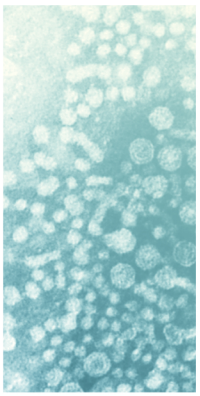
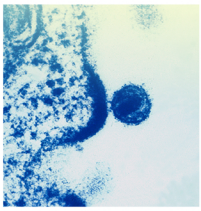
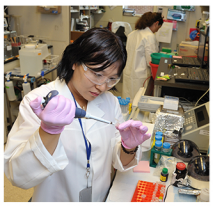
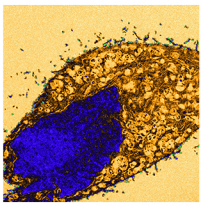
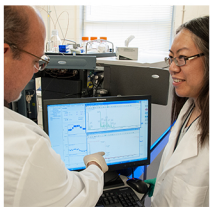
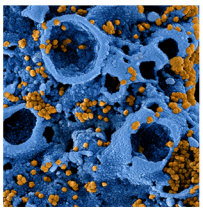
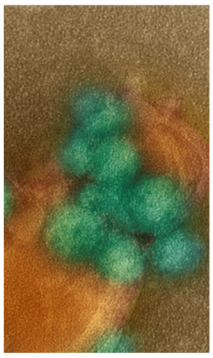
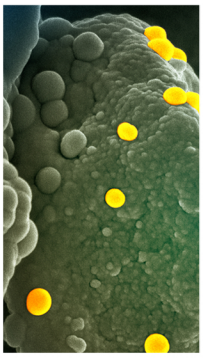
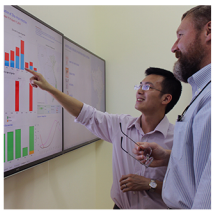
United States-Japan Cooperative Medical Sciences Program

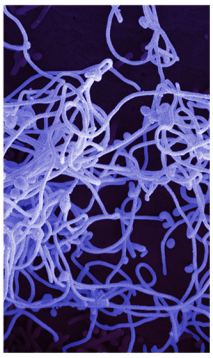
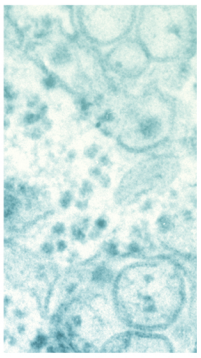










20th International Conference on Emerging Infectious Diseases in the Pacific Rim

and meetings of the AIDS (joint with Immunology Board), Acute Respiratory Infections, Cancer, Hepatitis, and Viral Diseases panels



January 8-11, 2018

The Coli Hotel
Shenzhen, China

Ministry of Health, Labour and Welfare of Japan (MHLW)

Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT)

Ministry of Foreign Affairs of Japan (MOFA)

National Institutes of Health

Japan Agency for Medical Research and Development

深圳市第三人民医院
THE THIRD PEOPLE'S HOSPITAL OF SHENZHEN

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AGENDA

U.S.-Japan Cooperative Medical Sciences Program (USJCMSP)
20th International Conference on Emerging Infectious Diseases (EID) in the Pacific Rim

January 8-9, 2018
Venue: The Coli Hotel
Shenzhen, China

MEETING DESCRIPTION

The focus of this conference is on the pathogenesis and protective immunity of viral diseases of importance in the Asia-Pacific region. The objectives of this conference are to share current research findings and foster existing and potential international research collaborations that engage investigators and institutions in the Asia-Pacific region and the United States.

If you will be using Twitter or Instagram, please use the hashtag #CMSPEID.

Monday, 8 January 2018

7:00-8:00 Registration

8:00-8:30 **WELCOME REMARKS**

Master of Ceremony:
Gray Handley, Associate Director for International Research Affairs, National Institute of Allergy and Infectious Diseases (NIAID), U.S.A.

Norio Saito, Consul-General, Consulate-General of Japan in Guangzhou, China

Chris Smith, Chief of the Political-Economic Section, U.S. Consulate General in Guangzhou, China

Diane E. Griffin, University Distinguished Service Professor, Johns Hopkins Bloomberg School of Public Health, U.S.A. / U.S. Delegation Chair, USJCMSP

George Fu Gao, Director General, China CDC; Member, Chinese Academy of Sciences (CAS)

Aikichi Iwamoto, Managing Director, Department of Research Promotion, Japan Agency for Medical Research and Development (AMED), Japan

8:30-9:00 **La Montagne-Heilman Lectureship**
Introduced by Gray Handley, NIAID, U.S.A.
Mary K. Estes, Baylor College of Medicine, U.S.A.

9:00-9:30 **Shimao-Takeda Lectureship**
Introduced by Aikichi Iwamoto, AMED, Japan
Takaji Wakita, National Institute of Infectious Diseases (NIID), Japan

9:30-9:40 Announcements

9:40-10:00 Coffee Break

SESSION 1: Recent Trends of Viral Infections in Asia and the Pacific Rim
Moderators: ***Takaji Wakita**, NIID, Japan* and ***Christopher Walker**, Nationwide Children's Hospital, U.S.A.*
Rapporteurs: ***Sheikh Akbar***, *Toshiba General Hospital, Japan* and ***Rajen Koshy***, *NIAID, U.S.A.*

10:00-10:20 The Threat of Avian Influenza A H7N9
***Malik Peiris**, The University of Hong Kong, Hong Kong SAR China*

10:20-10:40 Endemic Prevalence of Hepatitis Viruses in Mongolia and Its Deadly Consequences
***Naranbaatar D. Dashdorj**, Onom Foundation, Mongolia*

10:40-11:00 Dengue: Current Situation and Challenges
***Usa Thisyakorn**, Chulalongkorn University, Thailand*

11:00-11:20 Alphavirus Vaccines: Novel Platform Based on the Insect-Specific Eilat Virus
***Scott C. Weaver**, University of Texas Medical Branch, U.S.A.*

11:20-11:40 CCHF Expansion in Asia
***Roger Hewson**, Public Health England, United Kingdom*

11:40-11:55 Q&A

11:55-13:35 Lunch Break and Poster Viewing

SESSION 2: Pathogenesis of Viral Infections

Moderators: ***Jiro Arikawa**, Hokkaido University, Japan* and ***Richard Kuhn,** Purdue University, U.S.A.*
Rapporteurs: ***Eun-Chung Park**, NIAID, U.S.A.* and ***David McDonald**, NIAID, U.S.A.*

13:35-13:55 Immune Recovery After Antiviral Cure of Chronic Hepatitis C
***Georg Lauer**, Harvard University, U.S.A.*

13:55-14:15 Coronavirus RNA Proofreading on is Critical for Coronavirus Replication, Virulence, and Pathogenesis
***Mark R. Denison**, Vanderbilt University Medical Center, U.S.A.*

14:15-14:35 Understanding the Pathogenesis of Enterovirus 71 Infection Based on the Receptor Identification
***Hiroyuki Shimizu**, NIID, Japan*

14:35-14:55 HIV: Cryo-EM Structures and Atomic Model of the HIV-1 Strand Transfer Complex Intasome
***Dmitry Lyumkis**, The Salk Institute, U.S.A.*

14:55-15:10 Q&A

15:10-15:30 Coffee Break

Moderators: ***Kouichi Morita**, Nagasaki University, Japan* and ***Harry Greenberg**, Stanford University, U.S.A.*
Rapporteurs: ***Eun-Chung Park**, NIAID, U.S.A.* and ***David McDonald**, NIAID, U.S.A.*

15:30-15:50 SFTS: Pathogenesis and Treatment
***Mi-Fang Liang**, National Institute for Viral Disease Control and Prevention, China*

15:50-16:10 Replication and Pathogenesis of a Fusogenic Bat-borne Orthoreovirus Associated with Acute Respiratory Tract Infections in Humans
***Takeshi Kobayashi**, Osaka University, Japan*

16:10-16:30 HBV Entry and Persistence: New Perspectives on an Old Pathogen
***Wenhui Li**, National Institute of Biological Sciences in Beijing, China*

16:30-16:50 Mother-to-Child-Transmission of Zika Virus
***Albert Ko**, Yale School of Public Health, U.S.A.*

16:50-17:05 Q&A

17:05-17:30 Break

17:30-18:00 Poster Session

17:30-19:00 Host-Sponsored Reception

Tuesday, 9 January 2018

SESSION 2: Pathogenesis of Viral Infections (CONTINUED)

Moderators: ***Kouichi Morita**, Nagasaki University, Japan* and ***Harry Greenberg**, Stanford University, U.S.A.*
Rapporteurs: ***Eun-Chung Park**, NIAID, U.S.A.* and ***David McDonald**, NIAID, U.S.A.*

8:00-8:20 HLA Class I-mediated HIV-1 Control in HIV-1 Infection
***Masafumi Takiguchi**, Kumamoto University, Japan*

8:20-8:40 Patriotic Health Campaign: China's Experience in Infectious Disease Control

***George F. Gao**, China CDC and CAS, China*

8:40-8:55 Q&A

SESSION 3: Hepatitis Virus Infection and Liver Cancer

Moderators: ***Tohru Kiyono**, National Cancer Center Research Institute, Japan* and ***Edward Trimble**, National Cancer Institute (NCI), U.S.A.*
Rapporteurs: ***Marie Ricciardone**, NCI, U.S.A.* and ***Yukari Totsuka**, National Cancer Center Research Institute, Japan*

8:55-9:15 Molecular Virology and Immunology of Hepatitis B Virus
***Jinlin Hou**, Nanfang Hospital, China*

9:15-9:35 Antibody Therapeutics Targeting GPC3 for the Treatment of Liver Cancer
***Mitchell Ho**, NCI, U.S.A.*

9:35-9:55 Genetic Landscape of Virus-associated HCC
***Tatsuhiro Shibata**, National Cancer Center Research Institute, Japan*

9:55-10:15 Coffee Break

10:15-10:35 Host Factors Related to Hepatitis Virus Infection
***Masashi Mizokami**, National Center for Global Health and Medicine, Japan*

10:35-10:55 Chemical-Viral Interaction Between Aflatoxin and Human Hepatitis B Virus in Induction of HCC
***John D. Groopman**, Johns Hopkins University Bloomberg School of Public Health, U.S.A.*

10:55-11:15 Integrated Genomics to Identify Drivers of Human Liver Cancers
***Xin Wei Wang**, NCI, U.S.A.*

11:15-11:30 Q&A

11:30-11:50 Break

SESSION 4: Vaccines

Moderators: ***Tetsuro Matano**, University of Tokyo, Japan* and ***Hideki Hasegawa**, NIAID, U.S.A.*
Rapporteur: **Kristina Lu**, *NIAID, U.S.A.*

11:50-12:10 Dengue
***Steve Whitehead**, NIAID, U.S.A.*

12:10-12:30 A Single-dose Live-attenuated Zika Vaccine
***Pei-Yong Shi**, University of Texas Medical Branch, U.S.A.*

12:30-12:45 Q&A

FLASH TALK SESSION: 2016 USJCMSP Collaborative Awards

12:45:13:00 Flash Talks from Recipients of a 2016 USJCMSP Collaborative Award
Introduced by Aikichi Iwamoto, AMED, Japan

(1) Mutations in Viral Capsid Modulate IFN-β Sensitivity of HIV-1
Akatsuki Saito, *Osaka University, Japan*
Joao Mamede, *Northwestern University, U.S.A.*
(2) Impact of Clinically Observed Integrase Mutations on Dolutegravir Resistance
Atsuko Hachiya, *National Hospital Organization Nagoya Center, Japan*
(3) A Balancing Act: Hepatic Antiviral Innate Immune Defense and Viral Evasion
Takeshi Saito, *University of Southern California, U.S.A.*
(4) CV Clearance by Antivirals Makes Changes in the Immune Related Cytokines
Tatsuo Kanda, *Nihon University, Japan*
Reina Sasaki, *St. Louis University, U.S.A.*

13:00-14:00 Lunch Break and Poster Viewing

SESSION 4: Vaccines (CONTINUED)

Moderators: ***Tetsuro Matano**, University of Tokyo, Japan* and ***Hideki Hasegawa**, NIAID, U.S.A.*
Rapporteur: **Kristina Lu**, *NIAID, U.S.A.*

14:00-14:20 Respiratory Syncytial Virus Vaccines are Approaching Clinical Trials
***Mark E. Peeples**, Nationwide Children's Hospital, U.S.A.*

14:20-14:40 An Acute HIV Infection Cohort in Bangkok, Thailand
***Eugène Kroon**, SEARCH, Thai Red Cross AIDS Research Centre, Thailand*

14:40-15:00 Catching a Moving Target: A Universal Influenza Virus Vaccine Strategy Based on the Conserved Stalk Domain of the Hemagglutinin
***Florian Krammer**, Icahn School of Medicine at Mount Sinai, U.S.A.*

15:00-15:20 Epidemiology Baseline of HFMD Caused by Multiple Serotypes of Enterovirus: Implications for Vaccine Development
***Qiaohong Liao**, CDC, China*

15:20-15:40 Phase 2 Clinical Safety and Immunogenicity of a Measles-vectored Chikungunya Vaccine
***Matthias Müllner**, Themis Bioscience GmbH, Austria*

15:40-15:55 Q&A

15:55-16:15 Coffee Break

SESSION 5: New Approach to Define Protective Immunity and Drug Discovery

Moderators: ***Hiroshi Kiyono****, University of Tokyo, Japan*
Rapporteurs: ***David McDonald****, NIAID, U.S.A.*

16:15-16:35 Norovirus
Kazuhiko Katayama, Kitasato University, Japan

16:35-16:55 Structure-Based Zika Drug Discovery
Yi Shi, Beijing Institutes of Life Science, Chinese Academy of Sciences, and Shenzhen Third People's Hospital, China

16:55-17:15 Mechanisms Controlling Innate Immune Responses to Nucleic Acids
Kensuke Miyake*, The University of Tokyo, Japan*

17:15-17:30 Q&A

17:30-18:00 **CLOSING REMARKS**

Jiro Arikawa, Hokkaido University, Japan

George Gao, China CDC and CAS, China

Diane E. Griffin, Johns Hopkins Bloomberg School of Public Health, U.S.A.

18:00 Acknowledgement of Appreciation/Adjournment

Wednesday, 10 January 2018

Concurrent USJCMSP Panel Meetings

8:15-17:30 Joint Meeting of the AIDS Panel and Immunology Board

8:20-17:20 Acute Respiratory Infections Panel Meeting

8:45-16:30 Cancer Panel Meeting

8:30-17:00 Hepatitis Panel Meeting

8:15-17:30 Viral Diseases Panel Meeting

12:00-14:30 Staggered Lunch Break

Thursday, 11 January 2018

Concurrent USJCMSP Panel Meetings

8:15-13:00 Joint Meeting of the AIDS Panel and Immunology Board

8:30-12:00 Cancer Panel Meeting

8:30-13:00 Hepatitis Panel Meeting

9:00-12:40 Viral Diseases Panel Meeting

9:00-12:00 Joint Committee Meeting (CLOSED SESSION)

12:30-14:00 Staggered Lunch Break

14:00-18:00 Optional Site Tour of BGI (formerly known as Beijing Genomics Institute), Shenzhen

6 Jan 2018

ABSTRACTS

(In Order of Agenda)

Translating Stem Cell Biology to Understand Gastrointestinal Virus Infection

Mary K. Estes, Ph.D.
Baylor College of Medicine, Houston, Texas, USA

Introduction: A limitation in translational research in the gastrointestinal tract has been the absence of models that recapitulate the diverse nature of the epithelium. Human intestinal enteroids (HIEs) contain the normal complement of intestinal epithelial cell types (stem, enterocyte, goblet, enteroendocrine, and Paneth cells).

Methods: We have established and utilized HIE cultures as pre-clinical models to study the response of the epithelium to common viral pathogens such as human rotavirus (HRV) and human noroviruses (HuNoV), which each kills almost 200,000 children annually by causing dehydrating gastroenteritis. Studies on HRVs have been limited because they are difficult to culture in transformed cell lines and do not infect small animals and HuNoVs were non cultivatable until 2016.

Results: We established HIEs derived from patient small intestinal tissue, showed they support HRV infection, and demonstrate previously unappreciated pathophysiologic and molecular responses to infection. Undifferentiated HIEs, consisting primarily of immature enterocytes, Paneth and stem cells, are less susceptible to infection compared to fully differentiated HIEs that consist predominately of mature enterocytes, confirming *in vivo* findings that the villus enterocyte is the primary target of HRV infection and replication. Enteroendocrine cells also are infected suggesting signaling that may be related to pathogenesis. Infection increases proliferation and cell death and alters cellular metabolism pathways and innate immune responses. Replication of the RV1 rotavirus vaccine is attenuated in HIEs. HIEs also are susceptible to infection with the previously noncultivatable human noroviruses (HuNoVs) providing the native intestinal milieu is mimicked. Host-specific phenotypes and strain-specific requirements, including addition of bile, needed for successful HuNoV cultivation have been identified. This cultivation system is useful to study virus inactivation and neutralization.

Conclusions: These findings establish HIEs as new models to understand the intestinal epithelial response to human gastrointestinal infections such as HRV and HuNoVs. HIEs allow us to address new questions about human host-pathogen interactions such as innate immune responses, stem cell activity, cell-cell communication within the epithelium and to identify and test new drug therapies to prevent/treat diarrheal disease.

Optimization of hepatitis C virus treatment and post-SVR syndrome

Takaji Wakita
National Institute of Infectious Diseases, Tokyo, Japan

Introduction: Since the discovery of the hepatitis C virus (HCV) in 1989, it has been recognized as a serious medical and public health problem worldwide. Research progress of HCV replicon and virus culture systems enabled various achievement in the HCV research field. Especially, introduction of new anti-HCV agents, so-called direct-acting antivirals (DAA), have greatly changed treatment for HCV, and a variety of choices for anti-HCV drug combinations are available. For better management and control of this worldwide infectious disease with anti-HCV agents, it is critical to develop a method for precisely profiling the antiviral efficacy of possible combination drug regimens and seek the "best treatment" based on scientific evidence.

Methods: We studied cell culture data in combination with a mathematical model and computer simulation to quantify the anti-HCV drug efficacy of different drug concentrations and combinations in a preclinical setting, to develop a quantitative basis for selecting drug combinations prior to costly clinical trials.

Results: HCV treatment with DAA can achieve a sustained virologic response (SVR) for almost 100% of treated patients. However, a significant number of patients who achieved SVR developed hepatocellular carcinoma. Therefore, carcinogenesis after SVR remains problematic. We analyzed liver pathology after SVR to improve the management of the increasing number of SVR patients.

Conclusions: We found abnormal organelles such as nuclear membrane disruption, cristae destruction in swollen mitochondria, and membrane vesicle formation of the ER in hepatocytes of SVR patients. Abnormal hepatocellular organelles in SVR patients indicate a persistent disease state (post-SVR syndrome). Thus, long-term follow-up of patients is necessary after achieving SVR.

The threat of avian influenza A H7N9

Malik Peiris
School of Public Health, The University of Hong Kong

A novel low-pathogenic avian influenza (LPAI) virus A (H7N9) causing zoonotic disease was detected in Shanghai in 2013. Since then, the virus has gradually spread to poultry in 31 municipalities, provinces and autonomous regions in mainland China with travel-associated cases reported from Hong Kong SAR, Macau SAR, Taiwan, Malaysia and Canada. The LPAI virus caused no symptoms of illness in poultry making it impossible to detect unless virological testing was carried out; thus human cases have often being the first clue to the presence of the virus. So far, 1622 human cases have been reported, leading to 619 deaths. The fifth wave of the outbreak in the winter of 2016 was the largest so far. The virus has still not adapted to efficient transmission between humans but risk assessment of current zoonotic viruses has identified H7N9 to be the most concerning pandemic threat. In 2017, one lineage of the virus has undergone mutation in the virus haemagglutinin leading to the emergence of a variant that is highly pathogenic (HPAI) in chickens. So far, 25 human cases with the HPAI variant have been detected; it is unclear if this is associated with increased pathogenicity for humans. However, emergence of resistance to oseltamivir has been reported in a number of these patients, posing a dilemma of how best to treat these patients. The Ministry of Agriculture has recently commenced vaccinating poultry with a H5/H7 bivalent vaccine and it remains to be seen what impact this intervention may have on the zoonotic wave this winter. In Hong Kong, interventions in live poultry markets such as “rest days,” where the market is empty of poultry, and banning of holding live poultry overnight within the live poultry market, have been effective at minimizing virus amplification and persistence, thus reducing zoonotic risk.

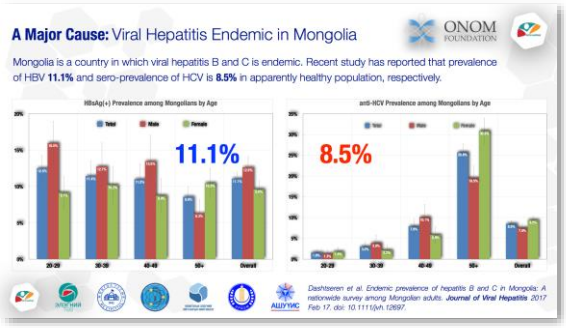
Endemic prevalence of hepatitis viruses in Mongolia and its deadly consequences

Naranbaatar D. Dashdorj[1,2,3,4,5,6], Andreas S. Bungert[1,5], Bekhbold Dashtseren[1,5,6], Odgerel Oidovsambuu[1,3,5], Dahgwahdorj Yagaanbuyant[1,5,6,7,8], Zulkhuu Genden[1,5,6], Batdelger Dendev[5,6,7], Davaadorj Duger[6,8,9],
Jeffrey S. Glenn[10], and Naranjargal J. Dashdorj[1,5,6]
[1]Onom Foundation, [2]Mongolian Academy of Sciences, [3]Mongolian Laboratory Network, [4]Medical Information Technology Association of Mongolia, [5]Liver Center, [6]Mongolian Society of Hepatology, [7]National Center for Communicable Diseases, [8]Mongolian National University of Medical Sciences, [9]Mongolian Gastroenterology Association, [10]Stanford University

Introduction: Mongolia has the world's highest rate of liver cancer mortality—nearly 12 times the global average. Prevalence of chronic hepatitis B, C, and D in Mongolia is at an endemic level and constitutes the main cause for Mongolia's world-leading liver cancer mortality rate. Liver cirrhosis and hepatocellular carcinoma mortality accounts for 15% of total annual mortality in Mongolia. In short, the hepatitis endemic is wreaking havoc in Mongolian society.

Methods: In this study, a representative group of Mongolian adults was tested for hepatitis B virus (HBV), hepatitis D virus (HDV), and hepatitis C virus (HCV). Screening was conducted at 17 different locations on a randomly sampled group, representing the Mongolian adult population. A total of 1158 adults, 500 (43.1%) men and 659 (56.9%) women, were included.

Results: The prevalence estimates of HBV and HCV amongst the general Mongolian adult population (20 years of age or older) were found to be 11.1%±1% (SE) and 8.5%±0.7% [207 418 (HBsAg+) and 160 228 (anti-HCV+) cases, respectively]. Subsequent screening of the HBV surface antigen-positive (HBsAg+) cohort revealed that a remarkable 57% were HDV-RNA+ and an additional 4% were positive on western blot alone. For HCV, the majority of cases are concentrated in older age groups with a prevalence of 25.8% amongst those aged 50 years and above, whilst the prevalence of HBV does not vary significantly amongst age groups. For both HBV and HCV, the data indicate a higher risk of infection and a higher mortality amongst men than amongst women.



Conclusions: This study represents the first nationwide estimate of the prevalence of HBV and/or HDV in Mongolia and is the first for HCV since 2005. Our data confirm the position of Mongolia as one of the hot-spots of chronic hepatitis infection in the world with about 19.4% of the adult population infected with either HBV or HCV. Even more alarmingly, our studies reveal an apparent 60% prevalence of HDV infection among the HBV-infected Mongolian population, the highest prevalence of HDV ever reported in a country in the world.

Dengue: Current situation and challenges

Usa Thisyakorn, Terapong Tantawichien, Chule Thisyakorn
Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Introduction: Dengue, the most common arboviral infection in humans in the world, is a re-emerging disease that affects millions of people annually. The increasing number of dengue cases over the last decades is associated with unplanned urbanization and lack of efficient health facilities, demographic transition, travel/commercial development, and limited efficacy of the vector control efforts.

Methods: A systematic literature search in regard to current situation of dengue virus was performed.

Results: The clinical presentations of dengue range from mild illness to the life-threatening severe forms of the disease associated with plasma leakage, shock, severe bleeding or multi-organ failure, which may be fatal. Although shock and plasma leakage seem to be more prevalent as age decreases, the frequency of severe bleeding or internal hemorrhage augments as age increases. Increase in liver enzymes, unlike conventional viral hepatitis, indicates liver involvement during dengue infections. Fatal cases were found to have significant frequencies of shock, altered consciousness, massive gastrointestinal bleeding, renal/hepatic failure and concurrent bacteremia. The early recognition of dengue infection, bleeding tendency, and signs of circulatory collapse would reduce mortality rate in patients with dengue infection.

Conclusions: Dengue is one disease entity with different clinical manifestations often with unpredictable clinical outcomes. Successful treatment, which is mainly symptomatic and supportive, depends on the early recognition of the disease and careful monitoring of disease severity. Proper management must consider the different age-specific clinical manifestations. The increasing number of dengue cases is a major global public health problem and implementation of effectively integrated vector management with efficacious dengue vaccines are the keys to success for disease control.

Alphavirus vaccines: Novel platform based on the insect-specific Eilat virus

Scott C. Weaver
Institute for Human Infections and Immunity and Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, USA

Introduction: Typically, vaccine development involves tradeoffs between immunogenicity and rapid, single-dose efficacy exemplified by live-attenuated vaccines and safety, maximized with replication-defective or inactivated platforms. To overcome this tradeoff, we capitalized on the recently discovered insect-specific alphavirus, Eilat virus, which is fundamentally restricted against replication in vertebrate cells.

Methods: By producing chimeric cDNA clones with the structural polyprotein gene of chikungunya, Venezuelan, and eastern equine encephalitis virus swapped into the Eilat backbone, we were able to rescue chimeric viruses for further testing.

Results: The resulting chimeric Eilat-based viruses were structurally indistinguishable down to 10Å resolution using cryo-electron microscopy from the vertebrate-pathogenic target alphaviruses, but retained the host range restriction of Eilat virus. They replicated highly efficiently in mosquito cells and induced rapid, long-lasting protective immunity in mice or macaques after a single dose.

