression analysis were carried out for determining potential risk factors of parasitic infections and only found significant associations between *S.mansoni* and age (P=0.03) and school location (P<0.001).

To identify the prevalence of *S.mansoni* varies by area, we separated the targeted schools based on locations and found significant difference between two areas, where one area showed three times higher infection rate compared to the other area【adjusted odds ratio: 2.99, 95% Confidence Interval (CI): 1.64-5.64, P<0.001】. Unexpectedly, prevalence of *S. mansoni* infection was not associated with distance from the lake. However, *S. mansoni* infection tended to decrease from town to rural settings. Though it is necessary to prove by cohort study, our preliminary analysis suggest that high population density is the one of risk factor for driving morbidity due to *S.mansoni* infection in Mbita area.
However,

P26

High Incidence and Mortality of Congenital Rubella Syndrome in Central Vietnam.

Michiko Toizumi[1], Hideki Motomura[2], Kensuke Takahashi[3], Pham Enga[4], Von Minh Hien[4], Lay Myint Yoshida[1], Le Huu Tho[5], Dan Duc Anh[6], Masahiro Hashizume[1], Koya Ariyoshi[3], Hiroyuki Moriuchi[2]

[1]Department of Pediatric Infectious Diseases, Institute of Tropical Medicine, Nagasaki University, Japan
[2]Department of Pediatrics, Nagasaki University Hospital, Japan
[3]Department of Clinical Medicine, Institute of Tropical Medicine, Nagasaki University, Japan
[4]Department of Pediatrics, Khanh Hoa General Hospital, Vietnam
[5]Office of Vice Director, Khanh Hoa Provincial Health Center, Vietnam
[6]Office of Deputy-Director of General, National Institute of Hygiene and Epidemiology, Vietnam

<Background>
Outbreaks of rubella and congenital rubella syndrome (CRS) still arise in countries where rubella-containing vaccine is not included in the national immunization programs, including Vietnam. Following a large-scale rubella outbreak in 2011, we experienced the emergence of CRS cases in Khanh Hoa Province, Vietnam. The aim of this study is to clarify clinical and epidemiological features of CRS in the region.
<Methods>
From October 2011 to September 2012, a prospective surveillance for CRS was conducted among infants aged <12 months with any manifestations suspected of CRS at Khanh Hoa General Hospital, the only referral hospital in the region. Study subjects underwent standard examinations, echocardiography, cranial ultrasonography, automated auditory brainstem responses and blood sampling for blood counts and rubella-specific antibodies. Mothers were asked their demographics as well as clinical information and knowledge of rubella through interview.
<Results>
A total of 38 children with CRS were enrolled. Clinical manifestations included low birth weight (71%), cardiac defects (72%), cataracts (11%), hearing impairment (93%), purpura (84%), hepatosplenomegaly (71%), and thrombocytopenia (76%). Patent ductus arteriosus was the most common cardiac complication, often associated with pulmonary hypertension. As of October 2012, 12 infants (32%) died. Most mothers experienced fever and rash in the first trimester and knew little about rubella and CRS.
<Conclusions>
Mortality and morbidity are still very high among CRS babies born following rubella outbreak in Vietnam. Introduction of rubella-containing vaccine in routine immunization program with catch-up vaccination to children and women of childbearing age will reduce CRS in Vietnam.

P27

IDENTIFICATION OF A BOVINE ROTAVIRUS TRANSMITTED DIRECTLY TO AND CAUSED DIARRHEA IN A HUMAN CHILD

Yen Hai Doan[1], Osamu Nakagomi[1], Toyoko Nakagomi[1],
Yair Aboudy[2], Ilana Silberstein[2], Esther Behar-Novat[3],
Lester M. Shulman[2,4]

[1]Nagasaki University, Nagasaki, Japan
[2]Central Virology Laboratory, Israel Ministry of Health, Tel Hashomer, Israel
[3]Stanford Medical School, Palo Alto, CA, USA
[4]Tel Aviv University, Tel Aviv, Israel