Conclusions: These Eilat virus-based chimeric vaccines appear to combine the safety of replication-defective platforms with the rapid and long-lasting protective immunity of a live-attenuated vaccine, justifying further development for human and equine use. They are also useful as diagnostic antigens and have been developed for chikungunya ELISAs that are now marketed nearly worldwide.

CCHF Expansion in Asia

Roger Hewson
Public Health England, United Kingdom

Crimean-Congo haemorrhagic fever virus (CCHFV) is a human pathogen of the utmost seriousness being both fast acting and highly lethal, with devastating disease symptoms that cause intense and prolonged suffering to those infected. Since its discovery over 60 years ago, this virus has repeatedly caused sporadic outbreaks responsible for relatively low numbers of human casualties, but with an alarming fatality rate of up to 60%. It is transmitted mainly by the bite of an infected tick, although zoonotic transmission from viremic animals including human-to-human transmission by direct contact is also common. The incidence of CCHFV-mediated disease closely matches the geographical range of permissive ticks, which are widespread throughout Africa, Asia, the Middle East, and Southeastern Europe. As such, CCHFV is the most widespread tick-borne virus on earth. Over recent years new focal epidemic centres have developed in previously non-endemic areas such as India and Mongolia and an increasing burden of CCHF has developed in countries such as Turkey, Iran, Afghanistan, Tajikistan, China and Pakistan. While the expanding geographic distribution of tick vectors, assisted by the disseminating activity of migratory birds, is thought to be an important factor in the expansion of CCHF in Asia, anthropogenic factors are expected to play an increasingly significant role in the near future. Thus classic disease control and intervention strategies such as vaccines and therapeutics to combat CCHF are becoming keys areas of research.

Immune Recovery After Antiviral Cure of Chronic Hepatitis C

Georg Lauer
Harvard University

Abstract to be distributed later.

Coronavirus RNA proofreading on is critical for Coronavirus Replication, Virulence, and Pathogenesis

James B Case, Xiaotao Lu, Kevin W. Graepel, Nicole R. Sexton, Everett Clinton Smith, Susan R. Weiss and **Mark R. Denison**
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Coronaviruses (CoVs) are endemic respiratory pathogens of humans. CoVs have demonstrated a profound capacity for host-species movement and zoonotic epidemics, as dramatically exemplified by severe acute respiratory syndrome coronavirus (SARS-CoV) and more recently, by the Middle Eastern respiratory syndrome coronavirus (MERS-CoV). Recent studies have implicated bats as probable reservoir hosts for host species movement and zoonotic infections. CoVs encode the largest and most complex genomes of any RNA viruses. We have discovered that CoVs encode the first known proofreading exonuclease (nsp14-ExoN), a function critical for high replication fidelity of CoVs. Genetic or chemical inhibition of ExoN results in virus lethal mutagenesis during treatment with RNA mutagens and attenuation of virulence *in vivo*. Targeting CoV proofreading in combination with inhibitors of the viral polymerase may hold promise as approaches to broadly effective CoV inhibitors.

Understanding the pathogenesis of enterovirus 71 infection based on receptor identification

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Introduction: Hand, foot, and, mouth disease (HFMD) is a common febrile disease occurring mainly in infants and children. The major causative agents of HFMD are coxsackieviruses A6 and A16, and enterovirus 71 (EV71). Large HFMD outbreaks, including cases with severe neurological diseases, mainly due to EV71, have been recently reported especially in the Asia-Pacific region, including in Malaysia, Taiwan, China, Cambodia, and Vietnam. EV71 infections cause a diverse range of neurological diseases and have resulted in thousands of deaths in young children, posing a serious threat to public health in the region. A number of cell-surface molecules are involved in the early stages of EV71 infection. By using different approaches, our group and Dr. Koike and colleagues in Japan have identified two human transmembrane proteins, P-selectin glycoprotein ligand-1 (PSGL-1) and scavenger receptor class B, member 2 (SCARB2), respectively. SCARB2 is expressed in a broad variety of tissues; however, PSGL-1 is primarily expressed on leukocytes. Amino acid residue 145 of the capsid protein VP1 (VP1-145) defined PSGL-1-binding (PB) and PSGL-1-nonbinding (non-PB) phenotypes of EV71 and has been identified as a potential neurovirulence determinant in humans and experimental mouse models.

Methods: To elucidate the *in vivo* involvement of VP1-145 in PSGL-1-dependent replication and pathogenesis, we investigated viral replication, genetic stability, and the pathogenicity of PB and non-PB strains of EV71 in a cynomolgus monkey model. Cynomolgus monkeys were intravenously inoculated with cDNA-derived PB and non-PB strains of EV71, EV71-02363-EG and EV71-02363-KE strains, respectively.

Results: Mild neurological symptoms, transient lymphocytopenia, and inflammatory cytokine responses, were found predominantly in the 02363-KE-inoculated monkeys. Histopathological analysis of CNS tissues revealed that 02363-KE induced neuropathogenesis more efficiently than that induced by 02363-EG. After inoculation with 02363-EG, almost all EV71 variants detected in clinical samples, possessed a G to E substitution at VP1-145, suggesting a strong *in vivo* selection of VP1-145E variants and CNS spread presumably in a PSGL-1-independent manner. EV71 variants with VP1-145G were identified only in PBMCs from two PB-inoculated monkeys.

Conclusions: VP1-145E non-PB variants are mainly responsible for the development of viremia and neuropathogenesis in a non-human primate model, suggesting the involvement of amino acid polymorphism at VP1-145 in cell-specific viral replication, *in vivo* fitness, and pathogenesis in EV71-infected individuals. I will discuss the involvement of VP1-145 in viral replication and pathogenesis in EV71 infection, and reliable animal models to be established in the future.

Patriotic Health Campaign: China's experience in infectious disease control

George Gao
China CDC and Chinese Academy of Sciences

This year, China celebrated the 65th anniversary of its Patriotic Health Campaign. As evidenced in its successful disease control in China in the last several decades, this campaign has been playing a very important role for China and would serve as a good model for the developing countries in the world. This talk will discuss the key elements of the campaign. A strong government commitment is the key for this campaign and public understanding (education) of disease control is another key element. A good example of disease control is malaria. Through vector (mosquitos) control and people's active involvement, the malaria epidemic declined rapidly in China in the last decades. We believe this disease control model can be applied in Africa, esp., on the eve of establishment of the Africa CDC. China should play a key role in the aid of disease control in Africa, which will benefit the whole world.

HIV: Cryo-EM structures and atomic model of the HIV-1 strand transfer complex intasome

Dmitry Lyumkis
The Salk Institute, USA

Abstract to be distributed later.

SFTS: Pathogenesis and treatment

Mi-Fang Liang
National Institute of Viral Disease Control and Prevention, China

Abstract to be distributed later.

Replication and pathogenesis of a fusogenic bat-borne orthoreovirus associated with acute respiratory tract infections in humans

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Introduction: Nelson Bay orthoreoviruses (NBVs) are fusogenic orthoreoviruses with a genome that contains 10 segments of double-stranded RNA. The first NBV was isolated from a fruit bat in 1968, but was not associated with any disease. However, several NBV strains recently have been identified as causative agents of respiratory tract infections in humans. Isolation of these pathogenic bat reoviruses from human patients suggests that NBVs have evolved to propagate as zoonotic agents in humans.

Methods: Reverse genetics systems are powerful tools to study many aspects of virus biology and virus-host interactions and to rationally design viruses for use as vaccines or gene-transduction vectors. To date, no strategy has been developed to rescue infectious viruses from cloned cDNA for any fusogenic orthoreoviruses. In this study, we report the development of a plasmid-based reverse genetics system free of helper viruses and independent of any selection, for NBV isolated from humans with acute respiratory infection. The cDNAs corresponding to each of the 10 full-length RNA gene segments of NBV were co-transfected into cultured cells expressing T7 RNA polymerase, and viable NBV was isolated using a plaque assay. We used the reverse genetics system to generate viruses deficient in the cell attachment protein σC and the fusion-associated transmembrane (FAST) protein to define the biological functions of these proteins in the viral life cycle.

Results: Studies with σC-deficient viruses demonstrated that σC is dispensable for cell attachment in several cell lines, including murine fibroblast L929 cells but not in human lung epithelial A549 cells, and plays a critical role in viral pathogenesis. We succeeded in rescuing a replication-competent FAST-deficient virus, suggesting that FAST is not essential for NBV replication. However, the replication kinetics of the FAST-deficient virus were significantly slower than those of wild-type NBV, suggesting that FAST protein is dispensable but is required for efficient NBV propagation.

Conclusions: The reverse genetics approach described in this study can be exploited for studying fusogenic orthoreovirus biology and to develop vaccines, diagnostics, and therapeutics.

HBV entry and persistence: New perspectives on an old pathogen

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Introduction: Hepatitis B virus (HBV) has likely been circulating in human populations for thousands of years and its infection remains an important public health problem worldwide. Currently several nucleos(t)ide analogs can efficiently suppress HBV replication; however, there is no curative treatment for chronic HBV infection. The infection mechanisms of HBV, in particular how the virus specifically enters into liver hepatocytes and how the it establishes persistent infection, were only partially understood. Elucidating these critical steps could deepen our understanding of the virus and help to develop novel treatments against the infection.

Methods: We combine virology, biochemistry and animal studies to investigate molecular mechanisms of HBV infection.

Results: A liver-specific bile-acid transporter, sodium taurocholate co-transporting polypeptide (NTCP), was identified as a key receptor for human HBV and its satellite hepatitis D virus (HDV). Sequence analysis and experimental data indicate that NTCP's orthologs likely act as common cellular receptors for all known primate hepadnaviruses. Modification of three amino acids in NTCP rendered mice susceptible to infection with HDV *in vivo*. Molecular determinants critical for HBV/HDV entry overlap with those for bile-salt uptake by NTCP. HepG2-NTCP cells support the whole life cycle of HBV infection; genetic studies using these cells revealed that some cellular enzymes including DNA polymerase Kappa contribute to the establishment of the viral reservoir for persistent HBV infection.

Conclusions: NTCP acts a specific receptor for human HBV and HDV, it is likely required for viral entry of all primates HBVs. NTCP is a primary restriction of cross-species infection of HBV. NTCP-based cell cultures have become a backbone platform of studying HBV/HDV infection. Significant advances have been made in understanding molecular mechanisms of HBV infection since the discovery of HBV receptor, and new interventions are also being developed toward the ultimate goal of curing HBV.

Mother-to-Child transmission of Zika Virus

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Abstract to be distributed later.

Immunopathogenesis in dengue virus infections

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Introduction: Dengue hemorrhagic fever (DHF), characterized by plasma leakage and bleeding diathesis, occurs in a small percentage of dengue virus (DENV) infections. DHF is strongly associated with secondary DENV infections and there is a close temporal correlation between plasma leakage and clearance of viremia, both of which suggest an immunological basis for the disease.

Methods: We have studied the evolution of immune responses and their relationship to disease through prospective studies of Thai children with acute febrile illness. Blood samples collected daily from early in the febrile period through the critical phase of defervescence have been analyzed by flow cytometry to determine the timing of immune activation and cell subsets affected.

Results: Activation of various T-cell subsets and plasmablasts was detected during the febrile phase, and peaked during the critical phase of illness. Immune activation was significantly higher in secondary DENV infections than in primary infections and higher in children with DHF than in those with milder illness (dengue fever).

Conclusions: Our data indicate that immune activation is associated with the occurrence of plasma leakage both in its timing and magnitude. These findings support a role for T- and B-lymphocyte activation in the pathogenesis of severe dengue.

HLA class I-mediated HIV-1 control in HIV-1 infection

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Introduction: HLA-B*57 and -B*27 are associated with successful control of HIV-1 or slow progression to disease in Caucasians and Africans, but are very rare HLA alleles (<1%) in Japan. HLA-B*52:01-C*12:02, which is found in approximately 20 percent of Japanese and associated with autoimmune diseases such as ulcerative colitis and Takayasu arteritis, is a protective haplotype in HIV-1-infected Japanese individuals. We investigated the control of HIV-1 by these HLA-mediated immune responses.

Methods: We identified T-cell epitopes restricted with HLA-B*52:01-C*12:02 in treatment-naïve Japanese individuals chronically infected with HIV-1. The role of T cells restricted by HLA-B*52:01 or C*12:02 in suppression of HIV-1 replication was evaluated in Japanese individuals infected with HIV-1 subtype B. We further analyzed the cross-recognition of these T cells for mutant epitopes detected in epidemic HIV-1 and the mutant viruses to clarify the ability of these T cells to suppress replication of the mutant viruses.

Results: We found that the individuals having HLA-B*52:01-restricted T cells specifc for four HIV-1 epitopes or HLA-C*12:02-restricted ones specifc for two HIV-1 epitopes had significantly lower pVL and higher CD4 counts than those not having them. These T cells exhibited a strong ability to suppress HIV-1 replication *in vitro.* Most of these epitopes are in a conserved region of HIV-1 though some mutations in these epitopes were detected in small number of epidemic viruses. The T cells cross-recognized most of the mutant epitopes and cells infected with these mutant viruses.

Conclusions: Our findings suggested that both the HLA-B*52:01-restricted and the HLA-C*12:02-restricted T cells contribute to the supression of HIV-1 replication in the individuals having this haplotype. Our recent study showed that HLA-C*12:02 together with KIR2DL2 contributed to NK cell-mediated control of HIV-1, suggesting that both CTLs and NK cells contribute to suppression of HIV-1 in the individuals having this haplotype. These T cells may be useful as effector T cells for cure treatment and AIDS vaccine in Asian countries.

Molecular virology and immunology of hepatitis B virus

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Abstract to be distributed later.

Antibody therapeutics targeting GPC3 for the treatment of liver cancer

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Introduction: Hepatocellular carcinoma (HCC) is the most common liver cancer and the fifth most common cancer worldwide. Glypican-3 (GPC3) is a cell surface heparan sulfate proteoglycan protein highly expressed in more than 70% of HCC but not in adult normal tissues. To pursue its role as a target for antibody-based therapies in liver cancer, we have developed (*a*) immunotoxins, (*b*) antibody-drug conjugates (ADCs) and (*c*) chimeric antigen receptors (CARs) targeting GPC3 and evaluated their potential for the treatment of HCC using multiple cell and mouse models.

Methods: First, we have generated two human and humanized antibodies (HN3 and hYP7) targeting GPC3. Both are fused to a fragment of Pseudomonas exotoxin A (PE) to create immunotoxins. Second, we selected duocarmycin and pyrrolobenzodiazepine dimer (PBD) as the payload to construct the hYP7-DC and hYP7-PC ADCs. Third, we have engineered the GPC3-specific CARs (CAR.HN3 and CAR.hYP7) that contain CD3ζ chain and the 4-1BB costimulatory endodomain along with a truncated human EGFR polypeptide (huEGFRt), the cetuximab binding site for visualizing or removing CAR T cells. We used a set of GPC3-positive liver cancer cell lines including Hep3B, HepG2 and Huh7 to evaluate anti-tumor activity of the immunotoxins, ADCs and CAR T cells.

Results: The immunotoxin based on HN3 (HN3-PE) has superior anti-tumor activity, compared to YP7 (YP7-PE) both *in vitro* and *in vivo*. Intravenous administration of HN3-PE induces regression of Hep3B and HepG2 liver tumor xenografts in mice via dual mechanisms: inactivation of cancer signaling via the antibody and inhibition of protein synthesis via the toxin. GPC3-specific ADCs showed anticancer effect in peritoneal and subcutaneous tumor models. Interestingly, hYP7-PC (single-dose, 5mg/kg) induces tumor regression in Hep3B liver cancer xenograft model. The CAR T cells (CAR.hYP7) based on the hYP7 antibody showed highest cytolytic activity against GPC3-positive HCC cells. CAR.hYP7 significantly induced the production of IFN-γ after exposure to HCC tumor cells. A single treatment with CAR.hYP7 cells exhibited sustainable antitumor efficacy in the Hep3B model and all mice survived after 9 weeks post CAR T-cell treatment.

Conclusions: Our work suggests that GPC3-targeting immunotoxins, ADCs and CAR T cells are a promising group of antibody therapeutics for the treatment of liver cancer. Ongoing clinical development will help define the utility of GPC3 as a target in liver cancer.

Genetic landscape of virus-associated HCC

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Division of Cancer Genomics, National Cancer Center Research Institute

Hepatocellular carcinoma (HCC) is prevalent worldwide, mainly associated with virus infection, and its underlying etiology and genomic structure are heterogeneous. We performed whole-genome sequencing and elucidated landscapes of somatic alterations in 300 Japanese HCC. Our comprehensive analysis identified point mutations in non-coding regions, structural variations, and virus integrations, in addition to coding mutations. We discovered novel recurrently mutated coding and non-coding regions, such as lincRNA NEAT1/MALAT1, promoters, and regulatory regions.

Somatic mutations in the cancer genome are generated by multiple carcinogenic causes that challenge DNA replication/repair fidelity. Interestingly and importantly every mutagenic factors or processes leave unique marks on the DNA probably due to their own physical characteristics, that are called mutational signatures. In this presentation, I will also report de-coding of mutational signatures and their associations with clinical/biological/epigenetic features in HCC.

Host factors related to hepatitis virus infection

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Abstract to be distributed later.

Chemical-viral Interaction between aflatoxin and human hepatitis B virus in induction of HCC

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Liver cancer, including hepatocellular carcinoma (HCC) and cholangiocarcinoma, accounts for 9.1% of all reported cancer deaths and is the second most common cause of cancer mortality worldwide. The incidence of liver cancer varies enormously globally and unfortunately the burden of this nearly always fatal disease is much greater in the less economically developed countries of Asia and sub-Saharan Africa. Each year more than 750,000 new cases and more than 300,000 deaths occur in the People's Republic of China. More than 90 percent of common cancers in developed countries are diagnosed after the age of 45, but in high-risk regions for liver cancer, onset begins in both men and women by 20 years of age and peaks at 40-49 years of age in men and 50-59 years of age in women. This earlier onset of HCC might be attributable to exposures that are both substantial and persistent across the life span.

Concurrent with the early aflatoxin research were a series of studies describing a role for the hepatitis B virus (HBV) in HCC pathogenesis. A number of investigations found that chronic carriers of HBV, as indicated by sequential hepatitis B surface antigen (HBsAg) positivity at six-month intervals, were at increased risk of developing HCC. Approximately 90% of HBV infections acquired in infancy or early childhood become chronic, whereas only 10% of infections acquired in adulthood become chronic, and less than 50% of chronic carriers progress to HCC. The global burden of HBV infection varies widely and China, Southeast Asia, and sub-Saharan Africa have some of the highest rates of chronic HBV infection in the world, with prevalence of over 10%. The public health significance of HBV as a risk factor for HCC is staggering with the consideration that there are over 400 million viral carriers and between 10-25% of these individuals are likely to develop HCC. The biology, mode of transmission and epidemiology of this viral infection continues to be actively investigated and has been recently reviewed.

Significant etiological factors associated with development of HCC in developing countries include infection in early life with hepatitis B virus (HBV) and lifetime exposure to high levels of aflatoxin B_1 (AFB_1) in the diet. Indeed, the multiplicative interaction between HBV and AFB_1 has been documented in two separate cohorts at high risk for HCC. Over the past 25 years, an appreciation for the role of the hepatitis C virus (HCV) has also emerged. HCV is contributing to HCC being the most rapidly rising solid tumor in the US and Japan. Detailed knowledge of the etiology of HCC has spurred many mechanistic studies to understand the pathogenesis of this nearly always-fatal disease. Fortunately, the successful development of some highly effective new drugs that cure HCV infection is a major advance and will hopefully diminish the role of this virus in liver cancer.

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Integrated genomics to identify drivers of human liver cancers

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Genomic analyses of most solid tumors reveal a complex mutational landscape with vast inter-tumor heterogeneity (sample to sample) and intra-tumor (within each tumor) heterogeneity. Each histological tumor type and tumor cells within each tumor type display striking molecular and biological variations. Tumor molecular heterogeneity represents a major obstacle for early diagnosis and effective treatment. This is especially relevant to hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) where various etiological factors may elicit different molecular mechanisms to initiate carcinogenesis, leading to different molecularly distinct subtypes and complex tumor cell communities. Such heterogeneity poses a major challenge to define drivers responsible for early stage hepatocarcinogenesis and to develop diagnostic tools and effective treatment modalities for HCC and ICC. New strategies must be exploited to change the current dire situation. The establishment of patient populations with associated well-annotated biobanks and well-characterized molecular features is essential to better define unique tumor subtypes. Moreover, understanding molecular features of tumor cells at a single cell level may provide a better understanding of tumor cell communities and help define key drivers responsible for tumor initiation and progression. Molecular-based technologies such as integrated genomics, transcriptomics and metabolomics provide a superior resolution to distinguish tumor subtypes, which allow for stratification of patients with greater homogeneity and can assist in molecular re-staging. These various genome-based signatures also delineate critical gatekeepers of cancer initiation and progression, which can be further honed by integrated genomics to identify key driver genes and functionally linked networks in HCC and ICC. With its knowledge, we may effectively identify biomarkers and actionable targets for early liver cancer intervention.

Dengue

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Abstract to be distributed later.

A single-dose live-attenuated Zika vaccine

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Introduction: Zika virus (ZIKV) infection during pregnancy can result in devastating congenital abnormities or fetal demise. The unique persistence of ZIKV in the male reproductive system poses a risk of sexual transmission. Ideally, an effective vaccine should prevent ZIKV viremia during pregnancy and *in utero* transmission to developing fetuses, as well as prevent severe disease in adults including Guillain-Barré syndrome and damage to the testes.

Methods: We have rationally engineered live-attenuated ZIKV vaccine candidates containing deletions in the 3' untranslated region of the ZIKV genome (ZIKV-3'UTR-LAV). Such ZIKV-3'UTR-LAV candidates were recently shown to confer sterilizing immunity in a mouse model after a single-dose immunization. The current study aims to further characterize the vaccine candidates in mouse and non-human primates for their safety and efficacy.

Results: ZIKV-3'UTR-LAV prevents viral transmission during pregnancy and testis damage in mice, as well as infection of non-human primates. After a single-dose vaccination, pregnant mice challenged with ZIKV at embryonic day 6 (E6) and evaluated at E13 showed markedly diminished levels of viral RNA in maternal, placental, and fetal tissues. Vaccinated male mice challenged with ZIKV were protected against testis infection, injury, and oligospermia. Furthermore, a single immunization of rhesus macaques elicited a rapid and robust antibody response, leading to complete prevention of viremia upon challenge with an epidemic strain of ZIKV. The ZIKV-3'UTR-LAV vaccine candidates have a desirable safety profile, including a transient, low-level replication in A129 mice, no mortality after intracranial infection of 1-day-old outbred CD-1 mice with 10^3 focus-forming units, and lack of mosquito vector infection after large oral doses.

Conclusions: A single-dose ZIKV-3'UTR-LAV vaccine prevents ZIKV infection in non-human primates, diminishes mouse *in utero* transmission, and protects male reproductive damage. These results suggest that further development of ZIKV-3'UTR-LAV is warranted for humans.

Respiratory syncytial virus vaccines are approaching clinical trials

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Introduction: Respiratory syncytial virus (RSV) was first identified as a human virus 60 years ago and is now known to infect nearly every child by the age of two. RSV has a predilection for the bronchioles where inflammation, mucus hyper-production and infected cell death cause constriction. RSV causes the deaths of an estimated 100,000 children each year, primarily in developing countries. In developed countries, RSV is the top cause for hospitalization of children and a major cost to health care systems. RSV is also an important pathogen in elders, rivaling influenza virus. The fusion (F) glycoprotein is the main RSV neutralizing antigen. The F protein is an unstable protein because it must refold dramatically to cause virus membrane to fuse with the membrane of a target cell. The first RSV vaccine, a formalin inactivated virus preparation similar to the successful Salk poliovirus vaccine, was tested in children in the 1960s, but instead of protecting the young vaccines, they developed more severe disease upon natural challenge.

Methods and Results: Over the intervening years, steady progress has been made toward a live-attenuated RSV, primarily by NIH in collaboration with several pharmaceutical companies. Such a vaccine would be applied intranasally. The F protein, inserted into and expressed by several different virus vectors, also stimulates a protective immune response. Both attenuated and virus-vectored RSV would likely be given at six months of age at the earliest, leaving infants < six months of age unprotected. The functional F protein, in its "pre-fusion" form, induces higher levels of antibodies than its refolded, post-fusion form. Solution of the pre-fusion form of the F protein has enabled rational modifications to stabilize it for use as an antigen. This stabilized F protein is being developed as a maternal vaccine to raise the level of neutralizing antibody in infants, thereby protecting them for an additional month or two beyond that from the maternal antibody that they normally receive. In addition to stabilized pre-fusion F protein vaccines, post-fusion F protein and virus-like particle (VLP) vaccines that include the F protein are also in development. Some of these vaccines are formulated with traditional adjuvants and others with experimental adjuvants.

Conclusions: We are rapidly moving toward clinical trials for live attenuated and virus-vectored RSV vaccines to protect children > six months of age, as well as for F protein RSV maternal vaccines to protect infants < six months of age. Protein or VLP vaccines are also being considered for elders. The number of academic institutions, biotechnology companies and pharmaceutical companies that are currently participating in these efforts have greatly expanded over the past 10 years. The outlook for successful RSV vaccines seems likely.

An acute HIV infection cohort in Bangkok, Thailand

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Introduction: Timing and extent of HIV insult during acute HIV infection (AHI) were largely unknown when the RV254/SEARCH 010 cohort was initiated. Main aims of the cohort are describing clinical, immunological, and virological characteristics of AHI, to limit HIV reservoir with very early antiretroviral therapy (ART), and to identify volunteers who may be candidates for future HIV remission and cure protocols.

Methods: Volunteers presenting for voluntary HIV counseling and testing with 4th generation immunoassay (IA) at the Thai Red Cross Anonymous Clinic in Bangkok gave informed consent and had blood samples screened by pooled HIV nucleic acid testing (NAT) and sequential IA. AHI was defined as either being 4th generation IA positive with positive HIV RNA and negative or indeterminate Western blot, or 4th generation IA negative with positive HIV RNA by qualitative (NAT) and quantitative (HIV RNA) assays. ART is started immediately with clinical, immunological, and virological characteristics of the study subjects as primary endpoints. The study also collects descriptive demographic and behavioral data. Interventions include immediate ART, three- monthly follow-up with blood draws, physical and neurological examinations, questionnaires and behavioral counseling. Optional procedures include brain MRI/MRS, lumbar puncture, inguinal lymph node biopsy, sigmoid biopsy and leukapheresis at baseline and further set time-points.

Results: From April 2009 to October 2017, 269,529 samples were screened to identify 646 acutely infected participants and 503 were enrolled. Longest follow-up in the study is eight years. Median age is 26 years (range 18-70), 89% are MSM, and 77% (384/496) have HIV subtype CRF01_AE . Median CD4 and VL at baseline are 364 cells/mm^3 (range 7-1302) and 5.9 log copies/ml (range 2.2-8.2). 39% enroll during Fiebig stages I and II. Nine volunteers have been lost to follow-up (1.8%), two died (0.41%), and eight withdrew (1.6%). 452 have VL < 20 copies/mL while 23 recent recruits have declining VL. Seronegativity post-ART is high in Fiebig I treated participants, 53% (29/55) at 24 weeks after treatment. The cohort has resulted in 32 peer reviewed publications showing that that early ART in acute HIV infection restricts the HIV reservoir size and replication and immune activation in blood, gut and CNS compartments in all Fiebig stages but

does not fully restore CD4 depletion in the gut lamina propria . Additional publications address viral kinetics; early CNS invasion; the effect of early ART on HIV DNA set point and HIV serology; and ART resistance trends in the Bangkok MSM population. Cohort data modeling suggests that early ART leads to an 89% reduction in onwards transmission among the MSM participants. Cohort participants are now being enrolled into HIV cure studies employing latency reversing agents, broadly neutralizing antibodies, and therapeutic vaccines. An independent behavioral and ethics study into decision making by participants consenting to analytical treatment interruption is embedded into the cure studies.

Conclusion: With appropriate planning, stakeholder engagement and resources we have successfully established a large single site AHI cohort, contributing to elucidating AHI and now to HIV remission and cure research.

Catching a moving target: A universal influenza virus vaccine strategy based on the conserved stalk domain of the hemagglutinin

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Introduction: Influenza virus infections remain a significant cause of morbidity and mortality worldwide. Current vaccines show acceptable efficacy against antigenically matched viruses by inducing strain specific antibodies against the membrane-distal globular head domain of the viral hemagglutinin, but fail to protect against drifted and pandemic strains. The membrane-proximal stalk domain of the viral hemagglutinin exhibits a high degree of both sequence and structural conservation across influenza virus subtypes and monoclonal antibodies directed against this region typically show broad neutralizing activity. However, these antibodies are rare and usually not induced/boosted by regular seasonal vaccines. We hypothesize that a vaccine strategy that stimulates a robust immune response towards this region of the hemagglutinin could provide universal influenza virus protection.

Methods: We developed a universal influenza virus vaccine based on the conserved stalk domain of group 1 and group 2 hemagglutinins. By sequential vaccination of mice and ferrets with chimeric hemagglutinin constructs that share the same stalk domain but have divergent head domains we were able to specifically boost broadly neutralizing antibody titers against conserved epitopes in the hemagglutinin stalk.

Results: Animals vaccinated with these constructs were protected from morbidity and mortality induced by infection with a panel of heterologous and heterosubtypic influenza A viruses. Additionally, chimeric hemagglutinin vaccination also impacted on virus transmission in the ferret model. In the light of emerging viruses in Asia it is of note that our vaccination regimen also protected animals from H5N1, H6N1 and H7N9 virus challenges and reduced lung titers upon H10 virus infection. Finally, we showed that stalk-reactive antibodies were boosted in individuals that received an H5N1 vaccine in clinical trials. This supports the hypothesis that exposure to hemagglutinins with divergent heads but conserved stalk induces such antibodies in humans.

Conclusions: The present data suggest that this vaccine strategy has the potential to provide broad influenza virus protection in humans and clinical trials are currently ongoing. A universal influenza virus vaccine, which requires a single or only a few immunizations, would represent a major advance towards the control of influenza worldwide and would significantly enhance our pandemic preparedness.

EV71 – Development of vaccine and to market

Hongjie Yu
Fudan University, China

Abstract to be distributed later.

Phase 2 clinical safety and immunogenicity of a measles-vectored chikungunya vaccine

Katrin Ramsauer[1], **Matthias Müllner[1]**, Sabrina Schrauf[1], Andrea Pfeiffer[1], Patrick Czar[1], Christa Firbas[2], Judith Aberle[3], Scott C. Weaver[4], Erich Tauber[1]

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Introduction: Themis is developing a safe and effective preventive vaccine platform against priority pathogen diseases such as chikungunya infection by using a "plug-and play" vaccine technology. The technology is based on a measles vaccine vector (MV) that can be genetically modified to express immunoprotective proteins for designated pathogens. This platform technology has already demonstrated proof of principle in large animal models and in humans. Non-human primates were immunized with MV-CHIK and subsequently challenged with a relevant infectious dose of chikungunya virus. All animal were fully protected against CHIKV symptoms and viremia. A Phase 1 clinical trial conducted in Austria demonstrated the safety and immunogenicity of the vaccine in healthy human subjects . Here, we present clinical Phase 2 data that demonstrate the safety and immunogenicity of MV-CHIK.

Methods: The blinded and placebo controlled Phase 2 clinical trial in healthy, adult, volunteer subjects is currently ongoing (NTC02861586). The 263 eligible subjects were randomized to receive one of two vaccine doses at one or five months interval. The main objectives of the study are to determine the safety and tolerability of the vaccine and vaccine immunogenicity, which is determined by plaque reduction neutralization test (PRNT) at four weeks after the primary vaccination schedule and until up to five months after the last treatment. Furthermore, the role of measles pre-existing immunity is addressed in detail by including a study group that received a measles vaccine prime prior to the first chikungunya vaccine administration.

Results: The study treatment phase is completed and final analysis is currently ongoing. Here, we present Phase 2 interim findings on humoral and cellular responses elicited by the recombinant chikungunya vaccine MV-CHIK. In addition, we show that the vaccine has an excellent safety and tolerability profile.

Conclusions: The clinical safety and immunogenicity findings clearly support the further clinical development of the chikungunya vaccine candidate.

[1] Ramsauer K, Schwameis M, Firbas C, Müllner M, Putnak RJ, Thomas SJ, Desprès P, Tauber E, Jilma B, Tangy F. Immunogenicity, safety, and tolerability of a recombinant measles-virus-based chikungunya vaccine: a randomised, double-blind, placebo-controlled, active-comparator, first-in-man trial. Lancet Infect Dis. 2015 May;15(5):519-27.

Norovirus

Kazuhiko Katayama
Kitasato University, Japan

Abstract to be distributed later.

Structure-based Zika drug discovery

Yi Shi
Beijing Institutes of Life Science, Chinese Academy of Sciences, and Shenzhen Third People's Hospital, China

Abstract to be distributed later.

Mechanisms controlling innate immune responses to nucleic acids

Kensuke Miyake
The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Toll-like receptor 7 (TLR7) in the endolysosome is a sensor for single-stranded RNA (ssRNA) from viruses and it induces antiviral immune response. In addition, this receptor also responds to synthetic small molecules such as R848 and Imiquimod. However, it remains unclear how TLR7 senses these two distinct ligands. There are two ligand-binding sites in the structure of TLR7: the first site binds to small chemical ligands, a guanosine (G) or deoxyguanosine (dG), whereas the second site binds to a uridine-containing oligoribonucleotides (U-ORN). We have found that TLR7 recognizes guanosine (G) or deoxyguanosine (dG) in the presence of ssRNA. With ssRNA, G/dG activates TLR7 and induces cytokine production in macrophages, cDCs and pDCs. These results strongly suggest that TLR7 recognizes degradation products of ssRNA, rather than ssRNA, and raise the possibility that TLR7 senses degradation products of genomic DNA as well. Recently, we found that accumulation of G/dG in the endolysosomes of macrophages resulted in an autoinflammatory disease with hepatosplenomegaly and histiocytosis. This talk focuses on the pathophysiological roles of nucleoside-sensing by TLR7 in inflammatory disorders.

U.S.-Japan Cooperative Medical Sciences Program (USJCMSP)

AIDS and Immunology Joint Panel Meeting Abstract Book

Shenzhen, China
January 10-11, 2018

U.S.-Japan Cooperative Medical Sciences Program (USJCMSP)
20th International Conference on Emerging Infectious Diseases (EID) in the Pacific Rim

AIDS and Immunology Joint Panel

January 10-11, 2018
Multifunction Hall I, 2nd Floor, Coli Hotel, Shenzhen, China

The focus of the AIDS and Immunology Joint Panel conference is on HIV/AIDS molecular virology, immunology, and preclinical research toward clinical studies in the Asia-Pacific region. The objectives of this conference are to share current research findings and foster existing and potential international research collaborations that engage investigators and institutions in the Asia-Pacific region and the United States. If you will be using Twitter or Instagram, please use the hashtag #CMSPEID.

Wednesday, 10 January 2018

7:00-8:15	Registration
8:15-8:20	**Announcements and Housekeeping** David McDonald, NIAID
8:20-8:30	**Welcome** Thomas Hope and Tetsuro Matano, US-Japan AIDS Panel Co-Chairs
8:30-8:45	**NIH Office of AIDS Research** Maureen Goodenow, NIH Office of AIDS Research

Session 1: Clinical Impact of HIV in the Asia-Pacific Region

8:45-9:15	**HLA class I-mediated HIV-1 Control in Vietnamese Infected with HIV-1 Subtype A/E** Masafumi Takiguchi, Kumamoto University
9:15-9:45	**HIV-1 Prevalence and Syphilis Incidence of MSM Cohort in Mongolia** Shin-ichi Oka, National Center for Global Health & Medicine
9:45-10:15	**HIV Cure Studies in Thailand** Eugene Kroon, SEARCH, Thai Red Cross AIDS Research Centre
10:15-10:30	Coffee Break

Session 2: HIV Molecular Virology

10:30-11:00	**Synergistic Anti-HIV Effect of Combination of a Novel Entry Inhibitor and NAbs** Shigeyoshi Harada, National Institute of Infectious Disease
11:00-11:30	**Live Cell Imaging of HIV-1 Reverse Transcription** João Mamede, Northwestern University
11:30-12:00	**Cryo-EM of HIV IN** (title not yet confirmed) Dmitry Lyumkis, Salk Institute
12:00-12:30	**Impact of Clinically Observed Integrase Mutations on Dolutegravir Resistance** Atsuko Hachiya, National Hospital Organization Nagoya Center
12:30-1:45	Lunch Break, The Coli Café, 1st floor

Session 3: HIV, Host Cell, and Microbiome Interactions

1:45-2:15 **The Tumor Suppressor APC Promotes HIV-1 Assembly Via Interaction with Gag Protein**
Kei Miyagawa, Yokohama City University

2:15-2:45 **Mutations in Viral Capsid Modulate IFN-β Sensitivity of HIV-1**
Akatsuki Saito, Osaka University

2:45-3:15 **Fibroblast-like Hematopoietic Cells, Fibrocytes, as Novel HIV-1 Latently Infected Cells**
Shinya Suzu, Kumamoto University

3:15-3:45 **APOBEC3H Variants Induce HIV-1 Fitness in Animal Model and Humans**
Yoshio Koyanagi, Kyoto University

3:45-4:00 Coffee Break

4:00-4:30 **Microbial Interactions in HIV Pathogenesis and Transmission**
Nichole Klatt, University of Washington

4:30-5:00 **Drug Distribution at SHIV Infection Sites in the Macaque Female Reproductive Tract**
Thomas Hope, Northwestern University

Thursday, 11 January 2018

Session 4: Mucosal Immunology and Vaccine Responses

8:15-8:45 **Mucosal Immunology**
Hiroshi Kiyono, University of Tokyo

8:45-9:15 **Virome Analysis in Intestine**
Satoshi Uematsu, University of Tokyo

9:15-9:45 **Peripheral Immune Protection Against Genital Herpes Infection**
Norifumi Iijima, NIBIOHN

9:45-10:15 **Mucosal Immunology in Acute HIV Infection and Cure Studies in Thailand**
Alexandra Schuetz, AFRIMS

10:15-10:45 **Optimization of Novel Vaccine Vector Approaches for HIV/AIDS**
Pablo Penaloza-MacMaster, Northwestern University

10:45-11:00 Coffee Break

11:00-11:30 **Dynamics of Wild-type and Mutant Epitope-Specific CD8+ T-cell Responses in SIV Controllers**
Tetsuro Matano, University of Tokyo

11:30-12:00 **Functional Mechanisms of Memory and Memory-like NK Cells in HIV/SIV and CMV infections**
R. Keith Reeves, Harvard University

12:00-12:30 **Strategies to Suppress SIV Replication in B-cell Follicle Sanctuary Sites**
Yoshinori Fukazawa, Vaccine and Gene Therapy Institute

12:30-1:00 **The Human IL-15 Superagonist ALT-803 Drives SIV-specific CD8+ T cells Into B Cell Follicles**
Jonah Sacha, Oregon Health & Science University

1:00 **Closing Remarks**
Thomas Hope and Tetsuro Matano, US-Japan AIDS Panel Co-Chairs

Abstracts
(Alphabetized by Last Name)

Strategies to Suppress SIV Replication in B-cell Follicle Sanctuary Sites

Yoshinori Fukazawa[1,2], Richard Lum[1,2], Bryan E Randall[1,2], Jin Young Bae[1,2], Alden Ho[1,2], Joseph Clock[1,2], Scott Hansen[1,2], Haesun Park[1,2], Alfred W Legasse[1,2], Michael K Axthelm[1,2], Jeffrey D Lifson[3], Afam A. Okoye[1,2] and Louis J Picker[1,2].

[1]Vaccine and Gene Therapy Institute, Oregon Health & Science University, Beaverton, OR 97006; [2]Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR 97006; [3]AIDS and Cancer Virus Program, Leidos Biomedical Research, Inc., Frederick National Laboratory, Frederick, MD 21702.

Introduction: We have reported that rhesus macaques (RM) with elite control of pathogenic SIV infection show exquisite restriction of replication-competent virus to CD4+ follicular helper T cells (TFH) resident within B cell follicles of secondary lymphoid tissues, suggesting that the highly effective anti-viral CD8+ T cell responses in these RM are able to almost completely clear and/or suppress productive SIV infection in extra-follicular T cell zones but not within B cell follicles. Here we evaluated whether disruption of this B cell follicular sanctuary can facilitate the clearance of persistent virus replication in elite controllers (EC).

Method: A total of 7 SIV-infected EC RM (plasma viral load: less than 1,000 copies/ml) received multiple doses of a B cell depleting anti-CD20 antibody (Ab) subcutaneously for 1st dose, then intravenously for 2nd and 3rd doses. Next we asked whether disruption of B cell follicles in SIV-infected RM on antiretroviral therapy (ART) would facilitate the clearance of reactivating virus in TFH and enhance virological control after ART cessation. A total of 21 RM determined to be mamu B*08^{+} (n=9) or B*08^{-}/B*17^{-}/A*01^{-} (n=11) were intravenously inoculated with SIVmac239 and placed on ART 12 days later. Once all RM achieved stable virus suppression, they were randomized into 2 groups and received multiple doses of anti-CD20Ab or control Ab starting ~2 weeks before and up to 6 weeks after ART cessation.

Results: Anti-CD20Ab treatment decreased plasma viral load by 1-2 log. This result indicates that disruption of B follicles by B cell depletion improves overall CD8+ T cell-mediated viral control in EC. In ART treated RM, anti-CD20Ab treated RM showed enhanced control of virus replication immediately after ART cessation and a trend towards lower viral load set points.

Conclusion: Overall these data suggest that the B cell follicular sanctuary must be overcome to fully evaluate the ability of virus-specific CD8+ T cells to reduce viral reservoirs or control viral rebound to achieve durable viral remission.

Impact of Clinically Observed Integrase Mutations on Dolutegravir Resistance

Atsuko Hachiya[1, 2], Karen A. Kirby[3], Urara Shigemi[1], Masakazu Matsuda[1], Reiko Okazaki[1], Mai Kuboya[1], Junji Imamura[1], Stefan G. Sarafianos[3], Yoshiyuki Yokomaku[1], Yasumasa Iwatani[1, 4]

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[3]Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine
[4]Department of AIDS Research, Graduated School of Medicine Nagoya University

Background: Dolutegravir (DTG), a second-generation integrase strand transfer inhibitor (INSTI), displays potent antiretroviral effects and superior resistance profiles. To date, HIV-1 integrase (IN) mutations associated with high-level DTG resistance have not yet been reported in the clinical settings. To explore potential DTG resistance mutations, we analyzed the impact of IN mutations detected in clinical isolates.

Methods: We isolated raltegravir (RAL)-resistant viruses from clinical samples during virological failure under a RAL-based regimen. The clinically suspected resistance mutations were introduced into the IN region of an HIV-1 DNA clone by site-directed mutagenesis. Drug susceptibility of the recombinant viruses was evaluated in a single-round replication assay using TZM-bl cells. Structural analyses of the IN-INSTI complexes were performed *in silico*.

Results: Genotypic and phenotypic analyses demonstrated that over the time course of clinical samples, a novel combination of L74F/V75I mutations in the IN region conferred resistance to first-generation INSTIs. Next, we evaluated whether the addition of L74F alone or L74F/V75I to the major resistance mutations impacts the level of INSTI resistance. The results showed that the addition of L74F to the major mutations, G140S/Q148H, increased the DTG resistance level (15-fold). In contrast, L74F/V75I drastically enhanced the level of DTG resistance when combined with either N155H (>385-fold) or G140S/Q148H (100-fold). Notably, these combinational mutations also increased the resistance magnitude to cabotegravir (CAB), which is currently an investigational second-generation INSTI.
DTG efficiently chelates two divalent metal ions of the IN catalytic center that are coordinated by the DDE motif (D64-D116-E152). The recently reported cryo-electron microscopy structure of the HIV-1 intasome

shows that L74 and V75, located in the β2 strand, are juxtaposed to the β1 strand containing D64 at the active site. This suggests an indirect structural impact of the L74F/V75I mutations, which may affect metal coordination, leading to high resistance to second-generation INSTIs.

Conclusions: This is the first report to demonstrate that clinically detected L74F/V75I mutations enhance the resistance level of the major mutations to all four currently available INSTIs. These findings will help our understanding of the superior resistance profiles of second-generation INSTIs and provide insights into the rational design of the next generation INSTIs.

Synergistic Anti-HIV effect of Combination of a Novel Entry Inhibitor and NAbs

Shigeyoshi Harada[1], Tetsuro Matano[1,2], Kazuhisa Yoshimura[1]

[1]AIDS Research Center, National Institute of Infectious Diseases
[2]Institute of Medical Science, University of Tokyo

Introduction: Neutralizing antibodies (NAbs) remain a critical contributor for HIV-1 vaccine and novel therapy. CD4 mimetic small compounds (CD4mc) act as a bifunctional entry inhibitor of HIV-1 with respect to both NAb activation and entry inhibition. Recently, we have developed novel bifunctional entry inhibitors that target non-CD4-binding site of HIV-1 envelope (Env) based on oleanolic acid derivatives. In addition, we also performed structure-based *in silico* screening of a chemical library containing over 5 million compounds to identify novel bifunctional entry inhibitors. In this study, we demonstrated the antiviral potency of hit compounds.

Methods: The susceptibility of the infectious HIV-1 clones (NL4-3, 89.6, YU2, JR-FL and the primary isolate, KP-5mvcR) to the hit compounds and neutralization sensitivity to NAbs were determined using the TZM-bl assay. Furthermore, to investigate synergistic effect of hit compounds in combination with NAbs, we calculated Combination Indexes (CIs) using the Chow and Talalay method.

Results: First, we synthesized and tested 48 oleanolic acid derivatives and found novel bifunctional entry inhibitors NAT-078 and NAT-080r, which could inhibit HIV-1 infection in IC_{50} values ranging from 0.17 to 19 μM. Second, we coupled structure-based in silico screening to an TZM-bl assay and selected 3 compounds (50b, 16b and 01c) as novel entry bifunctional inhibitors, which exhibited significant activity against HIV-1 with IC_{50} values ranging from 0.80 to 45 μM. All five hit compounds interfered with viral infection at the entry step. Furthermore, we examined synergistic effect between the hit compounds and eight NAbs targeting different domains in gp120 and gp41 (b12, PG9, PG16, 2G12, 447-52D, KD-247, 2F5 and 4E10). Almost all combinations showed synergistic effect in CI values from 0.31 to 0.90. The results demonstrated that the hit compounds NAT-078, NAT-080r, 50b, 16b and 01c were markedly more potent and broadly active than the previous CD4mc.

Conclusions: In the present work, we show that the novel bifunctional entry inhibitors NAT-078, NAT-080r, 50b, 16b and 01c can inhibit HIV-1 infection and also greatly enhance NAb activities. This study supports further investigation into the NAb based therapeutics.
(335/250-500 words)

Drug Distribution at SHIV Infection Sites in the Macaque Female Reproductive Tract

Katarina Kotnik Halavaty[1], Adina K. Ott[2], Danijela Maric[1], Jonathan T. Su[2], Edgar Matias[1], Lara Pereira[3], James M. Smith[4], Patrick F. Kiser[2], Thomas J. Hope[1]

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Introduction: Worldwide, sexual HIV transmission in young women is double that in young men. Prevention of HIV transmission in the female reproductive tract (FRT) by using a tenofovir disoproxil fumarate (TDF)-eluting intravaginal ring (IVR) is under investigation. Here we demonstrate the ability to locate the initial sites of infection, and to measure the drug distribution within a TDF-IVR-protected macaque FRT. Specifically, we are examining the impact of drug transport on viral spread within the FRT of these animals.

Methods: Six pigtail macaques were treated with TDF-IVRs for 28 days, and vaginally challenged with a high dose (~10^5-10^6) of a single round non-replicative SIV-based vector expressing HIV env and Luciferase and mCherry reporter genes. The FRT was scanned using *in vivo* imaging (IVIS), fluorescent microscopy and nested PCR (mCherry region) to detect early infection events. TFV tissue concentrations were quantified using LC-MS/MS, with ^{13}C-labeled TFV used as an internal standard.

Results and Conclusions: IVIS revealed infection events in the ovaries of two animals. Fluorescent microscopy revealed transduced cells in ovaries of five of six TDF-IVR animals. PCR data demonstrated frequent viral infections in the vagina and cervix of four of six animals. However, tenofovir levels were variable throughout the FRT, with the highest concentrations in the upper vaginal/lower cervical area (10^4-10^5 ng/g of tissue), near the site of the ring.

Utilizing reporter viruses allows us to detect the initial infection sites within the FRT. We performed complete pharmacokinetic and pharmacodynamic studies at both anatomical and cellular levels. In this ongoing study we are further investigating the IVR drug delivery efficiency and drug transport in tissues compared to the orally administrated TDF in challenged macaques. This novel research is critical in understanding the complex interplay between viral infection events and protective drug levels at the site of initial infection.

Peripheral Immune Protection Against Genital Herpes Infection

Norifumi Iijima

National Institutes of Biomedical Innovation, Health and Nutrition, Laboratory of Adjuvant Innovation and Immunology Frontier Research Center, Osaka University

The mucosal tissues of the respiratory, intestinal and genital tracts are major portals of entry into the body for many pathogens. Among causative organism of a disease, sexually transmitted pathogens including Human immunodeficiency virus (HIV), herpes simplex virus (HSV) and high-risk human papilloma virus (HPV) are known to cause severe illness through reproductive tract. HSV-2 is one of the most common sexually transmitted infections (STIs) with a high prevalence of 417 million in the world. HSV-2 is primarily transmitted through genital epithelial cells, which leads to the establishment of latency in the sacral ganglia. So far, several pharmacological interventions are available to inhibit virus replication. However, HSV-2 has not been able to be completely cured by them. Furthermore, it's been known that neonatal HSV-2 infection is seriously lethal without the treatment and HSV-2 infection in adult increases the susceptibility of high-risk HPV and HIV infection. Therefore, preventative vaccines or curative medicines are required for this disease. In 2012, HSV-2 vaccine trial announced that the vaccine was ineffective to prevent HSV-2 disease or infection despite inducing anti-HSV-2 immunity in the circulation. Towards developing vaccines to prevent HSV-2 transmission, a further understanding of the mechanism by which immune responses mediated within the relevant infection site (e.g. genital mucosal site and neuronal tissues) is necessary. Currently the immune mechanism of protection within the female genital mucosa and dorsal root ganglia (DRG) are poorly understood. Here, we found that 1) genital tissue-resident immune response by establishing memory lymphocyte cluster (MLC) including HSV-2-specific T cell populations was required for preventing the spreading of HSV-2 from genital mucosal site (the site for virus entry) to DRG (the site for virus latency) and 2) IFN-□ rapidly produced by circulating memory $CD4^+$ T cells in DRG increased vascular permeability in blood-nerve barrier to allow the entry of anti-HSV antibody within neuronal tissues. I would like to introduce these findings at this conference in detail.

APOBEC3H Variants Induce HIV-1 Fitness in Animal Model and Humans

Yoshio Koyanagi[1], Yusuke Nakano[1] and Kei Sato[1,2]

[1]Institute for Frontier Life and Medical Sciences
[2]Kyoto University, CREST, Japan Science and Technology Agency

Introduction: APOBEC3 (A3) is a group of proteins that inhibits retroviral replication. In humans, there are seven A3s, A, B, C, D, F, G, and H, that act as cytidine deaminases. However, HIV-1 Vif counteracts A3-mediated anti-viral action. A3D, A3F, and A3G elicit robust anti-HIV-1 effect in an *in vivo* model, human hematopoietic stem cell transplanted mice (humanized mice). Although *A3H* gene is polymorphic and can be categorized into seven haplotypes: stable (II, V, and VII) intermediate (I), and unstable (III, IV, and VI), the anti-viral effect of A3H has yet to be evaluated in animal model.