Multiple genotypes occur for each genome segment of rotaviruses, allowing to speculate the host species of an isolate from the genotype constellation. Albeit rarely, interspecies transmission occurs either by genetic ressortment or as a whole virion. A G6P[1] rotavirus, Ro8059, was isolated from a one-year old boy in Israel in 1995. Since genotype G6P[1] is generally associated with bovine rotaviruses, and the child developed diarrhea within days of his first contact with calves at an urban farm, this study was undertaken to characterize the whole genomic RNA constellation of Ro8059 and four G6P[1] bovine strains by RNA–RNA hybridization and full genome sequencing. The genome constellations of all four bovine G6P[1] strains were G6-P[1]-I2-R2-C2-M2-A3-N2-T6-E2-H3 for VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5, respectively. Ro8059 shared the same genotype constellation with these bovine strains, with high nucleotide sequence identities for each of the 11 genome segments, indicating that Ro8059 represented a direct transmission of a rotavirus from a calf to a human child as a whole virion. To our knowledge, this was the earliest example with a complete epidemiological link in which an entirely bovine rotavirus directly infected a human child and caused diarrhea. Thus, all bovine rotaviruses may not always naturally be attenuated to humans.

P28

Establishing an outbreak alert system for priority diseases in Kenya

Mitsuru Toda, Ian Njeru, Matilu Mwau, Shingo Inoue, Kouichi Morita

Department of Virology, Institute of Tropical Medicine, Nagasaki University
NUITM Africa Research Station, PO 19993-00202
mitsuru.toda@gmail.com

Background

Arbovirus such as yellow fever and Rift Valley fever are serious public health concerns in sub-Saharan countries. The JST-JICA SATREPS project aims to tackle these issues by developing an affordable and domestically producible rapid test kits, enhancing reference laboratory capabilities, and establishing a model for an outbreak alert system in Kenya.

One of the key components of this project is to enhance the social technology on disease prevention and control. In partnership with the Division of Disease Surveillance and Response (DDSR) at the Ministry of Public Health and Sanitation (MOPHS), a pilot study of SMS-based outbreak alert system will be implemented in 2013. The poster will summarize the research methodologies and plans for the pilot.

Methods

A 6-month pilot will be conducted using randomized control trial methods. A total of 153 facilities in 12 districts will be followed closely in Kenya. Of those, half will be randomly selected receive the intervention. The study will measure the time spent between disease detection, notification, and response at peripheral facilities and national and district level surveillance and response coordinators. We will also measure the data quality of outbreak notifications. Because outbreaks may not occur during the study period, mock outbreak simulations will be conducted to measure improvements in the key indicators. We expect to observe improvements in the timeliness of suspected outbreak notification and response in our intervention facilities.

Conclusion

The pilot study will help us guide discussions on whether the model will be rolled out to wider geographical areas in the country and or the region. We expect that this new innovative system will help enhance the disease surveillance and response mechanisms in Kenya.

(Total abstract word count: 273)

Direct Evidence for Genetic Reassortment between Co-circulating Human Rotaviruses by Full Genome Sequencing of a G3P[4] Rotavirus Strain Isolated in India

T. N. Hoa Tran, Toyoko Nakagomi, Osamu Nakagomi

Department of Molecular Microbiology and Immunology, Graduate School of Biomedical Sciences, and The Global Center of Excellence, Nagasaki University, Nagasaki, Japan

Rotavirus A is the single most important etiological agent of severe diarrhea in infants and young children and is the major cause of child mortality in many resource-poor countries. Unlike in developed countries, a myriad of unusual combinations of G and P genotypes have been observed in rotaviruses detected in developing countries, raising a concern about their becoming a threat to the existing vaccines. Mixed infection of a single individual with more than one strain is thought to be a mechanism by which genetic reassortants are formed with unusual G and P combinations. However, few studies provided direct evidence that such unusual strains were formed as a result of co-infection of locally co-circulating strains. Here, we re-analyzed by employing full-genome sequencing techniques a G3P[4] strain, 107E1B, detected in India in 1993, and revealed that the genome of 107E1B had virtually an identical nucleotide sequence with that of a co-circulating G2P[4] strain, 116E3D, except their VP7 genes. Phylogenetic analysis identified the VP7 gene of 107E1B as of typical human rotavirus origin and showed it was 99.3% identical with that of another Indian G3 strain. Thus, this study provided robust evidence that the G3P[4] strain was formed through genetic reassortment in which a G2P[4] strain with a typical DS-1 genogroup background acquired the VP7 gene from a locally co-circulating G3 human rotavirus strain. Thus, this study founded a basis on which to facilitate full genome sequence analysis of an increasing number of G3P[4] strains in China and elsewhere in the world.

P30

Salmonella Stn regulates membrane composition and integrity

Masayuki Nakano and Toshiya Hirayama

Department of Bacteriology, Institute of Tropical Medicine, Nagasaki University, Japan

It has been shown that *Salmonella* enterotoxin (Stn) is considered a *Salmonella* virulence factor and a responsible for the enterotoxicity of *Salmonella*. However, the role of Stn in *Salmonella* virulence is still debated because it had been reported that the contribution of Stn in the virulence is variable in each strain. In this study, to evaluate the functions of Stn, we examined the relationship between Stn and *Salmonella* virulence using the *stn* gene-deleted mutant in *in vitro* and *in vivo* models.

S. Enteritidis strain 171, a clinical isolate from Thailand, was used as a standard strain. The mutant strain was constructed by homologues recombination. To evaluate the functions of Stn, we examined the virulence phenotypes of mutant strain using cultured mammalian cells and by a murine ligated ileal loop model. In addition, we also performed the protein profiles of mutant strain.

Although we examined *Salmonella* virulence in *in vitro* and *in vivo* models, we did not observed remarkable difference of virulence phenotypes between wild-type and mutant strain. We next characterized the phenotypes of mutant strain in various *in vitro* conditions. When we analyzed the proteomic profile of total *Salmonella* cell membrane, it was remarkable for the absence of a protein signal in the mutant strain, which was identified as OmpA. To verify this result, the morphology of *Salmonella* was examined by transmission electron microscopy and OmpA was localized by immuno-gold labeling. In this system, Stn, affected membrane morphology. These results indicate that Stn, via regulation of OmpA localization, functions in the maintenance of membrane integrity.

P31

Dipeptide production and assimilation in *Porphyromonas gingivalis* by four dipeptidyl peptidases

Shakh M. A. Rouf, Yuko Ohara-Nemoto, and Takayuki K. Nemoto

Department of Oral Molecular Biology, Course of Medical and Dental Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8588, Japan

Porphyromonas gingivalis, a Gram-negative anaerobe, is an asaccharolytic periodontal pathogen, and is recently reported to closely relate to systemic diseases, such as cardiovascular diseases, decreased kidney function, and rheumatoid arthritis. The bacterium solely utilizes amino acids as energy and carbon sources, which are incorporated mainly as dipeptides rather than free amino acids into the cell. Although gingipains, potent endopeptidases, initially producing oligopeptides, and dipeptidyl peptidase (DPP) IV, DPP7 and DPP11, which thereafter release dipeptides, have been biochemically well characterized, the mechanism of dipeptide production is yet to be fully understood. We here define the fourth enzyme DPPV, which complements the repertoire not covered by DPPIV, 7 and 11. **[Methods]** Recombinant DPPs were expressed in an *Escherichia coli* expression system. DPP-disrupted *P. gingivalis* strains were constructed by homologous recombination using erythromycin-, tetracycline-, and ampicillin-resistant genes. Peptidase activity was measured with dipeptidyl MCA substrates. **[Results]** Most dipeptide productions were lost in a ΔDPPIV-7-11 triple knock out strain, suggesting that dipeptide production was largely explained by these three DPPs. However, some of the activities releasing Lys-Ala, Gly-Phe, Met-Leu, and Ser-Tyr were still maintained in the mutant. Interestingly, this substrate preferential profile was similar to that of *P. gingivalis* DPPV, and moreover, these activities were shut down in a ΔDPPIV-V-7 mutant. Although DPPV shared the hydrophobic P1-position specificity with DPP7, these two DPPs seemed to be allocated to respective substrates by the difference in the P2-position specificity. **[Conclusion]** DPPIV, V, 7, and 11 are committed to efficient dipeptide production in *P. gingivalis*.