Methods: We utilized humanized mice transplanted with either stable (II, V, and VII) or intermediate (I) *A3H* haplotypes donors and inoculated wild type or *vif*-mutant HIV-1s. The amount of HIV-1 RNA in plasma was quantified qPCR and the proportions of naïve and memory $CD4^+$ cells in human $CD45^+$ leukocytes of peripheral blood mononuclear cells (PBMCs) were routinely analyzed by flow cytometry. RT-PCR product was obtained from the plasma of infected mice at 6 wpi and cDNA was prepared. The resulting cDNA fragments were cloned and the sequence was analyzed. The HIV-1 Vif sequences were obtained from the Los Alamos National Laboratory HIV-1 sequence database (https://www.hiv.lanl.gov/components/sequence/HIV/search/search.html). The information of *A3H* haplotypes of individuals was extracted from the 1000 Genomes Project (http://www.internationalgenome.org).

Results: We found that stable *A3H* haplotypes specifically affect HIV-1 fitness and that viral fitness is determined by the two amino acid residues at positions 39 and 48 of HIV-1 Vif. This observation suggests that HIV-1 is frequently adjusted through acquiring the ability to counteract stable A3H during its replication under the pressure of stable A3H. In contrast, the selection pressures mediated by intermediate A3H were not detected. Next global transcriptome analysis indicated that HIV-1 infection induces the expression of immune associated genes including *A3H*. Subsequent molecular phylogenetic analysis using more than 3,000 *vif* sequences including all subtypes obtained from the HIV-1 sequence database and cell culture experiment using several transmitter/founder HIV-1 Vifs suggest that the A3H polymorphism may influence the efficacy of HIV-1 dissemination at individual and population levels. We also provide evidence suggesting that anti-viral protein tetherin is a critical factor in HIV-1 transmission.

Conclusions: Our analyses indicate that stable A3H may control HIV-1 dissemination in both intra- and inter-individual scales.

HIV Remission Studies in a Thai Cohort Treated Since Acute HIV Infection

Eugène Kroon, on behalf of the SEARCH 010/RV254 study team.

SEARCH, The Thai Red Cross AIDS Research Centre, Bangkok, Thailand

Introduction: Study SEARCH 010/RV254 is a cohort of predominantly (89%) Thai MSM infected with HIV-1 subtype CRF01_AE (77%) treated with antiretroviral therapy (ART) since the earliest stages of HIV infection (Fiebig I-IV). Currently 503 subjects have enrolled in the cohort, longest follow-up is 8 years, and 452 have VL < 20 copies/mL. Seronegativity is 53% in Fiebig I participants after 24 weeks on ART.

We have shown that there is a significant decline in HIV reservoir size with early ART in this population, that the inducible reservoir in these participants is low, and that effector CD8+ T cells are associated with smaller reservoir size. For these reasons these individuals are considered good candidates for HIV remission strategy studies.

Methods: Since January 2015, eligible and consenting participants from SEARCH 010/RV254 have been enrolling into HIV remission studies. Protocols are developed by multidisciplinary study teams and undergo review by the study site IRB in Thailand and multiple IRBs internationally. All protocols to date require participants to have longer term viral suppression, include a placebo or control arm, and include analytic treatment interruption (ATI) with safety and time to viral rebound as the studies' main endpoints. Viral loads were monitored every 3-7 days and ART resumed if viral load > 1000 copies/ml. Protocols conducted to date are 1) ATI without intervention in participants who started ART in Fiebig 1 (n=8); 2) latency reversal + ART with vorinostat/maraviroc/hydroxychloroquine in volunteers treated since Fiebig 3/4 (n=15); and 3) broadly neutralizing antibody VRC-01 + ART in volunteers treated since Fiebig 1-3 (n=19). A study with a therapeutic vaccine combination is currently ongoing. RV254 participants invited for remission studies, both decliners and joiners, are interviewed by independent social scientists to explore expectations and motivations to decline or join.

Results: All participants (n=40) had viral load rebound (range 9 days to 10 months; median 22 days). Time to VL rebound in Thai AHI participants was longer than in US chronic HIV cohorts: median 14 days in chronic US (n=14) vs. 26 days in Fiebig I Thai (n=8; p=0.02) and 22 days in Fiebig III/IV Thai (n=14; p=0.003). Rate of viral load rise after ATI is less steep in ATI participants: chronic 0.65 log/day, Fiebig I 0.26 log/day, Fiebig III 0.18 log/day (p=0.02 and 0.002, respectively). HIV reservoir size by total HIV DNA increased during ATI and declined to pre-ATI levels after ATI resumption. Effector CD8+ T cells contribute to lower maximum viral loads after treatment interruption. We did not observe any acute retroviral syndrome, new resistance mutations by genotyping, nor virological failure after ART resumption. Study joiners and decliners are generally satisfied with their decision and this does not change over time.

Conclusion: HIV-remission studies using ATI appear safe in this population. ART alone minimally prolongs time to VL rebound and combination strategies including immune interventions are next step. Community participation and social science studies are critical to inform conduct of these trials.

Restoring the Vaginal Microbiome with Lactobacillus for Urogenital Health and HIV Prevention

Lagenaur, LA, Parks, TP, Marcobal, A, Nilsen, T, Swedek, I, Lee, P, Hemmerling, A and Cohen, CR.

Introduction: Preventing acquisition of HIV is a major global health challenge facing women today. Young women, especially in Africa, are disproportionally infected by HIV compared to their male counterparts. While this disparity is multifactorial, dysbiosis of the vaginal microbiome is a major factor in HIV susceptibility. Women with dysbiosis are at 4-fold greater risk of acquiring HIV-1 infection than women with *Lactobacillus*-dominant microbiomes. One solution is to deliver beneficial vaginal *Lactobacillus* as a live biotherapeutic to women to modify the microbiome, prevent dysbiosis and reduce HIV infection. To take this a step further, delivery of a genetically modified *Lactobacillus* expressing an HIV entry inhibitor could help prevent HIV in women who are most at risk. It is noteworthy that lactobacilli themselves are an important component of vaginal health in humans and act by increasing vaginal acidity, decreasing inflammation and reducing the incidence of vaginal infections. Live biotherapeutics represent a new class of products that are applicable to the prevention, treatment or cure of diseases in humans.

Methods: LACTIN-V (*Lactobacillus crispatus* CTV-05) is being evaluated in a Phase 2B clinical trial (NCT02766023) to prevent recurrence of bacterial vaginosis (BV). It is delivered intravaginally as a powder in a vaginal applicator to restore the vaginal microbiome after metronidazole treatment for BV. A next generation product, MucoCept-CVN, is a genetically modified *L. jensenii* expressing the HIV entry inhibitor, modified Cyanovirin-N (mCV-N). MucoCept-CVN is delivered as a vaginal tablet to both restore the vaginal microbiome and reduce HIV acquisition.

Results: LACTIN-V delivered as a powdered formulation appears to be safe and colonizes up to 78% of women using the product. Clinical trial results for LACTIN-V as a preventative for BV will be available in the last quarter 2018. MucoCept-CVN is in preclinical development. The *L. jensenii* expressing mCV-N, formulated as a vaginal tablet, colonized macaques and produced mCV-N protein *in situ*, on the vaginal mucosal surface. Macaques colonized with *L. jensenii* expressing mCV-N had a 63% reduction in Simian(S) HIV acquisition. No overt safety signals have been seen in the macaque or tissue models and no immune response to mCV-N was noted. An investigational new drug application is being filed for MucoCept-CVN with a first-in-human trial tentatively scheduled to begin in late 2018/early 2019.

Conclusions: Live biotherapeutics are a new class of biologic products to prevent dysbiosis of the microbiome. *Lactobacillus crispatus* CTV-05 can be safely administered to the vaginal mucosa in women to restore the vaginal microbiome to a *Lactobacillus*-dominant state. Recombinant Lactobacilli expressing HIV entry inhibitors will be the next generation of HIV prevention tools.

High-Resolution Cryo-EM Studies of HIV Integration

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Introduction: Like all retroviruses, HIV-1 irreversibly inserts a viral DNA (vDNA) copy of its RNA genome into host target DNA (tDNA). The intasome, a higher-order nucleoprotein complex composed of viral integrase (IN) and the ends of linear vDNA, mediates integration. Productive integration into host chromatin initiates with the formation of the soluble synaptic complex (SSC), an assembly of IN around vDNA oligonucleotides, and then proceeds through a multi-step reaction mechanism to result in the formation of the strand transfer complex (STC) containing catalytically joined vDNA and tDNA. HIV-1 intasomes have been refractory to high-resolution structural studies, despite considerable effort.

Methods: We have been using mutant IN enzyme containing a fusion protein at its N-terminal domain (NTD) to assemble and produce soluble HIV SSCs and STCs. More recently, we began using the WT construct, which is significantly more challenging to work with under in vitro conditions. Intasome structure is determined by high-resolution cryo-EM. All structural analysis is coupled to functional activity assays designed to evaluate the ability of the constructs to perform concerted and half-site integration into supercoiled target DNA.

Results: Cryo-EM analysis provided the first high-resolution structure of an HIV STC assembled with a mutant IN. We showed that, using the mutant IN, HIV intasomes can assemble into multiple compositional states. We have since derived high-resolution structures of SSCs and are working on improving conditions for routine cryo-EM structural analysis and for analyzing the WT specimen. Our data suggest that the higher-order intasome forms should be the functionally relevant type. All relevant forms contain an intact active site. I will discuss progress and current challenges pertaining to high-resolution cryo-EM studies of HIV intasomes.

Conclusion: The work highlights how HIV-1 can use the common retroviral intasome core architecture to accommodate different IN domain modules for assembly and provides a platform for developing better IN strand transfer inhibitors with improved resistance profiles.

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Live Cell Imaging of HIV-1 Reverse Transcription

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Introduction Following HIV-1 fusion with the cell membrane the conical capsid containing the viral RNA genome is delivered to the cytoplasm. There is general agreement that the process of reverse transcription facilitates the disruption of the conical core through a process known as uncoating. Ultimately, a double stranded DNA genome is generated through the completion of reverse transcription and the reverse transcripbed viral genome must reach host nuclear DNA where it will integrate to complete the early life cycle of HIV. The site of the completion of reverse transcription is a contentious issue. Some models suggest that reverse transcription is initiated and completed in the cytoplasm while others suggest that reverse transcription is (or can be) completed in the nuclear pore or nucleus. To gain insights into these processes by live cell imaging during infection we have developed a method to label viral dsDNA.

Methods The binding of tetR to dsDNA tetO sequences has been used extensively in inducible expression systems. We devised a dsDNA detection system where we have inserted an array of tet operons (tetO) into an HIV-1 viral vector that expresses a GFP reporter gene (HIVtetO-GFP). We also designed a panel of cell lines stably expressing tetR-tdTomato fusion proteins. The binding of tetR-tdTomato to the dsDNA tetO repeats recruits the tdTomato fluorescent protein to tag the DNA provirus. This DNA tethering allows the monitoring of formation and trafficking to the viral DNA genome by fluorescence microscopy of fixed or living cells. Here we used VSV-G pseudotyped particles carrying the tetO arrays that are also labeled with IN-GFP. This configuration makes it possible to follow nascent reverse transcribed viral DNA, from the moment when the double-stranded DNA encoding the tetO arrays becomes accessible to the expressed tetR-tdTomato, until integration. The specificity of the tetR-tdTomato interaction can be evaluated through the addition of tetracycline that disrupts tetR-tetO binding.

Results Initial validation studies revealed that cells stably transduced with HIVtetO-GFP contained a small puncta of nuclear tdTomato that was sensitive to the presence of tetracycline. Live cell microscopy detects the recruitment of the tetR-tdTomato to the IN-GFP labeled viral complexes in the cytoplasm approximately 2 hours post viral challenge. The recruitment of the tetR-tdTomato in the cytoplasm reveals that the viral dsDNA becomes exposed to cytoplasmic factors relatively early, consistent with the early uncoating model.

Conclusions This newly developed live-cell imaging reverse transcription system will allow answering many key questions of the HIV-1 early-steps of infection, such as the exact location of reverse transcription and the interaction of viral DNA with the innate sensing machinery.

Dynamics of Wild-Type and Mutant Epitope-Specific CD8$^+$ T-cell Responses in SIV controllers

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Introduction: aCD8$^+$ T-cell responses exert strong suppressive pressure on HIV replication and frequently select for viral escape mutations. Some of these mutations result in loss of epitope binding to MHC-I, while others do not diminish epitope presentation but inhibit CD8$^+$ T-cell recognition. Selection of the latter mutations may induce CD8$^+$ T-cell responses targeting the mutated epitope. In the present study, we investigated such virus-host CD8$^+$ T-cell interaction in a macaque AIDS model.

Methods: We examined dynamics of CD8$^+$ T-cell responses targeting a Mamu-A1*065:01-restricted viral Gag$_{241\text{-}249}$ (SSVDEQIQW) epitope and a mutant Gag$_{241\text{-}249}$.D244E epitope in SIVmac239 infection by using wild-type Gag$_{241\text{-}249}$-A1*065:01 and mutant Gag$_{241\text{-}249}$.244E-A1*065:01 tetramers. Mamu-A1*065:01-positive macaques that showed persistent viremia after SIVmac239 challenge elicited CD8$^+$ T cells dominantly detected by the wild-type tetramer, followed by selection of the viral escape GagD244E mutation with concomitant increases in CD8$^+$ T cells dominantly detected by the mutant tetramer. We then examined wild-type and mutant epitope-specific CD8$^+$ T-cell responses in SIV controllers.

Results and Conclusions: We previously reported two groups of SIV controllers, transient controllers and stable controllers (PLoS Pathog 11:e1005247, 2015). The former elicited CD8$^+$ T cells dominantly targeting the mutant Gag241-244.244E epitope in six months, which were not induced in the latter. The GagD244E mutation became dominant and detectable after one year in the former but not in the latter. These results suggest that appearance of CD8$^+$ T cells dominantly detected by the mutant tetramer can be an indicator of the beginning of viral control failure in SIV controllers.

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The Tumor Suppressor APC Promotes HIV-1 Assembly via Interaction with Gag Protein

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Introduction: Diverse cellular proteins and RNAs are tightly regulated in their subcellular localization to exert their local function. Likewise, proper recruitment and localization of virus components at specific sites in infected cells provides an important means of controlling efficient and dynamic viral spread. In our present study, we report that the tumor suppressor adenomatous polyposis coli protein (APC) directs the localization and assembly of HIV-1 Gag polyprotein at distinct membrane components to enable the efficient production and spread of infectious viral particles.

Methods and Results: A proteomic analysis and subsequent protein-protein interaction assay reveals that the carboxyl terminus of APC interacts with the matrix region of Gag. Ectopic expression of APC, but not its familial adenomatous polyposis-related truncation mutant, prominently enhances HIV-1 production. Conversely, the depletion of APC leads to a significant decrease in membrane targeting of viral components, resulting in the severe loss of production of infectious virions. We also find that APC regulates the plasma membrane localization of viral RNA to enhance the packaging of viral RNA into virions. Furthermore, APC promotes the directional assembly of viral components at virological synapses, thereby facilitating cell-to-cell viral transmission.

Conclusions: These results suggest that APC regulates cell-to-cell viral transfer in $CD4^+$ T cells by enhancing the targeting of viral components to the virological synapses. Our findings uncover a previously uncharacterized function of APC in HIV-1 replication and thus provide important new insights into the molecular mechanisms underlying HIV-1-host cell interactions.

HIV-1 Prevalence and Syphilis Incidence of MSM Cohort in Mongolia

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Introduction: Our molecular epidemiological study in Mongolia conducted in 2009 identified a hot spot of HIV-1 transmission in men who have sex with men (MSM). To control the infection, we have collaborated with NGOs that has provided safer sex promotion since mid-2010 and the treat-all strategy for MSM has been implemented since 2013. To monitor HIV infection, an MSM cohort has been organized since 2014.

Methods: MSM were anonymously registered by the deep finger vein authentication system at the NGOs. HIV and syphilis were examined repeatedly with rapid diagnostic test kits. Data of our cohort was compared with the National surveillance data. HIV+ blood samples were collected from the National surveillance and the second molecular study has done in 2017.

Results: By the end of 2016, 757 MSM were registered in this cohort and 1,692 tests were done. HIV-1 prevalence of this cohort was 8.9%. No HIV-1 seroconversion has occurred for 3 years. Newly diagnosed HIV-1 infection in 2014, 2015, and 2016 were 6/245 (2.44%), 2/327 (0.61%), and 1/471 (0.21%), respectively. Prevalence of syphilis was 15.5%. There were 27 syphilis seroconversions. Incidence of syphilis in 2014, 2015, and 2016 were 1.56/100PY, 4.74/100PY and 10.06/100PY, respectively. The second molecular study revealed two clusters of rapidly expanding HIV-1 infection. One cluster was belonged to subtype B that was the previously detected one in 2009, and the other was the newly identified one with CRF51_01B in the second molecular analysis.

Conclusions: According to the National data, HIV-1 infection among Mongolian MSM revealed a steep rise in this decade. In our cohort, no HIV-1 seroconversion and small number of new cases suggested effective activities of NGOs. However, incidence of syphilis was increasing, indicating they might keep doing risky sexual behavior for STIs.

Optimization of Novel Vaccine Vector Approaches for HIV/AIDS

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Introduction: The unprecedented challenges of developing protective vaccines against HIV, malaria and tuberculosis have motivated the pursuit of more rational approaches for vaccine design. Many efforts are aimed at improving candidate HIV vaccine regimens based on selection of vectors with low pre-existing immunity. However, little is known about what factors influence immunogenicity and overall immune protection. Herein, we evaluated the effect of vaccine vector replication and vaccine vector dose in determining immune responses following immunization of mice and cynomolgus macaques with vaccine vectors expressing simian immunodeficiency virus (SIV) antigens. We demonstrate that replicating vaccine vectors induce more potent adaptive immune responses than non-replicating vaccine vectors. In addition, we show that higher vaccine doses tend to favor effector memory T cell differentiation, whereas lower vaccine doses favor central memory T cell differentiation. Finally, we demonstrate a critical role for type I interferon signaling in regulating vaccine-induced SIV-specific T cell responses following immunization with replicating viral vaccine vectors.

Methods: Cynomolgus macaques or C57BL/6 mice were immunized intramuscularly (i.m.) with escalating doses of novel lymphocytic choriomeningitis virus (LCMV) vectors expressing SIVmac239 Gag and Env (LCMV-SIV), and animals were bled longitudinally to assess T cell responses by ELISPOT and ICS on PBMCs, and antibody responses by ELISA and TZM-bl neutralization assays on sera. We compared immunogenicity of replicating versus non-replicating viral vectors in different immunization settings. 100 μg of interferon type I receptor blocking monoclonal antibodies were administered at the time of vaccination.

Results: LCMV-SIV vaccine vectors are highly immunogenic in macaques and mice. Immunogenicity is substantially improved by: 1) vaccination with replicating vectors and 2) blockade of interferon type I signaling.

Conclusions: Although viral diversity is a major obstacle in HIV vaccine design, the field should investigate further the use of replicating vaccine vectors and the effects of innate immune modulation to induce optimal adaptive immune responses.

Functional Mechanisms of Memory and Memory-like NK Cells in HIV/SIV and CMV infections

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Background: Burgeoning evidence indicates a broader functional repertoire for NK cells beyond innate immunity including memory and other memory-like functions. One recent example is memory-like NK cells identified by lack of the FcR intracellular γ-signaling chain (FcRΔg-NK cells)which still require antibody to grant antigen-specificity, but are pre-sensitized and capable of rapid mobilization and more robust responses against viral antigens. Interestingly, FcRΔg-NK cells are initially expanded by CMV infection as part of innate-priming, but execute memory-like killing against other pathogens through incompletely understood mechanisms.

Methods: Fifty-three rhesus macaques were used: twenty-one specific pathogen-free, rhCMV–; 10 rhCMV+ but otherwise experimentally naïve; and 22 chronically SIVmac-infected macaques. Thirty-five human subjects were studied: 12 healthy controls; 12 HIV-infected/ART-suppressed individuals; and 11 HIV-infected/untreated individuals. NK cell analyses were performed using polychromatic, functional, and phospho-flow cytometry assays.

Results: FcRΔg-NK cells were systemically distributed in mucosal and secondary lymphoid organs, but increased two- and four-fold in CMV+ and HIV/SIV-infected individuals. FcRΔg-NK cells displayed little difference in binding affinity to virus-antibody immunocomplexes compared to traditional NK cells, but exhibited two-fold more robust IFN-γ secretion and cytotoxicity, suggesting disparate signaling or activation could account for improved function. To that end, FcRΔg-NK cells showed significantly reduced expression of Helios and Eomes and clustered independently from traditional NK cells in multidimensional t-SNE. The γ-chain adaptor, Syk, was reduced or inactively dephosphorylated in FcRΔg-NK cells, but expression of active ζ-chain, phosphorylated by increased adaptor Zap70, was significantly upregulated, suggesting these cells may exploit the ζ-chain/Zap70 pathway in the absence of γ-chain/Syk to achieve greater functional potency.

Conclusion: Collectively, our work presents the first description of a combinatorial mechanism of innate-priming and alternative signaling cascade to explain the functional potency of memory-like NK cells. This mechanism could explain, at least in part, the improved functional potency of NK cell-mediated ADCC in CMV+ individuals and impaired functions observed in chronic HIV/SIV infections. Future studies targeted at harnessing these pathways could open up new modalities for vaccine and curative therapy.

The Human IL-15 Superagonist ALT-803 Drives SIV-Specific CD8+ T Cells into B Cell Follicles

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Introduction: There is an urgent need for alternate approaches to activate and clear the HIV reservoir that do not negatively impact immune function. Purging the reservoir is further complicated by the exclusion of virus-specific CD8+ T cells from B cell follicles, anatomical sites that harbor latently-infected CD4+ T cells. IL-15 is a key cytokine for homeostatic maintenance, proliferation, and expansion of memory CD4+ T cells, the primary HIV cellular reservoir, as well as natural killer (NK) cells and virus-specific memory CD8+ T cells. Here, we explored the human IL-15 superagonist complex, ALT-803, as an immunostimulatory molecule in chronically SIV-infected rhesus macaques.

Methods: SIV-infected rhesus macaques were treated with a dose of up 100 ug/kg ALT-803 and subsequently assessed for intrafollicular migration of SIV-specific CD8+ T cells via *in situ* immunofluorescence staining of lymph nodes with MHC-class-I tetramers. Additionally, RNA *in situ* hybridization was employed to determine the number of SIV-producing cells within the follicles and extrafollicular regions of the lymph node.

Results: ALT-803 activated NK cells and memory T cells, causing them to undergo proliferation and home to secondary lymphoid tissues, an anatomical location of the viral reservoir. *In situ* MHC-class-I tetramer staining confirmed increased numbers of SIV-specific CD8+ T cells in lymph node, and revealed that the effector cells trafficked into B cell follicles. Accordingly, lower numbers of SIV-producing cells were found within B cell follicles in elite controllers post ALT-803 treatment indicating immune mediated clearance.

Conclusions: IL-15 superagonist, ALT-803, triggers massive proliferation of NK cells and CD8+ T cells and also reactivates quiescent SIV. ALT-803 also drives activated NK and CD8+ T cells into lymph nodes, and allows for SIV-specific CD8+ T cells to enter B-cell follicles harboring latently-infected CD4+ T_{FH} cells. The ability of ALT-803 to potentially mediate the “shock” and “kill” and to grant CD8+ T cells access to lymph node sanctuary sites makes it an appealing candidate for studies aimed at durable cART-free HIV remission.

Mutations in Viral Capsid Modulate IFN-β Sensitivity of HIV-1

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Introduction: HIV-1 infection causes robust immune activation characterized by elevated levels of proinflammatory cytokines and type I interferons (IFNs). IFNs induce the expression of a set of interferon stimulated genes (ISGs) to limit productive replication and establishment of viral reservoirs. Therefore, HIV-1 needs to equip IFNs resistance to disseminate in infected individuals. Recent studies suggest that viral capsid (CA) partly determines IFNs sensitivity of HIV-1. Specifically, it was reported that loss of CA interactions with CPSF6 or CypA lead to higher IFNs sensitivity. However, the molecular mechanism of CA mutations for IFNs sensitivity is largely unknown. We recently reported that one CA mutant, RGDA/Q112D (H87R, A88G, P90D, P93A and Q112D) virus, was highly resistant to cynomolgus monkey TRIMCyp, leading to loss of CypA binding. Accordingly, this virus was highly sensitive to IFN-β. To investigate whether RGDA/Q112D virus could evolve to overcome restriction by IFN-β, we performed adaptation of RGDA/Q112D virus in the presence of IFN-β. We identified two types of mutation (Q4R and G94D/G116R) in the CA region. In the present study, we examine the effect of these mutations on IFN-β sensitivity, interaction with host factors and uncoating kinetics.

Methods: To test IFN-β sensitivity, Jurkat cells treated with IFN-β were challenged with GFP-expressing viruses encoding CA mutations. To examine the interaction of CA mutants with host factors, MT4 cells infected with Sendai virus vector expressing host factors were challenged with CA mutants. We performed virion incorporation assay to test CA-CypA interaction. We also tested uncoating kinetics of these mutants using live-cell fluorescent imaging technique.

Results: We found that RGDA/Q112D viruses carrying Q4R or G94D/G116R were more IFN-β resistant than the original RGDA/Q112D virus, suggesting that these CA mutations modulated IFN-β sensitivity of RGDA/Q112D virus. Virus infection and virion incorporation assays revealed that these mutations altered interaction of HIV-1 with host factors including MxB, CPSF6 and CypA. Interestingly, RGDA/Q112D virus showed slower RT kinetics as compared with WT virus. We observed that while the Q4R mutation accelerated RT and uncoating of RGDA/Q112D virus, the G94D/G116R mutation did not change the kinetics.

Conclusions: We demonstrated that RGDA/Q112D virus could evolve to be IFN-β-resistant with mutations in CA. CA mutations emerged in this adaption indeed conferred IFN-β resistance, one of which also altered IFN-β sensitivity of WT virus. The Q4R mutation specifically altered RT and uncoating kinetics of RGDA/Q112D virus, suggesting that Q4R and G94D/G116R mutations modulated IFN-β resistance of RGDA/Q112D virus by different mechanism. Our finding would provide novel approach to understand complex interplay between host factors and HIV-1 CA.

Fibroblast-Like Hematopoietic Cells, Fibrocytes, as Novel HIV-1 Latently Infected Cells

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Background: Accumulating evidence has demonstrated that the spindle-shaped fibroblast-like cells, fibrocytes (fibroblastic leukocytes), are associated with diverse forms of tissue remodeling and fibrosis. Of interest, fibrocytes express markers of both hematopoietic cells and stromal cells, and are known to be differentiated from a subpopulation of $CD14^+$ monocytes, which are one of the major target cells of HIV-1. In this study, we therefore asked whether fibrocytes serve as HIV-1 latently infected cells, the major barrier to curing HIV-1.

Methods: We analyzed the viral integration, mRNA expression, and/or protein expression in fibrocytes in peripheral blood obtained from HIV-1-infected patients or lymph nodes obtained from SIV-infected rhesus macaques.

Results: The *in vitro* differentiated fibrocytes could be infected with R5-type HIV-1 viruses, which was evident by the presence of proviral DNA. This was further confirmed by *ex vivo* analyses: the proviral DNA was detected in the fibrocyte-enriched fraction ($CD3^-$ $CD14^+$ $CD16^-$ $CD34^+$) of all ART untreated chronically HIV-1 infected patients tested. However, in spite of a sufficient viral integration, the level of viral production of the *in vitro* differentiated fibrocytes was much lower than that of differentiated macrophages. Consistent with this, several latency reversal agents (LRA), in particular, JQ1, a bromodomain-containing protein 4 (BRD4) inhibitor, significantly upregulated HIV-1 production in the infected fibrocytes. Indeed, we also detected provirus in ART-treated patients. Moreover, in a rhesus macaque infected with HIV-1-related virus SIV, p27 SIV Gag protein was detectable in the fibrocyte fraction in both the mesenteric lymph nodes and peripheral blood, but their levels were much lower than that in $CD4^+$ T cells. Of importance, we found an elevated level of fibrocytes in both peripheral blood of HIV-1-infected patients and lymph nodes of SIV-1-infected rhesus macaque, as observed with the injury or chronic inflammation of various organs.

Conclusions: Our study identified fibrocytes as novel HIV-1 latently infected cells, and found their elevated levels in HIV-1/SIV infection. Thus, it will be important to clarify how fibrocytes are important in the overall pool of HIV-1 latently infected reservoirs.

HLA class I-mediated HIV-1 Control in Vietnamese Infected with HIV-1 Subtype A/E

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Introduction: HIV-1 subtype A/E (CRF01-AE) is the dominant subtype in Southeast Asia. Over 1.7 million individuals are living with HIV-1 subtype A/E and over 100,000 individuals are still newly infected every year in these countries. However, there is only one large scale study of HLA class I association with clinical outcomes in HIV-1 subtype A/E infection in Thailand. We here analyzed the association between the presence of HLA class I alleles and clinical outcomes in treatment-naïve Vietnamese individuals chronically infected with HIV-1 subtype A/E, and further investigated the role of HLA-associated mutations in rapid progression to AIDS.

Methods: 536 chronically HIV-1-infected, ART-naïve Vietnamese individuals (61% and 39% of these individuals are men and women, respectively) were enrolled at their first visit in National Hospital of Tropical Disease (NHTD) in Hanoi during October 2012-February 2017. Participant $CD4^+$ T-cell count (cells/μl) and plasma HIV-RNA (copies/ml) were measured at the first visit. The median (interquartile ranges 25%/75%) of plasma HIV-RNA (pVL) and $CD4^+$ T-cell count (CD4 count) are 48,550 (15,000/125,000) and 284 (171/428), respectively. Gag, Pol, and Nef genotyping was successful for 369, 359, and 372 individuals, respectively.

Results: We found that HLA-C*12:02 was significantly associated with lower pVL and higher CD4 count and that the HLA-A*29:01-B*07:05-C*15:05 haplotype was significantly associated with higher pVL and lower CD4 count as compared to individuals without these respective genotypes. Nine Pol and three Nef mutations were associated with the HLA-A*29:01-B*07:05-C*15:05 haplotype, where a strong negative correlation between the number of HLA-associated Pol mutations and CD4 count as well as a positive correlation with pVL in individuals with this haplotype were observed.

Conclusions: HLA-C*12:02 and HLA-A*29:01-B*07:05-C*15:05 haplotype had protective and detrimental effects, respectively, on clinical outcome in Vietnamese infected with subtype A/E virus. The

individuals with HIV-1 mutations associated with this deleterious HLA haplotype may not control HIV-1 since the mutations affect control of HIV-1 replication by speficic T cells. This study suggests the mechanism of HLA class I–mediated detrimental effects on HIV-1 control in Vietnamese infected with HIV-1 subtype A/E.

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Virome Analysis in Intesitne

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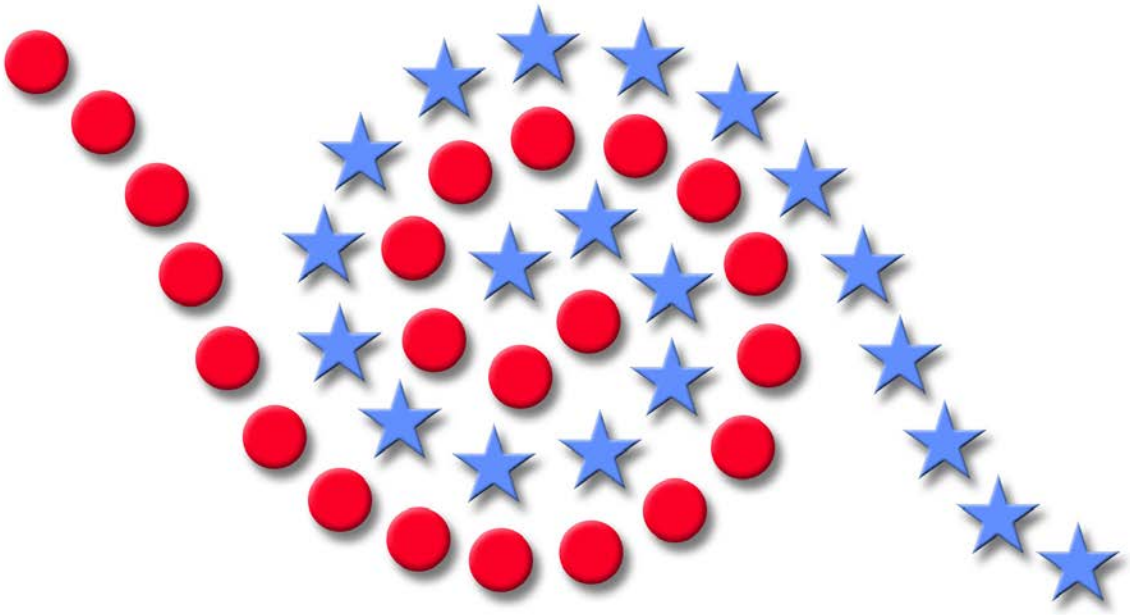
Our intestinal tract carries a lot of bacteria in the lumen as a resident microorganism. Conventionally, beneficial commensal bacteria present in our intestine have been thought to help our digestion and homeostasis, whereas putrid bacteria make harmful substances and promote aging and canceration. Recent studies have shown that commensal bacteria affects not only our health but also mental condition, as well as various diseases such as infectious diseases, inflammatory bowel diseases, obesity and diabetes. Interestingly, intestinal microbes surpass host human in number of both cells and genes. Therefore, it is not too much to say that the intestinal microflora is the second self. In considering the health condition and disease state, we should consider the existence of the commensal bacterial flora in addition to the influence of the host genes. In the background of these things, the development of the next generation sequencer has become a big turning point, resulting from the dramatic change in the research of the intestinal microflora. In classical analysis, each enteric bacterium was cultured one by one, and the sequence was confirmed. This requires the establishment of a difficult anaerobic culture method, which is very time consuming and labor intensive, and there was a limit to the analysis. However, by developing the next-generation sequencer, we can detect all the existing bacteria by extracting the whole genome from feces and exhaustively performing the sequence. 16s rRNA sequencing analysis is widely used as a method to conveniently examine the strain and classification of intestinal bacteria. However, since 16s analysis can only analyze up to the genus level, our institution conducts metagenome analysis by whole genome sequencing. We have constructed a pipeline that runs homologous search software capable of high-speed analysis on a supercomputer and performs metagenome analysis at super high speed. In this presentation we will outline the super high-speed pipeline constructed at our center, the intestinal bacteria analysis using it, and the analysis of intestinal virus flora.

United States-Japan Cooperative Medical Science Program:

20th Acute Respiratory Infections (ARI) Panel Meeting

10 January 2018

Shenzhen, China



Dear Distinguished Participant,

Thank you for your participation in the 20th U.S.–Japan Cooperative Medical Sciences Program (USJCMSP) Acute Respiratory Infections (ARI) Panel Meeting! This meeting is being held in conjunction with the 20th International Conference on Emerging Infectious Diseases (EID). The objectives for this conference are to share current research findings and to foster existing and potential international research collaborations that engage investigators and institutions in the Asia-Pacific region and the United States.

In January 1965, President Lyndon B. Johnson and Prime Minister Eisaku Sato met and issued a joint communiqué recognizing their mutual concern for the health and well-being of all peoples of Asia. The USJCMSP was founded in accordance with this communiqué. The United States and Japan agreed to undertake an expanded, cooperative research effort in the medical sciences, concentrating on health problems in Southeast Asia.

Following the Sato-Johnson summit, a series of meeting was organized between American and Japanese scientists, ultimately resulting in the formation of the U.S.-Japan Joint Committee. Committee members were appointed by the Japanese Ministry of Foreign Affairs and the U.S. Department of State to advise the respective governments about broad aspects of the program, set policy, and develop review procedures to ensure that program objects were met. The first delegates of the Joint Committee established a panel system applicable for each disease category, and appointed scientists to conduct an annual review of the scientific progress for each panel.

In 1965, the areas initially selected for study in the USJCMSP were cholera, leprosy, parasitic diseases, tuberculosis, and viral diseases. The Acute Respiratory Infections (ARI) Panel was established in 1996 to recognize the significance of acute respiratory infections worldwide including the United States, Japan, and Pacific Rim. Today, the scope of the program has expanded considerably, with nine panels and one board of scientific experts from each country overseeing research activities in the following areas: Acute respiratory infections, AIDS, Cancer, Cholera and other bacterial enteric infections, Genes, environment, and diseases, Hepatitis, Immunology, Nutrition and metabolism, Parasitic diseases, Tuberculosis and leprosy, and Viral diseases.

In 1996, the USJCMSP pioneered the scientific conference “Emerging Infectious Diseases (EID) of the Pacific Rim.” This addition to the structure and annual agenda presented a framework for the joint aspects of the program. The EID conference is held annually in alternating countries and provides a venue for panel meetings and discussion of cross-cutting topics related to infectious disease research.

The USJCMSP continues to thrive, evolve, and respond robustly to the new, emerging infectious disease challenges of Asia and the greater Pacific region under the leadership of the program’s delegation. This 20th ARI Panel meeting will facilitate the exchange of scientific and public health information on the diseases cause by acute respiratory infections. We seek to identify areas of ARI research that need additional emphasis or support. This forum will also provide opportunities to foster critical research collaborations.

Sincere gratitude to the ARI Panel Chairs, Drs. Keigo Shibayama and Florian Krammer.

Your participation in this meeting is greatly appreciated.

Warmest regards,

Kristina T. Lu
ARI Panel Secretariat

U.S.-Japan Cooperative Medical Sciences Program (USJCMSP)
20th Acute Respiratory Infections (ARI) Panel Meeting – Viral Diseases

10 January 2018
Venue: Coli Hotel
Shenzhen, China

Wednesday, 10 January 2018

8:20-8:30	Opening remarks – ARI Panel Chairs Keigo Shibayama, National Institute of Infectious Diseases Florian Krammer, Icahn School of Medicine at Mount Sinai
	Session - Viral Respiratory Diseases
8:30-8:55	The evolution of human metapneumovirus G gene Naganori Nao, National Institute of Infectious Diseases
8:55-9:20	Pathogen screening and prognostic factors in children with Severe Acute Respiratory Distress Syndrome of Pulmonary Origin Noriko Nakajima, National Institute of Infectious Diseases
9:20-9:45	Molecular and epidemiological analysis of enterovirus D68 in the Philippines Hitoshi Oshitani, Tohoku University
	Session - Respiratory syncytial virus
9:45-10:10	Molecular characterization of respiratory syncytial viruses detected from children with repeated infections Michiko Okamoto, Tohoku University
10:10-10:35	Respiratory Syncytial Virus Vaccines Are Approaching Clinical Trials Mark Peeples, The Ohio State University College of Medicine
10:35-10:55	Break
	Session - MERS coronavirus
10:55-11:20	Zoonotic threats from MERS coronavirus Malik Peiris, The University Hong Kong
11:20-11:45	Effect of Human DPP4 Expression on MERS Pathogenesis Chien-Te K. Tseng, University of Texas Medical Branch
	Session – Coronavirus – Antiviral therapeutics
11:45-12:10	GS-5734 is a Broadly Active Antiviral Against SARS-CoV, MERS-CoV and Potential Pandemic CoVs Mark Denison, Vanderbilt University

12:10-12:35	Utilizing yeast to identify genetic interactors of viral proteins and antiviral therapeutics Stuart Weston, University of Maryland
12:35-13:35	Lunch
	Session - Influenza – Transmission, Pathogenesis, & Immunity
13:35-14:00	Influenza transmission in animal models and at the human-animal interface Hui-Ling Yen, The University Hong Kong
14:00-14:25	Combination effect of Anti-high mobility group box-1 monoclonal antibody and Peramivir against influenza A virus (H1N1)- induced pneumonia in mice Masato Yashiro, Okayama University
14:25-14:50	Characterization of a highly pathogenic avian H7N9 influenza virus Masaki Imai, University of Tokyo
14:50-15:15	Protective Immune Responses during H7N9 infection Jianqing Xu, Fudan University
15:15-15:40	Promoter binding function of influenza virus RNA polymerase PB2 subunit Koyu Hara, Kurume Medical University
15:40-16:00	Break
	Session - Influenza – Vaccine & Inhibitors
16:00-16:25	Susceptibility of human influenza viruses to neuraminidase inhibitors from 2011-12 to 2016-17 Reiko Saito, Niigata University
16:25-16:50	The influenza virus neuraminidase as target for broadly protective vaccines and therapeutics Florian Krammer, Icahn School of Medicine at Mount Sinai
16:50-17:15	Mucosal vaccine and impact of secretory IgA antibody for influenza virus infection Hideki Hasegawa, National Institute of Infectious Diseases
17:15-17:20	Closing remarks – ARI Panel Chairs

Title: GS-5734 is a Broadly Active Antiviral Against SARS-CoV, MERS-CoV and Potential Pandemic CoVs

Maria L. Agostini, Erica L. Andres, Amy C. Sims, Rachel L. Graham, Timothy P. Sheahan, Xiaotao Lu, Everett Clinton Smith, James Brett Case, Joy Y. Feng, Robert Jordan, Adrian S. Ray, Tomas Cihlar, Dustin Siegel, Richard L. Mackman, Michael O. Clarke, Ralph S. Baric, Mark R. Denison

Vanderbilt University Medical Center

Coronaviruses (CoVs) have emerged from animal reservoirs to cause severe and lethal disease in humans, but no therapeutics or vaccines are available to combat these infections. A collaboration of Vanderbilt University of North Carolina, and Gilead Sciences has shown that GS-5734 (remdesivir), a prodrug of an adenine C-nucleoside analog GS-441524, potently inhibits multiple CoVs, including SARS-CoV, Middle East Respiratory Syndrome (MERS-CoV), Bat CoVs, and the model CoV murine hepatitis virus (MHV) in vitro with high activity and selectivity. In animal models of SARS-CoV, GS-5734 is efficacious both prophylactically and therapeutically. Passaging of MHV in vitro with drug selected substitution mutations in the RNA-dependent RNA polymerase which conferred partial resistance to GS-5734. Resistance mutants remained sensitive to higher concentrations of GS-5734 and demonstrated reduced competitive fitness compared to WT virus. Introduction of homologous substitutions into SARS-CoV conferred identical partial resistance to GS-5734, and was attenuating in a mouse model of SARS. The studies lend additional support to the development of GS-5734 for therapeutic use against known and emerging CoVs.

Promoter binding function of influenza virus RNA polymerase PB2 subunit

Koyu Hara, Takahito Kashiwagi, Nobuyuki Hamada and Hiroshi Watanabe
Kurume University School of Medicine

Introduction The influenza virus RNA-dependent RNA polymerase consists of PB1, PB2 and PA subunits. The PB2 subunit of influenza virus RNA polymerase is known to be involved in the initiation of transcription of the virus genome via cap-binding. However, other specific roles of PB2 for viral RNA synthesis are not well understood. Here, we investigated the pecise function of PB2 by mutagenic analysis.
Methods Since positively charged basic residues (R, K and H) have the potential to bind negatively charged viral RNA and are expected to be important for polymerase activity, we focused on clusters of basic residues that are highly conserved among influenza A, B, and C viruses. For *in vivo* experiment, the viral RNP was reconstituted in 293T cells by expressing NA vRNA, NP, PB1, PA, and PB2 (WT or mutants) of influenza virus A/WSN/33. Transcript products of viral mRNA, cRNA and vRNA were measured by primer extension. For *in vitro* experiment, the RNA polymerase was purified by the tandem-affinity purification (TAP) method and the promoter binding activity and replication activity was measured by UV cross-linking and ApG synthesis, respectively.
Results We found that six basic residues in the N-terminal half of PB2 (124R, 142R, 143R, 268R, and 331K/332R) are importnant for the polymerase activity. Notably, R124A mutation remarkably reduced the synthesis of mRNA, cRNA, and vRNA *in vivo*, which was in good agreement with the data obtained *in vitro*. Cross-linking studies suggested that a reduction of the polymerase activity in R124A mutant was due to a significant decrease in binding to the viral RNA promoter. In 3D structure of the polymerase, 124R is visible through the NTP tunnel and is closely located to the polymerase active site.
Conclusions A residue 124R in PB2 of influenza virus RNA polymerase plays a key role in promoter binding during the RNA synthesis.

Mucosal vaccine and impact of secretory IgA antibody for influenza virus infection

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The 2009 pandemic of influenza virus highlighted the difficulty in predicting the subtype and strain of influenza viruses which cause a coming pandemic. This fear of an emerging pandemic of new influenza underscores the urgency of preparing effective vaccines to meet the pandemic. One means to mitigate current concerns is to develop a flu vaccine that is fully functional against drift influenza viruses. In our current situation, in which we can't predict which strain will cause a pandemic, cross-protective immunity plays a particularly important role in preventing the spread of highly pathogenic influenza viruses.

Intranasal administration of a vaccine induces cross-protective secretory IgA (S-IgA) antibodies on the surface of nasal mucosa which is not induced by parenteral injection of the vaccine. Secretory IgA antibodies on the mucosal surface play an important role in protection against influenza virus infection. We have shown that secretary polymeric IgA antibodies induced by intranasal inactivated influenza vaccine have higher neutralizing and cross-neutralizing ability against homologous and heterologous influenza viruses compared to monomeric IgA antibodies in humans(PNAS112(25):7809-14). In the present study, we established the method for making polymeric monoclonal IgA antibodies in vitro. Similar to secretory IgA antibodies in the human nasal wash which is induced by intranasal vaccination, the produced recombinant IgA elucidated that polymerization of the antibody enhances the neutralizing activity. Mass spectrometry analysis of the recombinant monoclonal multimeric IgA antibodies produced by this method revealed that molecular weight is 720kDa suggesting mainly consist of tetramer form. These tetrameric IgA antibodies may be applicable for mucosal route antibody drugs for the prevention and treatment of influenza virus infection.

Characterization of a highly pathogenic avian H7N9 influenza virus

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[Introduction]

Low pathogenic avian H7N9 influenza viruses have recently evolved to become highly pathogenic, raising concerns of a pandemic, particularly if these viruses acquire efficient human-to-human transmissibility. In this study, we characterized a highly pathogenic avian H7N9 influenza virus isolated from an infected human and its recombinant derivatives *in vitro* and *in vivo*.

[Methods and Results]

We compared a low pathogenic H7N9 influenza virus (A/Anhui/1/2013) with a highly pathogenic H7N9 influenza virus (A/Guangdong/17SF003/2016) isolated from a human, and two of its variants that represent neuraminidase inhibitor-sensitive and -resistant subpopulations detected within the isolate. The highly pathogenic H7N9 influenza viruses replicated efficiently in mice and ferrets, and were more pathogenic in these animals than the low pathogenic H7N9 influenza virus, with the exception of the neuraminidase inhibitor-resistant virus, which showed mild-to-moderate attenuation. All viruses transmitted among ferrets via respiratory droplets, and the neuraminidase-sensitive variant killed several of the infected and exposed animals. Neuraminidase inhibitors showed limited effectiveness against these viruses *in vivo*, but the viruses were susceptible to a polymerase inhibitor.

[Conclusions]

These results suggest that the highly pathogenic H7N9 influenza virus has pandemic potential and should be closely monitored.

The influenza virus neuraminidase as target for broadly protective vaccines and therapeutics

Florian Krammer
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Introduction

Protection from influenza virus infection is typically associated with neutralizing antibodies that target the viral hemagglutinin. Less is known about the contribution of immunity to the second surface glycoprotein, the neuraminidase, to protection. Here we aimed at elucidating the potency, breadth and mechanisms of neuraminidase-based protection against influenza virus infection.

Methods

To assess the protective effect of anti-neuraminidase immunity mice were vaccinated with recombinant N1, N2 or influenza B virus neuraminidases. We then assessed antibody titers and challenged mice with matched or heterologous viruses. To further analyze the molecular basis for the breadth of antibodies against influenza B virus neuraminidase we generated murine monoclonal antibodies. These monoclonal antibodies were then characterized for their breadth and protective effect in the mouse model. Finally, the conserved epitopes recognized by these antibodies were mapped using escape mutants and electron microscopy.

Results

Vaccination with purified recombinant neuraminidase resulted in complete protection from weight loss and mortality in mice challenged with matched influenza viruses. For N1 and N2 vaccinated mice, challenge with heterologous viruses resulted in protection from mortality but weight loss was observed. Interestingly, vaccination with influenza B virus neuraminidase induced solid and broad protection against several challenge strains. Using monoclonal antibodies we then confirmed the protective breadth of anti-influenza B virus neuraminidase immunity and elucidated the conserved epitopes involved in protection.

Conclusions

Our results indicate that the neuraminidase should be considered as a target antigen for broadly protective influenza virus vaccines. In addition, broadly protective anti-influenza B neuraminidase monoclonal antibodies have the potential to be developed into future therapeutics to treat influenza B virus infections.

Bats as possible animal origin of MERS-CoV

Susanna K. P. Lau

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Bats are important reservoir for emerging viruses including coronaviruses. Although dromedary camels are believed to be the immediate animal source of the recent MERS epidemic, the evolutionary origin of MERS-CoV remains obscure. While horseshoe bats are the primary reservoir of ancestors of SARS-CoV, the possible role of bats in the emergence of MERS-CoV is less clear. When MERS-CoV was first discovered, it was found to be most closely related to *Tylonycteris* bat CoV HKU4 (Ty-BatCoV HKU4) and *Pipistrellus* bat CoV HKU5 (Pi-BatCoV HKU5) previously discovered in lesser bamboo bat (*Tylonycteris pachypus*) and Japanese pipistrelle (*Pipistrellus abramus*) respectively in Hong Kong. Subsequently, two other lineage C betacoronaviruses, BtVs-BetaCoV/SC2013 and Coronavirus Neoromicia/PML-PHE1/RSA/2011 (NeoCoV) were also detected in bats from China and Africa respectively. Interestingly, a lineage C betacoronavirus, Erinaceus CoV VMC/DEU, has also been found in European hedgehogs, which are phylogenetically closely related to bats, in Europe. Although NeoCoV represents the closest bat counterpart of MERS-CoV in most genome regions, the spike (S) protein, important for host receptor binding, is genetically divergent from that of MERS-CoV. On the other hand, Ty-BatCoV HKU4 possessed an S protein being most closely related to MERS-CoV. The spike of Ty-BatCoV HKU4, but not that of Pi-BatCoV HKU5, was able to utilize the MERS-CoV receptor, human dipeptidyl peptidase 4 (hDPP4) or CD26, for cell entry. These findings suggested that bats may be the primary host of the ancestor of MERS-CoV. To better understand the evolutionary path of MERS-CoV, we collected bat samples from various regions in China. Diverse CoVs were detected, including a potentially novel lineage C betacoronavirus. Compared to Ty-BatCoV HKU4 and Pi-BatCoV HKU5, the virus was even more closely related to MERS-CoV and NeoCoV in most regions of its genome. In contrast, the S1 region was less closely related MERS-CoV than Ty-BatCoV HKU4 but more closely related to MERS-CoV than Pi-BatCoV HKU5. To determine if this virus can utilize hDPP4 as receptor, binding experiments using S1-receptor-binding domain (RBD), cell entry studies using pseudovirus assays and structural modelling of the RBD-hDPP4 interphase were performed. The results suggested a stepwise evolutionary process among lineage C betacoronaviruses in gaining the ability to bind hDPP4, and support a bat origin of MERS-CoV.

Keywords:
Bats, animal, origin, MERS, coronavirus

Pathogen Screening and Prognostic Factors in Children with Severe Acute Respiratory Distress Syndrome of Pulmonary Origin

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Abstract

Introduction: Acute respiratory distress syndrome (ARDS) is one of the most lethal diseases encountered in the pediatric intensive care unit (PICU). In this study, the etiological pathogens and prognostic factors of severe ARDS of pulmonary origin in children with respiratory virus infections were prospectively investigated. The primary aim of the study is to examine the pathogens that can contribute to severe ARDS of pulmonary origin in children. The secondary aim is to investigate the prognostic factors for the progression of ARDS of pulmonary origin and a fatal outcome using multivariate analysis.

Methods: This study was carried out from December 2013 to May 2015 at the PICU in the National Children's Hospital, Hanoi, Vietnam. The enrolled children fulfilled: i) PICU admission, ii) 1 month to 16 years old, iii) with infectious pneumonia and a respiratory virus infection, and iv) complicated with severe ARDS within 72 hours after PICU admission. Pathogens were detected in the blood and tracheal lavage fluid using molecular techniques and a conventional culture system. The serum levels of inflammatory mediators on the day of PICU admission were examined.