P32

Low-density lipoprotein receptor-related protein-1 (LRP-1) mediates autophagy and apoptosis caused by *Helicobacter pylori* VacA

Toshiya Hirayama and Masayuki Nakano

Department of Bacteriology, Institute of Tropical Medicine, Nagasaki University,

In *Helicobacter pylori* infection, vacuolating cytotoxin (VacA)-induced mitochondrial damage leading to apoptosis is believed to be a major cause of cell death. It has also been proposed that VacA-induced autophagy serves as a host mechanism to limit toxin-induced cellular damage. Apoptosis and autophagy are two dynamic and opposing processes that must be balanced to regulate cell death and survival.

Here we identify the low-density lipoprotein receptor-related protein-1 (LRP1) as the VacA receptor for toxin-induced autophagy in the gastric epithelial cell line AZ-521, and show that VacA internalization through binding to LRP1 specifically regulates the autophagic process including generation of LC3-II from LC3-I, which is involved in formation of autophagosomes and autolysosomes. Knockdown of LRP1 and Agt5 inhibited generation of LC3-II as well as cleavage of PARP, a marker of apoptosis, in response to VacA, whereas caspase inhibitor, Z-VAD-FMK, and necroptosis inhibitor, Necrostatin-1, did not inhibit VacA-induced autophagy, suggesting that VacA-induced autophagy via LRP1 binding precedes apoptosis. Other VacA receptors such as RPTPα, RPTPβ, and fibronectin did not affect VacA-induced autophagy or apoptosis.

Therefore, we propose that the cell surface receptor, LRP1, mediates VacA-induced autophagy and apoptosis.

P33

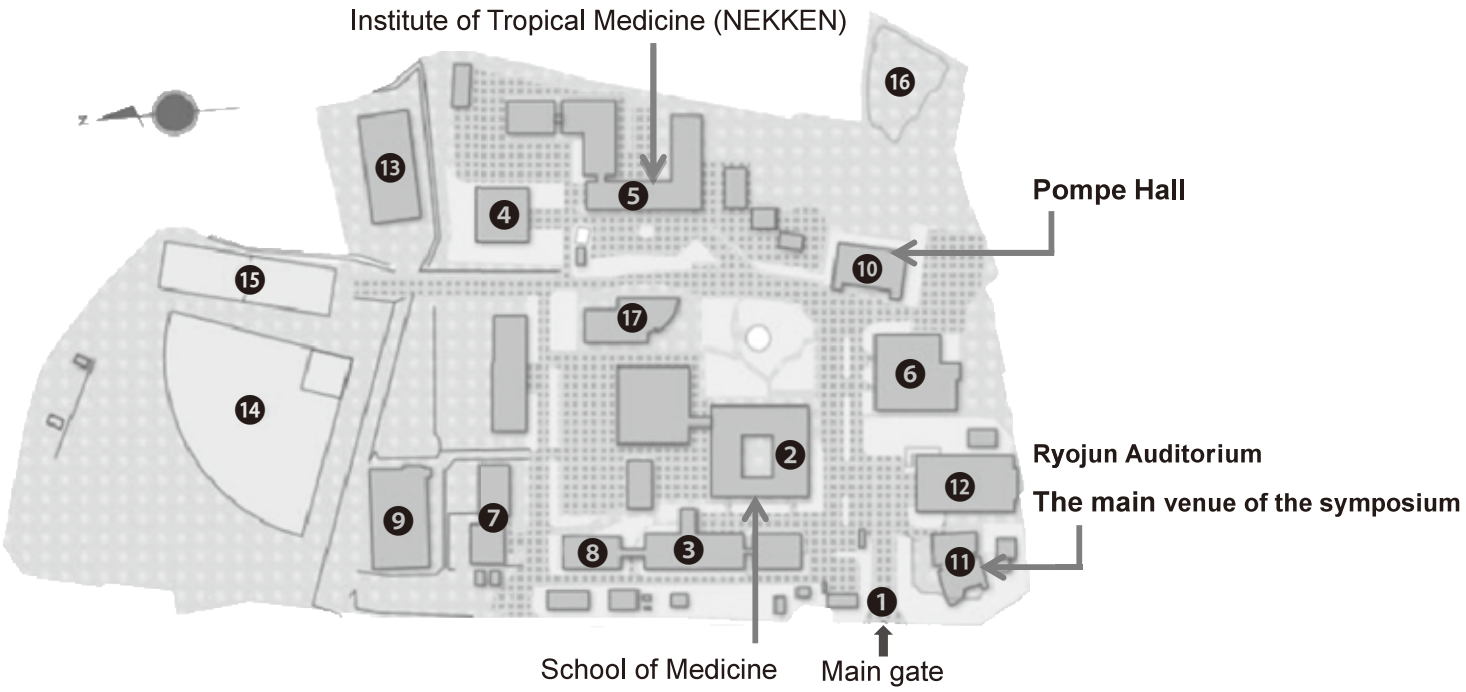
Role of SAM domain of SAMHD1 in its function as an innate immune factor against HIV-1 infection