Results: Fifty-seven patients (32 boys; median age, 9 months) were enrolled. Multiple virus infections, co-infection with bacteria/fungus, and bacteremia/fungemia were observed in 60%, 49%, and 32% of children, respectively. Adenovirus-B, measles virus, and cytomegalovirus were detected predominantly in tracheal lavage fluid. There were no statistically significant differences between non-survivors and survivors regarding the types of pathogen, incidence of multiple virus infection, gender, age, clinical features, and treatment. The serum levels of interferon (IFN)-γ and the IFN-γ/ interleukin (IL)-10 ratio were higher in non-survivors.

Conclusions: IFN-γ upregulation as detected on the day of PICU admission was found to be one of the possible prognostic factors affecting a fatal outcome. These results suggest that modulation of inflammatory responses is critical for the clinical management of children with ARDS.

The evolution of human metapneumovirus G gene

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3 Yokohama City Institute of Public Health, Yokohama, Japan

[Introduction]

Human metapneumovirus (HMPV), a member of the family Pneumoviridae, was first isolated in 2001 in the Netherlands. Seroepidemiological studies have shown that HMPV has been a major etiological agent of acute respiratory infections in humans for more than 6 decades. The viral genome is a non-segmented negative-sense RNA, which encodes nine proteins, including three surface glycoproteins: F (fusion), SH (small hydrophobic), and G(glyco-) proteins. Based on the antigenic and nucleotide sequence variations, HMPV is divided into two groups, A and B. Each viral group is further divided to two subgroups: A1 and A2 in group A, and B1 and B2 in group B. Detailed sequence analysis of HMPV strains revealed two clades A2a and A2b in the A2 subgroup. The G protein of HMPV is a highly glycosylated surface protein, which shows high diversity between subtypes. In this study, we identified unique HMPV A2b strains with 180- or 111- nucleotide duplication in the G gene (180nt-dup and 111nt-dup, respectively) (Miwako Saikusa et al, Frontiers in Microbiology, 2017; Miwako Saikusa et al, Microbiology and Immunology, 2017)

[Materials and Methods]

Clinical samples (throat swabs and nasal secretions) were collected in Yokohama and Sendai city in Japan between 2013 and 2017. The viral RNA was extracted from the clinical samples and cDNAs were synthesized using the viral gene specific primers. The cDNA of the viral genome was amplified by PCR and sequenced by sanger sequencing or next-generation sequencing. The sequence data were analyzed using MEGA (v7.0.20) and BEAST 2.

[Results]

Ninety HMPV A2b strains were detected: 47 and 43 strains in Yokohama and Sendai city respectively. In the 90 A2b strains, 35 and 3 strains had 180nt-dup and 111nt-dup, respectively. Phylogenetic analysis showed that these A2b strains with 180nt-dup and 111nt-dup were detected in the same cluster. The evolutionary rate of the G gene was estimated to be 3.5×10^{-3}/site/year (95% highest probability density: $1.9\text{-}5.5 \times 10^{-3}$ substitutions/site/year), and 180nt-dup were predicted to have occurred between 2010 and 2012.

[Conclusion]

HMPV A2b strains with 180nt-dup were also detected in Spain (María Piñana et. al., Future Medicine, 2017). Similarly, 60 and 72 nucleotide duplications in the G gene have been reported in human respiratory syncytial virus (RSV), another member of the family Pneumoviridae. Although the functional advantages of these nucleotide duplications are unclear, these RSV strains with nucleotide duplications have spread rapidly and globally, and are currently the predominant strains in many countries. HMPV A2b strains with 180nt-dup and 111nt-dup may also have potentials to spread rapidly and become one of the predominant strains.

Molecular characterization of respiratory syncytial viruses detected from children with repeated infections

Michiko Okamoto[1], Clyde Dapat[1], Raita Tamaki[1], Mariko Saito-Obata[2], Mayuko Saito[1], Socorro Lupisan[3], Hitoshi Oshitani[1]

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3. Research Institute for Tropical Medicine, Muntinlula, Philippines

Introduction

Human respiratory syncytial virus (RSV) is one of the most common causes of severe acute respiratory infection in infants and young children. While most children are considered to have been exposed to RSV before the age of three years, natural infection does not induce a life-long immunity, permitting repeated infection. However, a contribution of molecular change for repeated infections with the homologous subgroup of RSV is largely unknown.

Methods

A cohort study for children aged less than five years was conducted from May 2014 to January 2016 in a remote island in the Philippines. More than 3,800 children were enrolled in this cohort study and 1,802 children continued to participate throughout the study period. Nasopharyngeal swabs (NPSs) were collected from children with acute respiratory symptoms when they visited health facilities or during the biweekly household visit by trained study nurses when they presented acute respiratory symptoms within seven days after the onset. Viral RNA extraction, PCR, and sequencing were performed to compare F and G gene sequences between prior and subsequent RSV infections.

Results

A total of 438 (12.6%) samples were positive for RSV out of 3,471 NPSs collected during the study period. Two RSV epidemics were observed in this study with majority of RSV detected in the first epidemic was RSV-A (150/233, 64.4%), while RSV-B (183/205, 89.3%) was predominant in the second epidemic. All RSV-A and RSV-B strains were classified as ON1 and BA9 genotypes, respectively. Repeated infections were observed in 26 children including 4 children with homologous RSV-B reinfection. Sequence comparison of G protein between prior and subsequent homologous RSV-B infections showed three amino acid substitutions (A107T, R136T, and I254T) that led to potential changes in O-linked glycosylation patterns of G protein. While amino acid substitutions L173S and Q209K were detected in F protein, which are located in antigenic site VIII and site Ø, respectively and were reported as pre-fusion conformation-specific neutralizing epitopes.

Conclusions

The Q209K substitution in antigenic site Ø and L173S substitution in antigenic site VIII of F protein and changes in O-linked glycosylation pattern in G protein of RSV-B may contribute to the homologous reinfections observed in this study.

Molecular and epidemiological analysis of enterovirus D68 in the Philippines

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Introduction

Enterovirus D68(EV-D68) belong to the species *Enterovirus D*. Although EV-D68 is classified as one of enteroviruses, the virus has similar characteristics with rhinoviruses including acid sensitivity and the low optimal growth temperature (33 C). The virus has been detected mainly from children with acute respiratory symptoms with a considerable proportion of severe cases such as pneumonia and asthmatic bronchitis. The detection of EV-D68 had been extremely rare until recently. However, the reporting of EV-D68 have been increased from different parts of the world since 2010. Between 2014 and 2015, large outbreaks of EV-D68 in children with severe acute respiratory symptoms including some deaths were reported from North America, Europe, and Asia. The association between EV-D68 and acute flaccid paralysis (AFP) was also suspected in these outbreaks. However, the reasons for such increase in detection and a possible change in pathogenesis are not known.

Methods

From 2014 to 2016, we conducted a cohort study for children aged less than 5 years in a remote island of the Philippines (Biliran Island). Nasopharyngeal swabs were collected from children with acute respiratory symptoms at a hospital, rural health units (primary health care facility in the Philippines) and household. EV-D68 and other viruses were detected by real-time PCR and sequence analysis on 5'UTR and VP1 were conducted for EV-D68 positive samples. Sequence data were also compared with the sequences detected from our previous studies in the Philippines.

Results

A total of 6,142 samples were collected and 60 (0.98%) of them were positive for EV-D68. The sequence analysis of VP1 gene revealed that majority of the viruses (50/ 60, 83.3%) were classified as clade B (lineage 2), and the rest of them as clade A (lineage 3). Clade B viruses caused an apparent outbreak between September and October 2015, but clade A viruses were detected sporadically from July 2015 to April 2016. All clade B viruses were clustered in a single subclade and clade A viruses were classified in two distinct subclades. When compared with viruses detected in the previous studies, clade B viruses detected in the present study had amino acid substitutions in BC (T98A) and DE (M148V) loops of VP1, which are considered to be important antigenic sites. Patients infected with clade B virus had more severe clinical illness than those infected with clade A virus, although the number of patients was too small for clade A virus to have a statistical significance.

Conclusion

Molecular an epidemiological analysis of EV-D68 in the cohort study in the Philippines revealed that viruses in two clades (A and B) were co-circulating in the community and there was a significant epidemiological difference between two clades. Clade B viruses have also been causing major outbreaks in different parts of the world recently. Genetic changes of clade B viruses may have resulted in a change of virus characteristics including transmissibility and pathogenesis.

Respiratory Syncytial Virus Vaccines Are Approaching Clinical Trials

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Center for Vaccines and Immunity, Nationwide Children's Hospital, Columbus, Ohio
Department of Pediatrics, The Ohio State University College of Medicine, Columbus, Ohio

Introduction: Respiratory syncytial virus (RSV) was first identified as a human virus 60 years ago and is now known to infect nearly every child by the age of 2. RSV has a predilection for the bronchioles where inflammation, mucus hyper-production and infected cell death cause constriction. RSV is responsible for the deaths of an estimated 100,000 children each year, primarily in the developing world. In the developed world, RSV is the top reasons for hospitalization of children and a major cost to health care systems. RSV is also an important pathogen in elders, rivaling influenza virus. The fusion (F) glycoprotein is the main RSV neutralizing antigen. The F protein is an unstable protein because it must refold dramatically to cause virus membrane to fuse with the membrane of a target cell. The first RSV vaccine was tested in children in the 1960's, a formalin inactivated virus preparation, similar to the successful Salk poliovirus vaccine. But instead of protecting the young vaccinees, they developed more severe disease upon natural challenge.

Methods: Over the intervening years, steady progress has been made toward a live attenuated RSV, primarily by the NIH in collaboration with several pharmaceutical companies. Such a vaccine would be applied intranasally. The F protein, inserted into and expressed by several different virus vectors, has also been shown to stimulate a protective immune response. Both attenuated and virus-vectored RSV would likely be given at 6 months of age at the earliest, leaving infants < 6 months of age unprotected. The functional F protein, in its "pre-fusion" form, induces higher levels of antibodies than its refolded, post-fusion form. Solution of the pre-fusion form of the F protein has enabled rational modifications to stabilize it for use as an antigen. This stabilized F protein is being developed as a maternal vaccine to raise the level of neutralizing antibody in infants, thereby protecting them for an additional month or two beyond the maternal antibody that they normally receive.

Results: In addition to stabilized pre-fusion F protein vaccines, post-fusion F protein and virus-like particle (VLP) vaccines that include the F protein are also in development. Some of these vaccines are formulated with traditional adjuvants and others with experimental adjuvants.

Conclusions: We are rapidly moving toward clinical trials for live attenuated and virus-vectored RSV vaccines to protect children > 6 months of age, as well as for F protein RSV maternal vaccines to protect infants < 6 months of age. Protein or VLP vaccines are also being considered for elders. The number of academic institutions, biotechnology companies and pharmaceutical companies that are currently participating in these efforts have greatly expanded over the past 10 years. The outlook for successful RSV vaccines seems likely.

Zoonotic threat from MERS coronavirus

Malik Peiris

School of Public Health, The University of Hong Kong

Coronaviruses (CoV) are RNA viruses with capacity for generating genetic diversity by mutation and recombination. Human coronaviruses 229E, OC43, HKU1 and NL63, are known to be endemic in humans. Of these, 229E, OC43 and NL63 have crossed to from animals to humans within the past few hundred years; 229E from bats via camels to humans, OC43 from cattle and NL63 from bats. The precursor of SARS coronavirus is present in bats, emerged from game animal markets in Guangdong province, China, and led to an outbreak that spread globally to infect almost 8000 people in 25 countries. MERS-CoV is the most recent coronavirus of zoonotic and potential epidemic concern. It is endemic in dromedary (but not Bactrian) camels in the Arabian Peninsula as well as Africa and Central Asia. The virus is more commonly detected in camel calves but can re-infect previously sero-positive animals and can be detected at high prevalence in camel markets and abattoirs where animals from many sources are mixed together. Zoonotic infections have only been reported from the Arabian Peninsula and the reason for an apparent lack of zoonotic MERS in Africa remains to be elucidated. Viral genetic and phenotypic analysis of MERS-CoV from Africa provides some possible explanations. Zoonotic transmission events are often mild and unrecognized and become apparent when older persons with co-morbidities are involved. Zoonotic events can lead to transmission in humans within health care facilities, leading to large disease outbreaks. Late recognition and diagnosis and health care workers continuing to work with mild illness contribute to nosocomial outbreak. Molecular epidemiology can help elucidate transmission patterns within hospitals. It is possible that the greater stability of the virus in air-conditioned environments contributes to such outbreaks. Sero-epidemiological studies indicate that reported cases significantly under-estimate the true extent of infections taking place.

Susceptibility of human influenza viruses to neuraminidase inhibitors from 2011-12 to 2016-17

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Introduction: In Japan, four kinds of neuraminidase inhibitors, oseltamivir, zanamivir, peramivir, and laninamivir, are used for prevention and control of influenza virus infections. We assessed susceptibility of 1,214 influenza viruses collected by Niigata University under the collaboration of clinicians in Japan during December 2011 to April 2017.

Methods: Florescent based neuraminidase (NA) inhibition assay was used to determine 50% inhibitory concentration (IC_{50}) data for NA inhibitors, oseltamivir, zanamivir, peramivir and laninamivir, using World Health Organization (WHO) provided control viruses. The viruses showing reduced inhibition were subsequently performed of genetic sequencing of NA and analysis of enzymatic property using Michaelis constant (Km) to examine the affinity of NA to substrate (MUNANA).

Results: Of 212 influenza A/H1N1pdm09, 630 A/H3N2, 202 B/Victoria and 170 B/Yamagata tested, 7.5% (n=16) of A/H1N1pdm09 viruses showed highly reduced inhibition (HRI) to oseltamivir and peramivir according to WHO definition ($\geq$ 100 fold rise of IC_{50} value). In contrast, 0.48% (n=3) of A/H3N2, 1.49% (n=3) of B/Victoria and 1.76% (n=3) of B/Yamagata exhibited reduced inhibition (RI) to at least one of four NA inhibitors (10-100 fold rise with influenza A and 5-50 fold rise with influenza B). All of A/H1N1pdm09 viruses with HRI were collected during 2013-2014 and 2015-2016, possessing common H275Y mutation in NA. Enzymatic property assay revealed that the H275Y viruses had reduced NA affinity to MUNANA compared to the sensitive counterparts (42.0 versus 28.6 mM), suggesting limited transmission fitness of the H275Y viruses. Two of A/H3N2 viruses collected in 2017 showed reduced inhibition to zanamivir and laninamivir by ~20 fold and ~10 fold with multiple mutation sites in NA.

Conclusion: Although majority of influenza strains were susceptible to NA inhibitors, our data indicated importance of continuing drug susceptibility monitoring.

Effect of Human DPP4 Expression on MERS Pathogenesis

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Introduction: Middle East respiratory syndrome coronavirus (MERS-CoV) is a newly emerged cause of a severe acute respiratory disease similar to severe acute respiratory syndrome (SARS) caused by SARS-CoV in 2002-2003. Unlike the short-lived SARS epidemic that was controlled with extreme infection control measures, MERS-CoV infections persist in humans since emergence in 2012; as of, 9/27/2017, the virus has caused 2,102 documented cases of MERS and 733 deaths (34.8%). Like other human CoVs, MERS-CoV uses an exo-aminopeptidase, human dipeptidyl peptidase 4 (DPP4), as the receptor for uptake by permissive cells. Other effects of MERS-CoV-DPP4 interactions on MERS-CoV infection and disease are largely unknown. DPP4 plays a critical role in multiple physiological functions via its ubiquitous cellular presence and intrinsic enzymatic activity. Although expressed as a type II membrane glycoprotein, catalytically active soluble DPP4 (sDPP4) also exists in the circulation and other body fluids, and could participate in diverse functions. However, how the intrinsic DPP4 expression might affect the pathogenesis of MERS-CoV infection and disease has not been systemically investigated. Here, we report the effect of altered intrinsic levels of DPP4 expression on the MERS pathogenesis in heterozygous (+/-) versus homozygous (+/+) transgenic (Tg) mice globally expressing human (h) DPP4 viral receptor.

Methods: The 50% lethal dose (LD_{50}) was determined to compare the relative permissiveness of age- and sex-matched $hDPP4^{+/+}$ and $DPP4^{+/-}$ mice to MERS-CoV infection; whereas their prospective levels of human sDPP4 in the sera and complete blood cell counts were measured a commercially available ELISA kit and a hematology analyzer (Hemavet 950FS), respectively. Finally, the standard Vero E6-based micro-neutralization test and quantitative (q) RT-PCR-based assay were used for comparing the virus-neutralizing ability of naïve sera and the pulmonary innate antiviral responses of $hDPP4^{+/+}$ and $hDPP4^{+/-}$ Tg mice in response to MERS-CoV infection.

Results: As hDPP4 is the functional viral receptor, we would expect that $hDPP4^{+/+}$ mice would be more susceptible or at least equally susceptible to MERS-CoV infection and disease than their $hDPP4^{+/-}$ counterparts. We unexpectedly noted that $hDPP4^{+/+}$ mice were more resistant than $hDPP4^{+/-}$ mice to MERS-CoV infection, with a ~10-fold increase of the LD_{50} and reduced viral yields and sero-conversion rates. Additional studies revealed that $hDPP4^{+/+}$ mice had significantly higher serum levels of sDPP4 and virus neutralizing activity than $hDPP4^{+/-}$ mice, suggesting that higher levels of sDPP4 might function as viral decoys and enhance immunity to MERS-CoV infection. We also showed that treatment with exogenous recombinant hsDPP4 prevented MERS-CoV infection in $hDPP4^{+/-}$ Tg mice. In addition, naïve $hDPP4^{+/+}$ mice had elevations of all circulating leucocytes, particularly monocytes, eosinophils, and basophils, and were superior inducers of interferon (IFN)-related gene expressions than $hDPP4^{+/-}$ to acute MERS-CoV infection.

Conclusions: In addition to serving as a potential decoy of viral infection, elevated sDPP4 relation to increased components of innate immunity could reduce MERS-CoV infection. Additional studies are needed to further explore the mechanism of the increased resistance of elevated DPP4 expression to MERS-CoV infection.

Utilizing yeast to identify genetic interactors of viral proteins and antiviral therapeutics

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Introduction

Viral proteins must interact intimately with the host cell machinery for replication and pathogenesis. While yeast have been used extensively to study cell biology, there has been comparatively little use of this model system to identify how viral proteins interact with a cell. We have found that expression of various viral proteins can cause defective yeast growth. We hypothesize that the growth defect is a result of the viral protein interacting with, and disrupting cellular pathways. Therefore, the defect can be leveraged to identify novel genetic interactors of viral proteins in a eukaryotic cell. Moreover, drug screens can be performed allowing for an unbiased identification approach to find compounds targeting either cellular or viral proteins.

Methods

Genes encoding each viral protein are cloned into galactose-inducible expression plasmids and transformed into yeast. The optical density of transformed yeast is monitored over a 48 hour period to determine growth defects. For genetic studies, the expression plasmids are transformed into the yeast knockout library to screen for genes involved with the viral protein-mediated slow growth phenotype. For antiviral identification, yeast expressing the viral protein can be grown in the presence of drug libraries to identify compounds capable of reverting the slow growth phenotype.

Results

Proteins from multiple viruses have been tested for growth defects in yeast with at least one protein being found to inhibit growth from each virus. Work has focused on the Middle East respiratory coronavirus (MERS-CoV) ORF4a protein. Drug screens have been performed identifying multiple compounds that revert the slow growth phenotype. Work is ongoing to investigate the antiviral properties of these compounds *in vitro*. Additionally, ORF4a has been expressed in the yeast knockout library collection and yeast genes whose deletion was found to suppress the slow growth phenotype have been identified. Of these, *SIR2* has been the focus of study as the human homologue *SIRT1* has been suggested to have antiviral function. Work is ongoing to test the role of SIRT1 in mammalian cell culture systems.

Conclusions

Expression of certain viral proteins in yeast can cause a growth defect, potentially providing a powerful model system to further understand the function of viral proteins in eukaryotic cells. The growth defect can be utilized to identify compounds with potential antiviral properties and novel genetic interactors. Using the CRISPRi system, work is ongoing to investigate the relevance of genetic hits identified using yeast in mammalian cell culture systems. Future work will be aimed at validating yeast hits in mammalian systems and investigating the relevance of any *in vitro* hits in appropriate mouse model systems to analyze their relevance to replication and pathogenesis. To date, every virus tested has been found to have at least one protein capable of inhibiting yeast growth, this system may therefore provide a broadly applicable tool to analyze the function of viral proteins.

Protective Immune Responses during H7N9 infection

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First H7N9 outbreak occurred in 2013 in Yangzi River area and caused a ~30% mortality. 18 H7N9-infected patients were hospitalized at our center, 12 out of 18 survived whereas the left 6 failed to do so. Blood samples were longitudinally collected from all patients, immune responses, including cellular and humoral immune responses and plasma inflammatory cytokines, were compared between survival and fatal groups. Early and high neutralization antibody responses, rapid CD8+ T cell responses and early antiviral treatment are associated with survival and early recovery, respectively. Interestingly, H7N9 infection broadly boosted both group 1 and group 2 influenza antibody responses.

Combination effect of Anti-high mobility group box-1 monoclonal antibody and Peramivir against influenza A virus (H1N1)- induced pneumonia in mice

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Abstract

Objectives: There is a pressing need to develop a novel strategy for the emergence of an influenza pandemic. Subsequently, we reported the therapeutic effects of anti-high mobility group box-1 (HMGB1) monoclonal antibody (mAb) treatment on influenza A virus (H1N1)-induced pneumonia in mice. Clinically, however, we don't treat influenza patients without anti-influenza drugs. Here we report the combination effect of anti-HMGB1 mAb and anti-influenza drug in this study.

Design: Prospective animal trial.

Setting: Research laboratory.

Subjects: Eight-week-old male C57BL/6 mice.

Intervention: The mice were inoculated with H1N1, then Peramivir were administered intramusculy on day2, 3 and 4 hours after H1N1 inoculation. Anti-HMGB1 mAb or control mAb were administered on day2 and 3. The survival rate was analyzed and the lung lavage and histopathological analysis were performed on day5 and 7.

Measurements and Main Result: Lung lavage and pathological analyses were performed on day5 and 7 after inoculation. Peramivir and anti-HMGB1 mAb significantly improved the survival rate of H1N1-inoculated mice, although anti-HMGB1 mAb did not affect virus propagation in the lung. The treatment also significantly attenuated the histological changes, neutrophil and macrophage infiltration. This was associated with inhibition of HMGB1 and suppression of inflammatory cytokine/chemokine and oxidative stress. Furthermore, we showed that anti-HMGB1 mAb inhibited the translocation of HMGB1 from alveolar epithelial I (AEC Ⅰ) cells.

Conclusion: The combination therapy of Anti-HMGB1 mAb and Peramivir may be a novel strategy for severe influenza virus infection.

Key Words: influenza; hmgb1; peramivir; mouse; acute lung injury; cytokine

Influenza transmission in animal models and at the human-animal interface

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Influenza A viruses infect a wide range of hosts and are transmitted by multiple non-mutually exclusive modes as a result of different virus-host interactions. Among humans and most mammalian species, influenza viruses are transmitted by virus-laden aerosols or secretions released from the respiratory tract. In avian species, influenza viruses may replicate in the gastrointestinal tract and be transmitted via fecal droppings. Understanding the major modes that mediate influenza transmission between humans or at the human-animal interface is essential for developing effective control measures. We have applied conventional and novel experimental designs to study influenza transmission in animal models. Using the conventional experimental setup, we assessed the transmission potential of highly pathogenic and low pathogenic H7N9 virus in chickens and in ferrets. Applying a newly designed transmission chamber, we defined the sizes of airborne particles that mediate seasonal H3N2 and pandemic H1N1 influenza transmission in ferrets. Ferret-to-ferret transmission was mediated by virus-laden droplets and droplet nuclei larger than 1.5 μm, consistent with the quantity and size of virus-laden particles released by the donors. At the human-poultry interface, we assessed the feasibility of airborne transmission of avian influenza virus at live poultry markets. A multidisciplinary research approach to study influenza transmission under laboratory and field settings will benefit the development of practical infection control measures.

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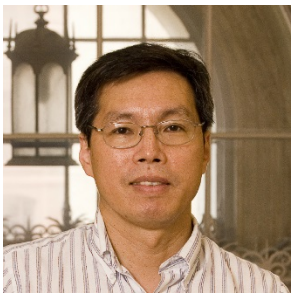
Pen Picture

Keigo Shibayama has been the chairman of ARI panel of Japan side since 2012. He is the chairman of Steering Committee of Japan Nosocomial Infections Surveillance (JANIS), which is the national surveillance of antimicrobial resistance in Japan (https://janis.mhlw.go.jp/english/index.asp). The mission is to tackle antimicrobial resistance by strengthening the surveillance program and basic research in Japan and Asian countries in cooperation with Ministry of Health, Labour and Welfare. Now JANIS helps Asian countries develop national surveillance program of AMR.



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Dr. Tseng presently is a Professor (tenured) in the Department of Microbiology and Immunology and the Director of the Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) P3 laboratories of the Galveston National Laboratory at the University of Texas Medical Branch. Dr. Tseng is an immunologist by training and accomplished investigator in the field of viral pathogenesis and translational research against emerging and re-emerging human viral pathogens, including SARS-CoV, MERS-CoV, Rift Valley fever virus, and influenza virus. His laboratory is most well-known for their expertise in establishing transgenic mouse models for studying pathogenesis of SARS-CoV and MERS-CoV infection and disease. Using his group's well-characterized transgenic mice that globally express human DPP4 receptor of MERS-CoV combined with the expertise of GNL veterinary staff in conducting NHP studies, Dr. Tseng has been very active in collaborating with his academic and industrial partners to develop effective preventive and therapeutic countermeasures against MERS. Examples of these include RBD or S1 protein-based subunit and various vector-based vaccines (Ad, VSV, and nanoparticle), RBD-specific human monoclonal antibodies, fusion inhibitors, sDPP4 receptor decoys, and nucleoside analogs. Dr. Tseng has been funded by a variety of federal and private agencies, including NIH, Ministry of Health, Kingdom of Saudi Arabia, and other Industrial Partners.

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I am currently a post-doctoral fellow in the laboratory of Matthew Frieman at the University of Maryland, Baltimore. The research interests of the lab are largely centered around the interaction between the SARS- and MERS-coronaviruses and the cells they infect, and how this translates to pathogenesis. My PhD was concluded in 2016 in the laboratory of Mark Marsh at the Laboratory for Molecular Cell Biology at University College London. That projected involved studying the cell biology and antiviral function of the human IFITM proteins, broad acting viral restriction factors that block entry into cells. The PhD training in a cell biology department helped me develop an interest in not only understanding virology, but also how understanding viral replication can inform about the normal function of a cell. This interest continues into my post-doctoral research. In the Frieman lab, my project is focused upon developing a novel system to use for further understanding how viral proteins interact with a eukaryotic cell, using the model system of the yeast *Saccharomyces cerevisiae.* We have shown that certain viral proteins are capable of inhibiting yeast growth when expressed in an inducible system. Using this phenotype, we are looking to find genetic interactors of these viral proteins using the well-established yeast knockout library collection, and screen for drugs that may have antiviral function. The project aims to uncover novel functions of viral proteins in modulating a cell for replication, with the potential to better understand the normal function of cellular pathways based on the way viral proteins interact with these.

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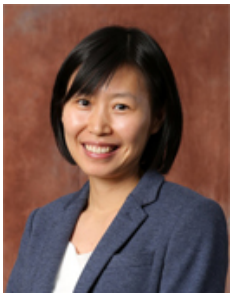

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U.S.-Japan Cooperative Medical Sciences Program

Cancer Panel Meeting

January 10-11, 2018

Shenzhen, China

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Agenda

Wednesday, 10 January 2018

08:15 **Registration**

08:45 **Welcome Remarks**
Dr. Tohru Kiyono (National Cancer Center of Japan, Tokyo)
Dr. Ted Trimble (U.S. National Cancer Institute, Bethesda)

Session 1 **Overview of Hepatitis and Liver Cancer**
Co-Chairs: Dr. Tohru Kiyono (Japan) and Dr. Xin Wei Wang (USA)

09:00 **Hepatocellular Carcinoma Burden, Epidemiology, Known Etiology**
Dr. John D. Groopman (Johns Hopkins University, Baltimore)

09:30 **Molecular Virology and Immunology of Hepatitis B Virus**
Dr. Jinlin Hou (Southern Medical University, Guangzhou)

10:00 **Break**

10:15 **Hepatitis-related Liver Diseases**
Dr. Masashi Mizokami (National Center for Global Health and Medicine, Tokyo)

10:45 **Viral Hepatitis and Hepatocellular Carcinoma in Mongolia**
Dr. Chinburen Jigjidsuren (National Cancer Center of Mongolia, Ulaanbaatar)

Session 2 **Molecular Biology of Liver Cancer**
Co-Chairs: Dr. Haruhiko Sugimura (Japan) and Dr. Marie Ricciardone (USA)

11:15 **Common Molecular Subtypes Among Asian Hepatocellular Carcinoma and Cholangiocarcinoma Among**
Dr. Xin W. Wang (U.S. National Cancer Institute, Bethesda)

11:45 **Characterization of the Hepatocellular Carcinoma Genomes**
Dr. Tatsuhiro Shibata (National Cancer Center of Japan, Tokyo)

12:15 **Lunch**

13:45 **Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma (The Cancer Genome Atlas Research Network)**
Dr. Jean Claude Zenklusen (U.S. National Cancer Institute, Bethesda)

14:15 **Molecular Subtypes of Hepatocellular Carcinoma: Prognosis and Stem/Maturational Status**
Dr. Taro Yamashita (Kanazawa University Hospital, Ishikawa)

14:45 **Break**

Session 3 Non-Surgical Treatment of Liver Cancer
Co-Chairs: Dr. Shuichi Kaneko (Japan) and Dr. Ann Chao (USA)

15:00 **High Affinity Antibodies Targeting GPC3 in Hepatocellular Carcinoma**
Dr. Mitchell Ho (U.S. National Cancer Institute, Bethesda)

15:30 **Current Status and Future of Targeted Therapy for Hepatocellular Carcinoma**
Dr. Ghassan K. Abou-Alfa (Memorial Sloan Kettering Cancer Center, New York)

16:00 **Big Data Intelligence Platform for Clinical Management of Liver Cancer**
Dr. YinYing Lu (Liver Cancer Center, 302 Hospital, Beijing)

16:30 **Adjournment**

Thursday, 11 January 2018

Session 4 Early Detection of Liver Cancer
Co-Chairs: Dr. Yukari Totsuka (Japan) and Dr. John D. Groopman (USA)

08:30 **Biomarkers for Hepatocellular Carcinoma among Hepatitis C Virus Patients**
Dr. Mei-Hsuan Lee (National Yang-Ming University, Taipei)

09:00 **Early Detection and Surveillance Strategies in Different Risk Groups**
Dr. Mindie Nguyen (Stanford University Medical Center, Palo Alto)

09:30 **Risk Score-Stratified Hepatocellular Carcinoma Screening in Patients with Cirrhosis**
Dr. Yujin Hoshida (Icahn School of Medicine at Mount Sinai, New York)

10:00 **Break**

Session 5 Summary and Discussion of Next Steps
10:30 Co-Chairs: Dr. Hitoshi Nakagama (Japan) and Dr. Ted Trimble (USA)

- Unanswered Research Questions
- Recommendations for Research Priorities
- Study Populations, Established Cohorts and Networks
- Opportunities to Work Together
- Next Steps

12:00 **Adjournment**

Abstracts

(*in order of presentation*)

Hepatocellular Carcinoma Burden, Epidemiology, and Known Etiology

John D. Groopman, Johns Hopkins University, USA

Collectively liver cancer, including hepatocellular carcinoma (HCC) and cholangiocarcinoma, account for 9.1% of all reported cancer deaths and is the second most common cause of cancer mortality worldwide (1). The incidence of liver cancer varies enormously globally and unfortunately the burden of this nearly always fatal disease is much greater in the less economically developed countries of Asia and sub-Saharan Africa (2). HCC is also the most rapidly rising solid tumor in the US and Central America and is overrepresented in minority communities, including African-Americans, Hispanic/Latino-Americans and Asian-Americans (1,3,4). Overall, there are more than 750,000 new cases each year worldwide and more than 300,000 deaths annually in the People's Republic of China (P.R.C.) alone (2). In contrast with most common cancers in the economically developed world where over 90% of cases are diagnosed after the age of 45, in high-risk regions for liver cancer onset begins to occur in both men and women by 20 years of age and peaks between 40-49 years of age in men and between 50-59 years of age in women (5-7). Gender differences in liver cancer incidence have also been well described and worldwide the number of cases among men were 554,000 and 228,000 among women in 2012 (8). These epidemiologic findings are also reflected in several experimental animal models where male rats have been found to have an earlier onset and higher incidence of cancer compared to female animals (9).

The significant etiological factors associated with development of HCC in the economically developing world are infection in early life with hepatitis B virus (HBV) and lifetime exposure to high levels of aflatoxin B_1 (AFB_1) in the diet (10,11). Indeed, the multiplicative interaction between HBV and AFB_1 has been documented in two separate cohorts at high risk for HCC (12-14). Over the past 25 years, an appreciation for the role of the hepatitis C virus (HCV) has also emerged. HCV is contributing to HCC being the most rapidly rising solid tumor in the US and Japan (15).

Alcohol is a recognized human carcinogen and has been causally linked to HCC. Alcoholic cirrhosis and heavy alcohol use have been repeatedly associated with an increase in HCC risk (16). However, it is unclear if alcohol use in the absence of cirrhosis influences HCC development (17). In addition to the association of alcohol and HCC, in economically developed countries the dramatic rise in overweight and nonalcoholic fatty liver disease has also been related to increased HCC (18-20). Of major concern for the future are the role that obesity, diabetes and general underlying fatty liver disease will play in the development of liver cancer (21-23). While the historic risk factors for liver cancer described above are addressed through a spectrum of prevention methods, these new etiologic factors portend an increasing trajectory in the incidence of this disease. Both therapeutic and pre-disease interventions will need to be deployed now to blunt the impact of these risk factors in the decades to come.

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Molecular Virology and Immunology of Hepatitis B Virus

Jinlin Hou, Southern Medical University, China

Hepatitis-related Liver Diseases

Masashi Mizokami, National Center for Global Health and Medicine, Japan

Viral Hepatitis and Hepatocellular Carcinoma in Mongolia

Chinburen Jigjidsuren and Unenbat Gurbadam
National Cancer Center, Mongolia

Cancer is a disease that affects large numbers of population all over the world. Diagnosis of cancer induces fear both in the individual and families. When the patient receives a cancer diagnosis, it is frequently viewed as a death sentence in most developing countries.

Mongolia is the 5th largest country in Asia, with a land area of 1,567 million square kilometers and a population approximating 2.6 million, 39% of whom reside in sparsely populated areas. Cardiovascular disease, cancer and injuries have been leading causes of population mortality since 1995 and numbers of deaths due to these diseases have increased every year. The health care system in Mongolia provides three levels of services.

Primary health care is provided by family doctors through 334 health units distributed throughout Mongolia. Secondary health care is delivered through general hospitals, including 7 in the capital city, 18 in the provinces, and 3 regional health care centers. The cancer center offers a range of medical services, training and research support.

Liver cancer is the leading primary site in both genders. HCC is the leading cancer in Mongolia, comprising almost 40% of new cancer cases. In addition, Mongolia also has high prevalence of hepatitis B virus (HBV) (10%) and hepatitis C virus (HCV) (15.6%) infection, as well as co-infection with the hepatitis delta virus (HDV) (8%). Reports have shown that among liver cancer patients in Mongolia, 46% have hepatitis C, 34% have hepatitis B, and 14% have co-infection with more than one hepatitis virus. New cases of HCC diagnosed were 2176. All patients suffering with Liver cancer were 2792. The number of patients died in 2015 were 1578. The percentage of HCC pts were in late stage at the time of the diagnosis 81.2%. 5-year survival for all HCC patients: 19.5%.

At the end of 2015, new direct-acting antiviral (DAA) medications for hepatitis C were introduced in Mongolia. Most recently, the Mongolian Government endorsed *The Healthy Liver Programme 2017-2020* in April 2017 under the Government Resolution. The Programme was also included in the Government Action Plan for 2016-2020 and launched in May 2017. The *Healthy Liver Programme* aims to reduce mortality rate caused by liver cancer and cirrhosis by early detection and treatment of viral hepatitis infection and to eliminate the spread of viral hepatitis infection, especially HCV infection among the Mongolian population by 2020.

The screening, prevention, diagnosis and treatment also a health education on oncology pose great challenges in resource-limited country like Mongolia. Major improvements in the diagnosis and treatment of cancer are being witnessed since the 1961 the establishment of Cancer Center of Mongolia. However, innovating new technologies in cancer diagnosis, surgical and radiation treatment will place substantial and diverse pressure on underfunded health delivery system, and therefore requires careful planning, funding and continuous help from foreign health systems by technology and education.

Common Molecular Subtypes Among Asian Hepatocellular Carcinoma and Cholangiocarcinoma

Xin Wei Wang, National Cancer Institute, USA

Primary liver cancers have a complex mutational landscape with vast inter-tumor heterogeneity, which poses a major challenge to define actionable drivers. They consist of two main histologically-distinct subtypes, i.e., hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) confined within the liver, whose diagnoses and treatment decisions are uniquely based on their baseline clinical features. However, both HCC and ICC are genetically, etiologically and biologically heterogeneous, which makes them highly resistant to treatment, ranking them as the second most lethal malignancies worldwide. Since a well-annotated biobank is key to improving our understanding of disease susceptibility and progression as well as patient outcomes, we established the Thailand Initiative in Genomics and Expression Research for Liver Cancer (TIGER-LC) consortium to create a comprehensive biorepository with biospecimens linked to etiologies and clinical features from 3,000 patients with liver cancer, and 3,000 high risk and healthy individuals who reside in Thailand. Here, by characterizing the first sequential 199 enrolled Thai patients, we demonstrate the presence of common molecular subtypes linked to similar prognosis among Thai HCC and ICC patients through systems integration of genomics, transcriptomics, and metabolomics. While HCC and ICC share recurrently mutated genes, including TP53, ARID1A, and ARID2, mitotic checkpoint anomalies distinguish the C1 subtype with key drivers PLK1 and ECT2, whereas the C2 subtype is linked to obesity, T-cell infiltration and bile acid metabolism. These molecular subtypes are found in 582 Asian, but less so in 265 Caucasian patients. Thus, Asian HCC and ICC, while clinically treated as separate entities, share common molecular subtypes with similar actionable drivers. Our results indicate that HCC and ICC, while clinically treated as separate entities, share common molecular determinants, suggesting that a unified molecular landscape of liver cancer is required to improve diagnosis and precision therapy.

Characterization of the Hepatocellular Carcinoma Genomes

Tatsuhiro Shibata, National Cancer Center, Japan

Hepatocellular carcinoma (HCC) has been increasing globally, but its prognosis is still unsatisfactory. Hepatitis virus infections with hepatitis virus B and C are major risk factors for HCC, and non-viral epidemiological factors including alcohol intake, obesity and diabetes have also play important roles on its carcinogenesis. Previously we conducted and reported more than 400 whole exome sequencing data and 300 whole genome sequencing data of Japanese HCC cases. These studies uncovered a list of HCC driver genes including potential therapeutic targets. Consistent with their heterogeneous epidemiological backgrounds, mutational signatures were also found to be complex. Further HCC genome sequencing data have been reported by TCGA, ICGC and other research groups. Therefore, we have compiled these public data with our data and finally collected 1,340 multi-ethnic HCC genomes. This HCC cohort, the largest one ever reported, uncovered a comprehensive landscape of HCC driver genes, which constitutes three core drivers (TP53, TERT and WNT signaling) and combination of infrequent alterations in various cancer pathways. Translating these comprehensive molecular-genetic data together with further basic research and international collaborations are highly expected for developing better treatments, precise diagnosis and effective prevention of HCC.

Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma (The Cancer Genome Atlas Research Network)

Jean Claude Zenklusen, National Cancer Institute, USA

Liver cancer has the second highest worldwide cancer mortality rate and has limited therapeutic options. We analyzed 363 hepatocellular carcinoma (HCC) cases by whole-exome sequencing and DNA copy number analyses, and 196 HCC cases by DNA methylation, RNA, miRNA, and proteomic expression also. DNA sequencing and mutation analysis identified significantly mutated genes, including LZTR1, EEF1A1, SF3B1, and SMARCA4. Significant alterations by mutation or downregulation by hypermethylation in genes likely to result in HCC metabolic reprogramming (ALB, APOB, and CPS1) were observed. Integrative molecular HCC subtyping incorporating unsupervised clustering of five data platforms identified three subtypes, one of which was associated with poorer prognosis in three HCC cohorts. Integrated analyses enabled development of a p53 target gene expression signature correlating with poor survival. Potential therapeutic targets for which inhibitors exist include WNT signaling, MDM4, MET, VEGFA, MCL1, IDH1, TERT, and immune checkpoint proteins CTLA-4, PD-1, and PD-L1.

Molecular subtypes of hepatocellular carcinoma: prognosis and stem/maturational status

Taro Yamashita, Kanazawa University Hospital, Japan

Introduction: Carcinogenesis could be characterized as deregulated malignant organogenesis mediated by abnormally proliferating and/or metastatic cancer cells and activated stromal cells that trigger angiogenesis, fibrosis, and inflammation at site. Liver cancer development may recapitulate fetal liver development in part in terms of emergence of cells expressing certain stem cell markers and the activation of signaling pathways during the liver development.

Methods: Gene expression profiles, Gd-EOB-DTPA enhanced MRI findings, whole exome data, and clinicopathological characteristics of hepatocellular carcinoma (HCC) in human and platelet derived growth factor C transgenic mice (PDGF-C Tg mice) were analyzed.

Results: Human HCC could be sub-classified at least into two groups, mature hepatocyte (MH)-like HCC and hepatic stem-cell (HpSC)-like HCC. HpSC-HCC was characterized by the activation of stem cell markers, Wnt and IGF signaling pathways, high frequency of vascular invasion, and poor prognosis after surgery. In contrast, MH-HCC was characterized by the expression of xenobiotic metabolism-related genes, uptake capacity of Gd-EOB-DTPA reagent at hepatobiliary phase of MRI, normal serum AFP levels, and good prognosis. MRI findings of HCC at hepatobiliary phase closely correlated with the HNF4α transcription program. Activation of HNF4α by polyprenoic acid treatment resulted in the recovery of Gd-EOB-DTPA uptake capacity and activation of hepatocyte function-related genes with less gene mutations in liver tumor developed in PDGF-C Tg mice.

Conclusions: Two different HCC subtypes closely related to the stem/maturational status of hepatocytes were identified with distinct gene expression patterns, activation of certain signaling pathways, and prognosis. HNF4α, one of the most ancient nuclear receptors and a master regulator of hepatocyte function, may induce the differentiation program of HpSC to MH, determine the stem/maturational status of HCC, and therefore could be a good drug target for the prevention and treatment of HCC.

High Affinity Antibodies Targeting GPC3 in Hepatocellular Carcinoma

Mitchell Ho, Wei Gao, Yen Phung, Mingqian Feng, Yi-Fan Zhang, Heungnam Kim, Nan Li, Dan Li, Hongjun Bai, Dimiter Dimitrov, Byungkook Lee, Jeffrey Rubin, Xin Wei Wang

National Cancer Institute, USA

Introduction: Glypicans are important modulators of signal transduction pathways including Wnt signaling in development and disease. However, the biochemical interaction between glypicans and Wnt molecules is not well characterized. Glypican-3 (GPC3) represents an attractive target for liver cancer therapy because it is highly expressed in hepatocellular carcinoma (HCC). We generated three human and humanized antibodies (HN3, HS20 and hYP7) that bind GPC3 with three distinct epitopes and used them as therapeutic antibodies for liver cancer treatment as well as research tools to study the interaction of GPC3 and Wnt molecules.

Methods: We established a combination method of synthetic GPC3 peptide immunization and high-throughput flow cytometry screening to isolate hybridomas for tumor cell binding. We humanized the anti-GPC3 mouse Fv antibodies by grafting combined KABAT/IMGT complementarity determining regions (CDR) into a human IgG germline framework. In addition, we used phage display technology to screen human antibodies specific for GPC3. We analyzed antibody activities in cell proliferation and luciferase-based Wnt reporter assay. We also analyzed their binding epitopes by modeling the GPC3 protein structure with mutagenesis and by mapping the heparan sulfate motifs with synthetic oligosaccharides.

Results: The antibody (YP7) recognizes the C terminal end of GPC3 close to the cell membrane. It binds cell surface-associated GPC3 with high affinity and specificity. The humanized anti-GPC3 antibody (hYP7) retains the binding properties of the original YP7 antibody. The HN3 human single domain antibody binds a cryptic Wnt binding site in the protein core of GPC3. The HS20 human monoclonal antibody preferentially recognizes the heparan sulfate (HS) chains of GPC3, disrupts the interaction of Wnt3a and GPC3 and blocks Wnt3a/β-catenin signaling. To determine the Wnt binding domain in HS chains of GPC3, we used a panel of synthetic HS oligosaccharides with distinct lengths and sulfation modifications. The oligosaccharides with the greatest competitive effect for HS20 binding are between six and eight saccharide residues in length. HS20 and Wnt recognize a HS structure containing IdoA2S and GlcNS6S, and that the 3-O-sulfation in GlcNS6S3S significantly enhances the binding of both HS20 and Wnt. We have also modeled the GPC3/Wnt complex and identified a potential Wnt site at the protein core of GPC3. Mutation of this site abolishes both Wnt and HN3 binding to GPC3 and reduces the effect of Wnt stimulation.

Conclusions: Our data reveals two Wnt binding domains on GPC3, the HN3 site on the core protein and the HS20 site on the HS chains. Our work supports the role of the glypican as a co-receptor for Wnt and suggests that the GPC3-targeting antibodies (hYP7, HN3 and HS20) are promising therapeutic antibodies.

Current Status and Future of Targeted Therapy for Hepatocellular Carcinoma

Ghassan Abou-Alfa, Memorial Sloan Kettering Cancer Center, USA

Hepatocellular carcinoma (HCC) has been perceived as a malignancy that is resistant to systemic chemotherapy for long period of time. Despite initial efforts that were supported by the NCI to help elucidate any potential role for systemic doxorubicin, no standard of care was approved and acknowledged for HCC until the advent of sorafenib. Sorafenib, a multi-kinase inhibitor with anti-angiogenic effect, was evaluated in a large phase III clinical trial versus placebo and showed a clear improvement of survival in its favor which helped establish it as a standard of care. This was back in 2007, and since then we lingered in a long hiatus of several genuine attempts of testing potentially more powerful anti-angiogenics to no avail. In 2017, things have changed markedly with 4 clinical trials showing improvements in survival or non-inferiority outcome. In the first line setting, lenvatinib, another multi-kinase inhibitor with anti-angiogenic and anti-FGF activity showed non-inferiority versus sorafenib. In the second line setting, regorafenib another mutlikinase inhibitor with ample similarity to soarafenib and some differences showed improvement in survival after progression on sorafenib when tested against placebo. Cabozabntinib, another multikinase inhibitor with anti c-met activity also showed an improvement in survival versus placebo. The advent of checkpoint inhibitors has already led to the approval of anti-PD-1 nivolumab in the second line setting based on a response rate of 15-20% despite the lack of survival data. This incredible increase in the number of targeted therapies and checkpoint inhibitors from 1 to 5 cannot go un-noticed, add to the already reported positive outcome data that led to the approval of FOLFOX as a first line therapy in China. The most crucial challenges are now the decision making of choosing a therapy based on this ample amount of positive outcome data. This is expected to be driven by the basis of biology that would help pick one therapy versus the other. Other important variables are ethnicity and etiology. Strategic planning suggestions of all those variables will be discussed.

Big-Data Intelligence Platform for Clinical Management of Liver Cancer From Surveillance, Treatment to Prognostic Prediction

Shanshan Lu, Ning Zhang, Ting Zhang, Cuihong Zhang, Yinying Lu
Comprehensive Liver Cancer Center, The 302 Hospital of PLA, China

Introduction: Hepatitis B virus (HBV) infection was the leading cause of Primary Liver Cancer (PLC) in China, while the morbidity and mortality of PLC triggered by alcoholic and nonalcoholic fatty liver diseases (NAFLD/NASH) are also increasing. To effectively monitor the high-risk population, improve the early diagnosis, carry out individualized systematic treatment and establish validated prognosis prediction model for Chinese PLC patients, 3 of multicenter specialized disease big data platforms conducted by the Comprehensive Liver Cancer Center of the 302 Hospital have been built since 2012, which cover chronic hepatitis and PLC patient population and help to improve PLC surveillance, diagnosis, treatment management. They are: the Real Word Research of Long-term Antivirus Therapy for Chronic Hepatitis B (CHB) (RWRABT2013), the Chinese Liver Cancer Clinical Survey (CLCS) and the Cholangiocarcinoma (CCA) Integrated Database (CCAID).

Method: On elastic net and univariate analysis of the primary cohort, independent factors associated with HCC or ICC for survival were selected using the Cox proportional hazards model. Receiver operating characteristic curves were employed to testify the capabilities of 1-year survival prediction among different models. The median survival rate of high, median and low risk groups was calculated and compared using Kaplan-Meier plot and log-rank ($p<0.001$).

Results: 1) RWRABT2013 plans to enroll 100,000 patients from over 200 hospitals with 10 years follow up, and has completed around 10,000 patients with CHB 3 year's consecutive data collection. A risk prediction model to identify high-risk individuals of developing hepatocellular carcinoma (HCC) among CHB patients based on Liver Stiffness Measurement (LSM) was generated. The model takes into account of LSM, age, gender, liver biochemical indexes, HBV DNA quantification, etc. which shows strong predictable efficiency (AUC=0.79). 2). Up to 2017, the CLCS project has enrolled total 5,000 PLC patients (which plans enroll total 20000 PLC patients by the end of 2020). We find out that HBV infection and/or alcohol consumption is the main etiological factor for PLC and patients were more likely at advanced stages when first diagnosed in China. Tumor staging and pathological grade are the two main factors correlated with the outcome; surgical or local tumor ablation therapies combined with comprehensive treatments are effective to improve prognosis and contribute to the improvement of the outcome of Chinese HCC patients in recent years. 3) Based on the CCAID containing the whole course of diseases data for 979 CCA patients, an accurate prediction model including 8 variables employed to test capabilities of 1-year survival for CCA patients was generated successfully with Elastic net's AUC reaching 0.845. In the validation cohort, median 1-year survival rates in high-risk, intermediate-risk and low-risk group were 17.8 %, 42.2% and 85.6% respectively.

Conclusion: The establishing of large scale and multifunctional database on CHB, HCC/CCA is essential to generate Chinese PLC risk prediction and clinical diagnosis as well as treatment guidance models. With the enlargement and development of databases, the big data integrated management platform will make greater contribution to improve the overall prognosis of PLC in China.

Biomarkers for Hepatocellular Carcinoma among Hepatitis C Virus Infected Patients

Mei-Hsuan Lee, National Yang-Ming University, Taiwan

Hepatitis C virus (HCV) is a RNA virus that chronically infects more than 185 million people. Chronic hepatitis C patients are asymptomatic and they do not aware their illness until severe liver diseases happen. Liver cirrhosis eventually occurs in 20 to 30% patients with chronic infection after 2 to 3 decades generally. Once cirrhosis occurs, hepatocellular carcinoma develops in 1 to 4% of the patients per year. The presentation included community-based and hospital-based cohorts to show biomarkers associated with hepatocellular carcinoma among HCV infected subjects with or without antiviral treatment. A community-based prospective cohort enrolled anti-HCV seropositives was followed. Age, serum levels of HCV RNA, HCV genotype, serum levels of alanine aminotransferase, and the presence of liver cirrhosis were significant determinants for the risk of hepatocellular carcinoma. The risk prediction model has ever been developed and been validated externally in another cohort with satisfactory accuracy. In addition to viral factors, host genetic variants were found to be associated with the risk of hepatocellular carcinoma by genome-wide approach. Another cohort consisted of clinical patients with interferon-based antivirals were recruited and followed. The treatment efficacy and the post-treatment seromarkers associated with risk of hepatocellular carcinoma among patients with a sustained virological response or non-sustained virological response were evaluated. The treatment of chronic hepatitis C patients before they developed cirrhosis showed a higher efficacy than did the treatment of those who had already developed cirrhosis. Among patients with sustained virological response, advanced age, male gender, cirrhosis, decreased platelet count, and increased aspartate aminotransferase and α-fetoprotein levels were associated with hepatocellular carcinoma. Additional biomarkers will be useful to monitor patients treated by antivirals successfully in the newly advanced antivirals with high response rates.