Sayuri Shigematsu, Hideki Hayashi, Toshifumi Matsuyama, Yoshinao Kubo
(Division of Cytokine Signaling, Graduate School of Biomedical Sciences, Nagasaki University, Japan)

SAMHD1 was originally isolated as a human homologue of mouse Mg11 which is an interferon γ (IFN-γ)-inducible gene. Mutations in SAMHD1 lead Aicardi Goutieres syndrome, mimicking a congenital viral encephalopathy. The patients have enhanced IFN-α production, suggesting that SAMHD1 inhibits inflammation signals. In addition, SAMHD1 has been reported to function as an innate immune factor against HIV-1 infection, and to be degraded by a HIV-2 accessory protein, Vpx. SAMHD1 is consistent with sterile alpha motif (SAM) histidine-asparadic acid containing (HD) domain. The HD domain converts deoxynucleotide triphosphate to deoxynucleoside and inorganic triphosphates. SAMHD1 inhibits reverse transcription of the viral RNA genome to DNA by reducing cellular concentration of deoxynucleotide triphosphate. However, the role of SAM domain is still unclear. Interestingly, SAMHD1 can suppress HIV-1 infection only in monocyte-derived macrophages, suggesting that the function of SAMHD1 to inhibit HIV-1 infection is regulated by unknown cellular factors, which may associate with the SAM domain. Searching many fibroblast cell lines, we identified a cell line in which SAMHD1 can suppress VSV-pseudotyped HIV-1 vector infection (designated SIH cells). To know the role of SAM domain for the SAMHD1-mediated inhibition of HIV-1 infection, two deletion mutants of SAMHD1 were constructed. The mutant HD1 defects the SAM domain, and the mutant SAM encodes only the SAM domain. To detect these mutant proteins by Western immunoblotting, C-terminally HA tagged mutants were also constructed. The HD and SAM mutants both inhibited the pseudotyped HIV-1 vector infection in the SIH cells as well as the wild type SAMHD1. The SIH cells endogenously expressed SAMHD1 analyzed by RT-PCR. On the other hand, expression level of the HD mutant was lower than those of the wild type SAMHD1 and the SAM mutant. In conclusion, these results suggest that the SAM domain stabilizes the SAMHD1 protein, and association of the SAM domain with an unknown cellular factor reduces the function of SAMHD1 to inhibit HIV-1 infection.

P34

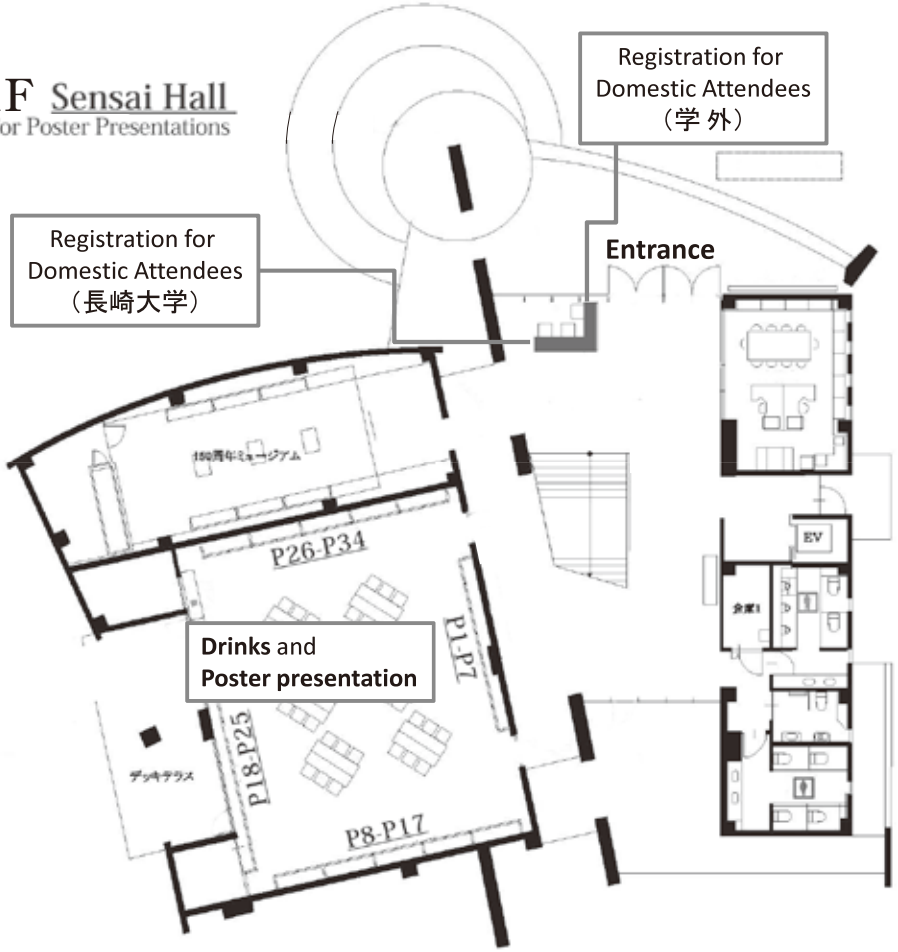
■Campus Map



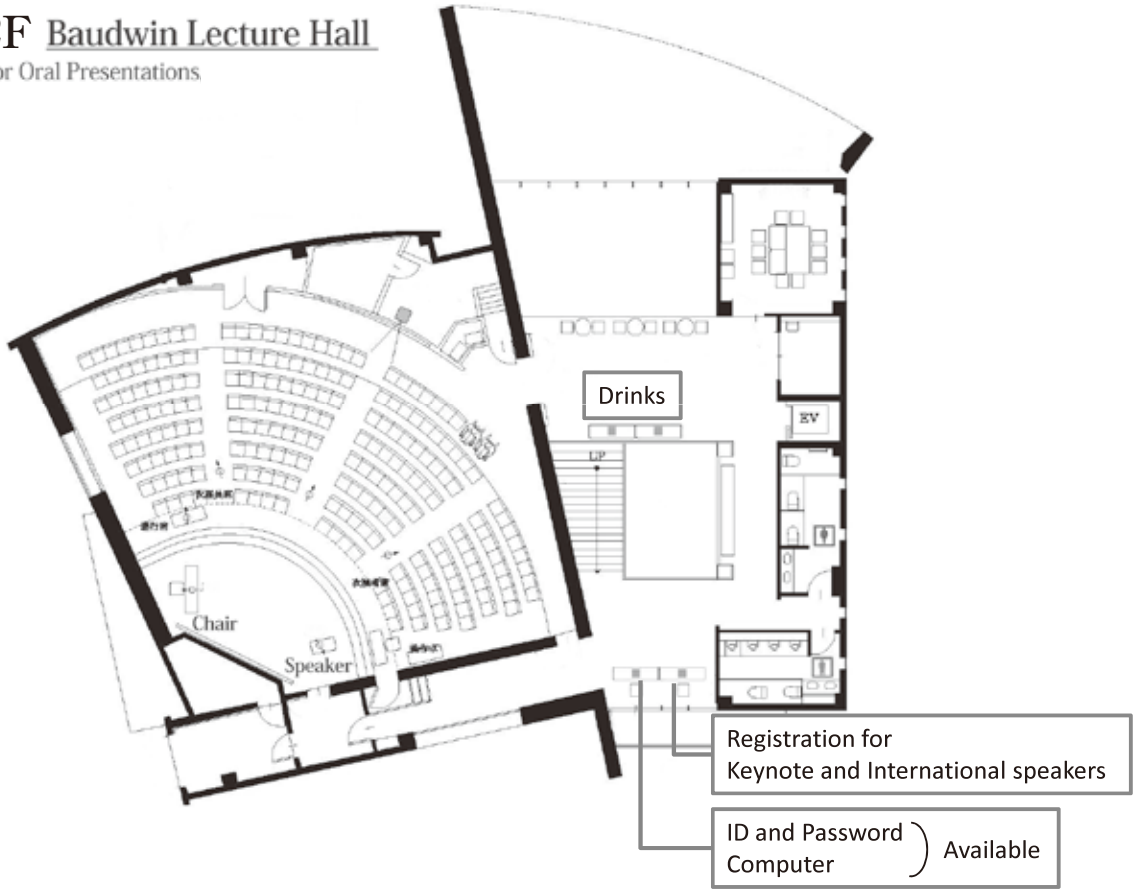
❶ Main Gate	★❷ School of Medicine	❸ Atomic Bomb Disease Institute
❹ Second Building of the Atomic Bomb Disease Institute	★❺ Institute of Tropical Medicine (NEKKEN)	❻ Medical Library
❼ Center for Frontier Life Sciences (Radioisotope Research Center)	❽ Center for Frontier Life Sciences (Gene Research Center)	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)	

Ryojun Auditorium

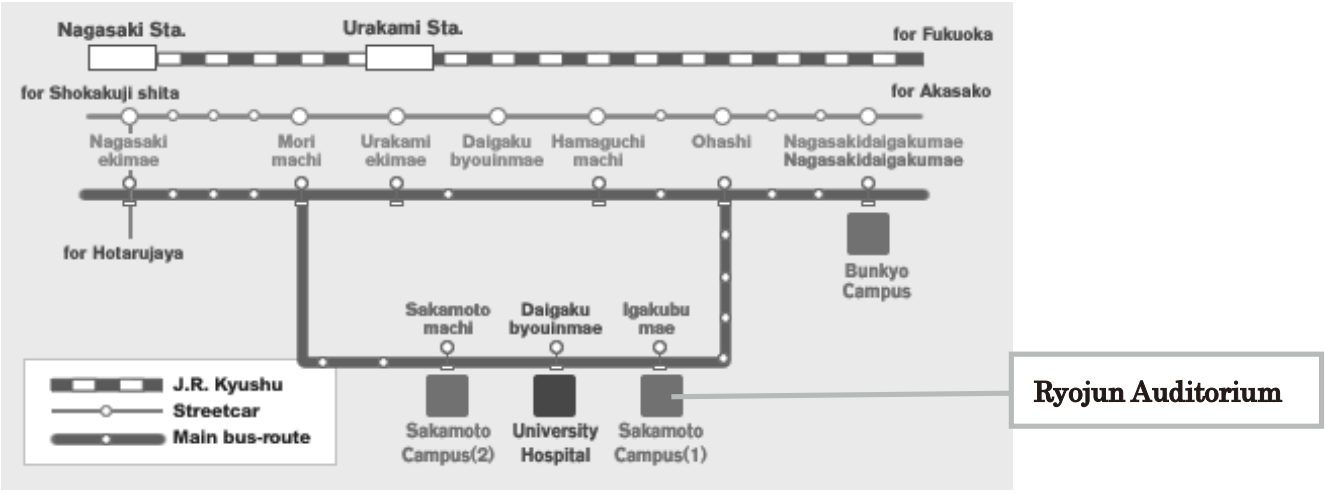
1F Sensai Hall
For Poster Presentations



2F Baudwin Lecture Hall
For Oral Presentations



Traffic Access to Sakamoto Campus



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**(150yen). Then walk for 10 minutes.