Hepatocellular Carcinoma – Early Detection and Surveillance Strategies

Dr. Mindie H. Nguyen, Stanford University Medical Center, USA

Hepatocellular carcinoma (HCC) is the seventh most common malignancy worldwide. HCC meets all the criteria established by the World Health Organization for performing surveillance on high-risk patients. The American Association for the Study of Liver Diseases (AASLD) currently recommends liver ultrasound every 6 months for HCC surveillance for patients with cirrhosis of any cause and for noncirrhotic but higher risk hepatitis B patients: Asian male aged > 40 years, Asian female aged > 50 years, Africans, and those with family history of HCC. However, although there are consensus guidelines in the U.S., Europe and Asia for HCC surveillance, adherence is poor, and the majority of patients with HCC are not diagnosed via screening or surveillance. Various patient, provider, and health care system factors may have all contributed to such nonadherence. Strategies to improve HCC screening and surveillance are urgently needed for early HCC detection and improved survival of HCC patients. Further research is needed to elucidate the various medical and/or cultural knowledge, belief, and practice patterns that can lead to barriers to HCC screening and surveillance at both patient and provider levels. Improved screening/surveillance tests are also needed. Effective tests need to be sensitive, accurate, affordable, and accessible. Patient selection strategies need to include emerging risk groups such as non-alcoholic fatty liver disease (NAFLD) patients without known cirrhosis. Strategies are also needed for patients on long-term viral suppressive therapy for chronic hepatitis B and patients following hepatitis C virus eradication.

Risk Score-Stratified Hepatocellular Carcinoma Screening in Patients with Cirrhosis

Yujin Hoshida, Icahn School of Medicine at Mount Sinai, USA

Introduction: Hepatocellular carcinoma (HCC) surveillance with biannual ultrasound with or without alpha-fetoprotein is currently recommended for all patients with cirrhosis. However, clinical implementation of this "one-size-fits-all" approach is challenging as evidenced by its low application rate. We aimed to evaluate the cost-effectiveness of risk-stratified HCC surveillance strategies in patients with cirrhosis.

Methods: A Markov decision-analytic modeling was performed to simulate a cohort of 50-year-old subjects with compensated cirrhosis. Risk-stratified HCC surveillance strategies was implemented, in which patients were stratified into high-, intermediate-, or low-risk groups by HCC risk biomarker-based scores and assigned to surveillance modalities tailored to HCC risk (2 non-risk-stratified and 14 risk-stratified strategies) and compared with non-stratified biannual ultrasound.

Results: Quality-adjusted life expectancy gains for biannual ultrasound in all patients and risk-stratified strategies compared with no surveillance were 1.3 and 0.9-2.1 years, respectively. Compared with the current standard of biannual ultrasound in all cirrhosis patients, risk-stratified strategies applying magnetic resonance imaging (MRI) and/or ultrasound only in high- and intermediate-risk patients, without screening in low-risk patients, were cost-effective. Abbreviated MRI (AMRI) for high- and intermediate-risk patients had the lowest incremental cost-effectiveness ratio (ICER) of $2,100 per quality-adjusted life year gained. AMRI in intermediate- and high-risk patients had ICERs <$3,000 across a wide range of HCC incidences.

Conclusions: Risk-stratified HCC surveillance strategies targeting high- and intermediate-risk patients with cirrhosis are cost-effective and outperform the currently recommended non-stratified biannual ultrasound in all patients with cirrhosis.

36th US-Japan Hepatitis Panel Meeting: New Approaches to HBV Therapy

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Shenzhen, China
January 10-11, 2018

36th US-Japan Hepatitis Panel Meeting
New Approaches to HBV Therapy
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Day 1. January 10. 8:30 AM.

8.30 - 8.40	**Welcome and Meeting Objectives.** Rajen Koshy Chris Walker Takaji Wakita
8:40-9:15	Setting the stage for new approaches to HBV treatment: How to define a cure for chronic hepatitis B in an era of emerging new therapies? **Raymond Chung, USA.**
Session 1:	**Chronic Hepatitis B in Asia: Scope of the problem and exploring new treatments.**
Moderators:	**Pei-Jer Chen**, Taiwan National University, Taipei. **Rajen Koshy**, NIAID, NIH, Bethesda.
9:15-9.35	The Hepatitis Prevention, Control, and Elimination Program in Mongolia: A Global Model for Hepatitis Elimination **Naranbaatar Dashdorj***,* Mongolia.
9:35-9:55	HBV-related liver diseases and development of new approaches to HBV therapy in China **Fusheng Wang,** China.
9:55-10:05	**Discussion**
10:05-10:30	**Break**
10:30-10:50	HBV prevalence in Vietnam. **Junko Tanaka, Japan.**
10:50-11:10	Chronic hepatitis B in Bangladesh: Challenges and successes in a trial of a therapeutic HBV vaccine. **Sheikh Akbar**, **Japan.**
11:10-11:20	**Discussion.**
Session 2:	**Markers of a functional cure for chronic hepatitis B.** *Objective:* To identify host and virological markers of a functional cure after treatment of chronic hepatitis B.
Moderators:	**Ray Chung**, Massachusetts General Hospital and Harvard University. **Fu-Sheng Wang**, China.
11:20-11:40	Immunity to HBV: Defining immunological markers of a cure. **Georg Lauer**, USA.
11:40-12:00	Linkage of immune response to functional cure of HBV infection. A comparative analysis with chimpanzees. **Tatsuya Kanto**, Japan

12:00-12:20	HBV pgRNA as a virological marker of HBV cure or rebound **Fengming Lu,** China.
12:20-12:30	**Discussion.**
12:30-2:00	**Lunch**
Session 2:	**Markers of a functional cure for chronic hepatitis B (continued).**
2:00-2:20	HBcrAg as a surrogate marker for cccDNA. **Akihiro Matsunoto**, Japan.
2:20-2:40	Host genome mutations and HBV integration in HCC patients with HBV infection **Y. Asahina,** Japan
2:40-3:00	NCGM:M2BP4. Markers of fibrosis in chronic hepatitis B? **Masashi Mizokami**, National Center for Global Medicine)
3:00-3:20	**Discussion**
3:20-3:40	**Break**
Session 3:	**Special Lectures.**
Moderators:	**Chris Walker** **Georg Lauer**
Topic 1:	**A balancing act: hepatic innate immune defense and viral evasion.**
3:40-3:55	**Speaker 1 (Aly lab)**
3:55-4:10	**Speaker 2 (Saito lab)**
Topic 2:	**Recovery of immune function after HCV eradication from patients.**
4:10-4:25	**Speaker 1 (Kanda lab)**
4:25-4:40	**Speaker 2 (Ray lab)**
4:40-5:00	**Discussion and adjourn.**
TBD	**Hepatitis Panel and guest dinner in the evening.**

<u>Day 2. January 11. 8:30 AM.</u>

Session 4:	**Preclinical animal models for testing HBV curative therapies.** Objective: to identify animal models to assess HBV therapies with host and viral targets.
Moderators:	**Lishan Su**, USA. **Kazuaki Chayama**, Japan.
8:30-8:50	Chimeric Liver and Acute Liver Failure mouse models **Kazuaki Chayama**, Japan

8:50-9:10	NTCP mediated HBV entry **Wenhui Li,** China.
9:10-9:30	Modeling HBV infection, immunity and therapy in mice. **Lishan Su**, USA.
9:30-9:50	A macaque model of HBV infection. **Benjamin Burwitz,** USA.
9:50-10:00	**Discussion.**
10:00-10:30	**Break.**
Session 4:	**Regulation of HBV replication and new targets for HBV therapy.** Objective: Review of basic research on HBV replication, integration and new targets for therapeutic intervention.
Moderators:	**Suichi Kaneko** **Haitao Guo**
10:30-10:50	HBV RNA splicing and integration. **Pei-Jer Chen**, Taiwan.
10:50-11:10	Role of the HBX protein in HCC. **Shuichi Kaneko**, Japan.
11:10-11:30	HBx mRNA degradation by RNA exosome which results X protein regulation in HBV infected cells. **Hussein Aly,** Japan
11:30-11:50	HBV cccDNA formation by a host repair factor, FEN1 **M. Muramatsu,** Japan
11:50-12:10	ccc DNA synthesis **Haitao Guo,** Indiana University
12:10-12:30	HBV capsid inhibitors **Baohua Gu**, China.
12:30-12:45	**Discussion.**
12:45-1:00	**Closing remarks and adjourn**

Abstracts
(Alphabetized by Last Name)

Acute HBV and HCV surveillance in Japan

Hideki Aizaki
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Introduction: There are between 1.1to 1.2 million HBV carriers and 1.0 to 1.5 million HCV carriers in Japan. The epidemiology of acute hepatitis B and C is poorly understood.

Methods: By the Infectious Disease Control Law from April 1999, acute HBV and HCV hepatitis has been monitored as a disease that requires notification of all the diagnosed cases in Japan. In this study, we determined the trend and distribution of acute hepatitis B and C nationwide in Japan.

Results: From April 1999, a total of 4,273 and 861 cases were reported with the diagnosis of acute hepatitis B and C, respectively. The annual number of acute HBV and HCV infections cases was 174 ~ 502 and 29 ~ 136, respectively. Data shows that the age peak for acute hepatitis B is between 25 and 29 years and for acute hepatitis C it is between 30 to 34 and 55 to 59 years. We also found that many HBV/HCV-positive people are unaware of their own infection until they receive a liver function check. The main transmission route of acute hepatitis B is sexual contact while that of acute hepatitis C is uncertain. In recent years the number of men infected with hepatitis B or C through sexual contact with other men (MSM) has increased. We analyzed the molecular basis of HCV transmission amongst HIV-infected MSM (HIVMSM). In 8 cases of acute hepatitis C in HIVMSM in 2012, 2014, and 2016, a genotype analysis suggested that the route of infection was shared among HIVMSM and that HIVMSM had repeated infection opportunities.

Conclusions: The data in this article indicated that diagnostic tests for hepatitis B and C viral infection is recommended for unchecked people. It is also suggested that 60% of the total acute hepatitis B cases are sexually transmitted. The vaccination against hepatitis B is recommended for high-risk group.

Molecular and Immunogenetic Study on Hepatitis B Patients

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Hepatitis B virus (HBV) infection is a major public health problem in Iraq. This work is carried out to evaluate some virological, immunogenetic and molecular parameters of Iraqi patients with chronic hepatitis B (CHB) and healthy HBV carriers, also to determine the associations of immuno status and chronic infection.

The study includes 50 patients with CHB and 50 healthy HBV carriers who have been referred to the Hepatology and Gastroenterology Teaching Hospital / Baghdad and Blood transfusion center in Baghdad with mean age of 45.6 years for CHB patients and 35.1years for healthy HBV carriers. In addition, blood samples were collected from fifty (50) apparently healthy blood donors and served as a control group.

The humeral and cellular immunity were evaluated, and the results clarified that the mean levels of IgG and IgA had showed a significant increase and the level of IgM was slightly increased in CHB patients than carrier group. The percentage of CD8+ was significantly decreased in CHB patients in comparison with carrier group, whereas the percentage of CD4+ T-cell had been slightly decreased. When some cytokines (gamma-interferon, IL-1 alfa, GM-CSF, IL-8, IL-6 and IL-2 receptor) had been measured in the sera of the studied groups. All these cytokines revealed a highly significant elevation among both groups in comparison with healthy control group.

The HLA is a crucial genetic factor that initiates or regulates immune response by presenting foreign or self-antigens to T-lymphocyte. The HLA- class I typing was conducted for three groups by using the serological method and the results showed that highly significant frequencies of HLA-A9, A10, B17, B18 and Cw6 antigens were observed in carrier group as compared to healthy group with P value (0.001), but the antigen frequencies of HLA–A3, A11, A26, B12, B14 and Cw3 were significantly higher ($P<0.001$) in chronic patients than healthy group. Additionally, when comparing between CHB patients and carrier group among HLA-class 1 (ABC) showed that a protective effect with carrier group as regard to HLA-A9, HLA 10, B17, B18 and Cw6 locus, whereas a positive association was found with chronic group as regard to HLA class -A3, A11, A26, B12, B14 and Cw3.

The PCR was used for detecting HBV DNA in sera of chronic patients and carrier group, gave rise a clear picture especially when used the Nested PCR. The data indicated a highly significant importance ($P<0.001$) was noticed between PCR direct protocol and Nested PCR protocol.

HBx mRNA degradation by RNA exosome which results X protein regulation in HBV infected cells.

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[2]Department of Gastroenterology and Metabolism, Institute of Biomedical and Health Sciences, Hiroshima University, Japan

Hepatitis B virus (HBV)-X protein is a transcriptional regulator for many cellular and viral genes. It is also involved in the pathogenesis of some HBV-induced complications like hepatocellular carcinoma and apoptosis. We previously showed that the Ski2/RNA exosome complex degrades HBV-X mRNA. In this study, we report the regulation of this system through the control of Ski2 expression. We performed cytokine screening and identified interleukin (IL)-1 as an inducer of the Ski2 promoter. We discovered the induction of transcription factor ATF3 expression by IL-We then found that ATF3 interacted with cyclic AMP responsive element sequence in the Ski2 promoter leading to the induction of Ski2 expression. We previously reported that Ski2 expression increases HBV-X mRNA degradation; in line with these data, we also showed HBV-X mRNA degradation in response to IL-1 treatment in ATF3-dependent manner. Interestingly, HBV-X significantly induced Ski2 expression in a seemingly negative feedback mechanism. Ski2/RNA exosome system was thought to be ubiquitous, this is the first report to show its activation to suppress HBV infection. Understanding the regulation of Ski2/RNA exosome system will help preventing HBV-X mediated complications through targeting the posttranscriptional degradation of its mRNA.

A balancing act: hepatic innate immune defense and viral evasion. Analyzing the role of exosomes secreted from HBV infected cells on Kupffer cell function.

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Department of Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

Hepatitis B (HBV) and C viruses (HCV) are serious health problems since infection with both viruses may end up into chronic hepatitis and its complications like liver cirrhosis, failure, and hepatocellular carcinoma (HCC).

The liver Kupffer cells constitutes the largest group of resident tissue macrophages in the body, and they play a crucial role in the liver immune response.

Exosomes released from HBV and HCV infected cells have been previously reported to carry inter-cellular signaling molecules and/or viral genome that suppressed the function of Natural Killer (NK) cells, and dendritic (DCs). This allows the virus to spread and establish chronic infection. In this work, we aimed to analyze the effect of these inter-cellular messengers secreted from HBV and HCV infected cells on Kupffer cells function. Our laboratory in Japan has isolated the extracellular exosomes from both HCV and HBV infected cells. Exosomes from HCV infected cells were sent to Dr. Takeshi Saito to perform proteomic analysis and identify the HCV-induced changes in the protein cargo of these exosomes. Co-culture experiments in our laboratory showed a suppression of Kupffer cells function upon exposure to exosomes derived from HBV infected hepatocytes.

Micro-RNA are small highly conserved non-coding RNA molecules involved in the regulation of gene expression. We performed NGS and identified a significant change induced by HBV on the miRNA cargo of the exosomes. Further analysis is undergoing to clarify the mechanism by which exosomes from HBV infected cells can suppress the function of Kupffer cells.

Host gene mutational profile and HBV integration in HCC patients with HBV infection

Yasuhiro Asahina
Tokyo Medical and Dental University, Tokyo, Japan

Hepatitis B virus (HBV) infection is a global epidemic that currently affects 250 million people worldwide. HBV infection is a common cause of chronic hepatitis, which can progress to liver cirrhosis and hepatocellular carcinoma (HCC) in many patients. During the last two decades, nucleotide/nucleoside analogues (NUCs) have been introduced as an anti-viral therapy for chronic HBV infection, persistent HBV suppression was achieved in many of the patients. However, risk of HCC still remains during sustained suppression of HBV replication by NUCs. Therefore, identification of the risk factors and understanding the genetic characteristics associated with HCC under suppression of HBV are important. The aim of the present study is to elucidate difference of gene profile of HCC among different HBV status such as persistently active, persistently suppressive by NUCs. By deep-sequencing analyses in patients with persistently infected with HBV, recurrent mutations were frequently found in *TP53* (67.9%), *TERT* (32.1%) and *CTNNB1* (10.7%), which was substantially different from those in HBV-unrelated HCC. HBV integration breakpoints were detectable in 92.6% patients and was frequently found in particular genes including *TERT*, *KMT2B*, and *MYO7A*. Frequency of *TERT* promoter mutations were significantly lower in patients who were treated with NUCs before HCC development compared with untreated patients (14.2% versus 50.0%, $p = 0.04$), although frequency of HBV integration was not significantly different between them. Our deep-sequencing analyses demonstrated that mutational profile of cancer gene is different among conditions of HBV infection, and virus suppression by NUCs may lead to decrease rate of *TERT* promoter mutations but not HBV integration.

Title: Expression of human NTCP enables HBV infection of rhesus macaques

Authors: Jochen M. Wettengel[2], Martin Mück-Häusl[2], Katherine B. Hammond[1], Marc Ringelhan[2,3], Chunkyu Ko[2], Jason S. Reed[1], Reed Norris[4], Byung Park[5], Sven Moller-Tank[6], Knud Esser[2], Justin M. Greene[1], Helen L. Wu[1], Shaheed Abdulhaqq[1], Gabriela Webb[1], William F. Sutton[4], Alex Klug[4], Tonya Swanson[4], Alfred W. Legasse[4], Aravind Asokan[6], Nancy L. Haigwood[4], Jonah B. Sacha[1,4], Ulrike Protzer[2,7], Benjamin J. Burwitz[1]

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Introduction: Hepatitis B virus (HBV) infection is a major global health concern, and the development of novel curative therapeutics is urgently needed. This requires a better understanding of virus persistence via covalently-closed circular DNA (cccDNA), disease pathogenesis and immune control of HBV infection. However, such efforts are severely impeded by the lack of a readily available, immune-competent, pre-clinical animal model that supports HBV infection. Macaques are regularly utilized in infectious disease research and could provide a physiologically relevant HBV model, but they are not naturally susceptible to HBV infection.

Methods: We created adeno-associated virus (AAV8) and helper-dependent adenovirus (HDAd) vectors expressing the HBV entry receptor, human sodium-taurocholate co-transporting polypeptide (hNTCP), and transduced rhesus macaque hepatocytes with these vectors *in vitro* and *in vivo*. We then challenged with HBV and monitored for markers of productive infection, including HBV DNA quantitative PCR, HBV surface antigen (HBsAg) ELISA, and intracellular HBV core antigen (HBcAg) staining.

Results: Expression of hNTCP on rhesus macaque and baboon primary hepatocytes *in vitro* rendered them permissive to HBV infection, and all replicative intermediates including cccDNA were present. Electron microscopy revealed spherical subviral particles and Dane particles in the supernatant of infected cells. Furthermore, AAV8 and HDAd vector-mediated expression of hNTCP on hepatocytes *in vivo* rendered rhesus macaques permissive to HBV infection. These in vivo macaque HBV infections were characterized by longitudinal detection of HBV DNA in serum, and identification of HBV DNA, RNA, and HBcAg in hepatocytes. Clearance of HBV infection was associated with elevated liver enzymes, anti-HBV T cell responses, and anti-HBc seroconversion.

Conclusions: Taken together, these results show for the first time that the simple expedient of expressing hNTCP on hepatocytes renders macaques susceptible to HBV infection, thereby establishing a physiologically relevant model of HBV infection to understand control of viral replication and test novel curative approaches.

Abolishment of Hepatitis B Virus Spliced RNAs Impairs Viral Infectivity in the Humanized Mouse Model

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In addition to the four well-known major continuous transcripts, hepatitis B virus (HBV) expresses significant amount of spliced RNAs. Despite discovered for more than 25 years, their biological function in viral life cycle remains undefined. These spliced RNAs are dispensable for HBV replication or virion formation in transfection-based systems, both *in vitro* and *in vivo*. However, spliced RNAs from HIV exert important roles in viral infection. Therefore, we used a humanized chimeric mouse model to address whether HBV spliced RNAs are involved in viral infection, like HIV spliced RNAs.

We first constructed splicing-deficient HBV mutant (sd-HBV) by substituting the conserved AG sequence at the two dominant splicing acceptor sites to CG (A487C and A280C) without affecting any protein sequence. The absence of spliced RNAs in sd-HBV-transfected cells was confirmed by Northern blot and RT-PCR. Equivalent amount of virions, harvested from mice hydrodynamically transfected with either wild-type or sd-HBV DNA, were inoculated to FRG human hepatocyte chimera mice. In wt-HBV infected humanized mice, serum HBV DNA continued to rise up to 10^8 copies/ml at week 12 post-infection. In contrast, serum HBV DNA and HBsAg were about 100-1000 fold less in sd-HBV infected mice, indicating the infectivity of sd-HBV virions to be severely impaired. A decline of infectivity was also observed for the virions produced by the mutant carrying only mutation at one dominant splice acceptor site (A487C). Furthermore, when the most abundant and frequently detected spliced variant , SP1 (with splicing from position 2447 to 489), was expressed at the time of mutant HBV A487C virion production and in the liver of humanized chimera mice before infection, the impaired infectivity of HBVA487C mutant could be rescued. These findings supported a role of HBV spliced RNAs, at least SP1 or its encoded proteins, in the viral infection cycle and started making sense of HBV spliced RNAs.

The Hepatitis Prevention, Control, and Elimination Program in Mongolia: A Global Model for Hepatitis Elimination

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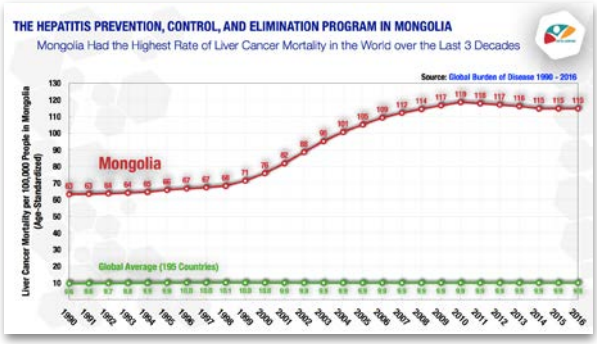
Introduction

Mongolia has the world's highest rate of liver cancer mortality—nearly 12 times the global average. Prevalences of chronic hepatitis B, C, and D in Mongolia are at an endemic level and constitute the main cause for Mongolia's world-leading liver cancer mortality rate, which, in fact, has been leading the world over the last three decades. At the moment, liver cirrhosis and hepatocellular carcinoma (HCC) mortalities account for 15% of total annual mortalities in Mongolia. In short, the hepatitis endemic is wreaking a havoc in Mongolian society.



Methods

To tackle this overwhelming burden of hepatitis in Mongolia, the Onom Foundation, the Mongolian Gastroenterology Association, and the Mongolian Society of Hepatology initiated the Hepatitis Prevention, Control, and Elimination (HPCE) Program on September 8, 2014. The HPCE Program in Mongolia is a comprehensive national hepatitis program that consists of three intrinsically inter-dependent campaigns with specific focuses on prevention, screening, and treatment. In addition, the HPCE Program includes the Comprehensive Research Component as a critical program element that facilitates usage of data-driven approaches in its implementation and enables an evidence-based decision-making in formulating right policies and regulations.

Results

Thanks to the persistent and unwavering effort of the above-mentioned organizations, the Parliament of Mongolia officially adopted the HPCE Program - ЭЛЭГ БҮТЭН МОНГОЛ ҮНДЭСНИЙ ХӨТӨЛБӨР into the 2016 - 2020 Action Plan of the Government of Mongolia (GoM) on September 9, 2016. The MISSION 2020 of the HPCE Program is to eliminate HCV in Mongolia by 2020 and to significantly reduce hepatitis-induced liver cirrhosis and HCC mortalities, and these two overarching goals were explicitly stated in the 2016 - 2020 GoM Action Plan. Following this very concrete mandate from the Parliament of Mongolia, the Cabinet of Ministers approved the detailed plan for the HPCE Program on April 12, 2017 using a blueprint that was developed by the Onom Foundation. In doing so, the GoM allocated MNT226Billion or US$96MM for the HPCE Program through 2020. It is the largest amount of funding ever allocated for a national program, demonstrating the full commitment of the GoM.

Conclusions

On May 5, 2017, the GoM organized the nationwide public launch of the HPCE Program. The Prime Minister opened the main event in Ulaanbaatar, the capital city. With this nationwide opening, the General Population Hepatitis Screening was launched to identify hepatitis infection status of every individual 15 years of age or older by the end of 2018 and to register his or her infection status into the national database. As of November 30, 2017, over 330 thousand people have been screened for HBV and HCV infections. The Public Health Insurance Fund subsidizes hepatitis B and C medicines and provides two free-of-charge HCV viral load testing under the HPCE Program. According to analysis of randomly selected HCV patients received treatment, SVR in real-life situations is around 98%. Finally, it should be emphasized that the HPCE Program in Mongolia is serving as a model for other countries in their fight against viral hepatitis.

The Role of Host DNA ligases in Hepadnavirus Covalently Closed Circular DNA Formation

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Introduction: Hepadnavirus covalently closed circular (ccc) DNA is the *bona fide* viral transcription template, which plays a pivotal role in viral infection and persistence. In terms of human hepatitis B virus (HBV), cccDNA is the basis for viral rebound after the cessation of therapy, as well as the elusiveness of a cure with current medications. Upon infection, the non-replicative cccDNA is converted from the incoming and *de novo* synthesized viral genomic relaxed circular (rc) DNA, presumably through employment of the host cell's DNA repair mechanisms in the nucleus. Therefore, the elucidation of molecular mechanism of cccDNA formation will aid HBV research at both basic and medical levels.

Methods: We screened a total of 107 cellular DNA repair genes for their potential roles in cccDNA biosynthesis through lentiviral shRNA knockdown in HBV stable cell line HepDES19. The prioritized preliminary hits were validated in nuclear extract-based in vitro cccDNA formation assay, and in DHBV/HBV stable cell lines and HepG2-NTCP HBV infection system, through chemical inhibition, shRNA knockdown, and CRISPR/Cas9 knockout approaches. cccDNA was analyzed by Southern blot and qPCR.

Results: We herein report that the cellular DNA ligase (LIG) 1 and 3 play a critical and overlapping role in cccDNA formation. Ligase inhibitors or functional knock down/out of LIG1/3 significantly reduced cccDNA production in an *in vitro* cccDNA formation assay, and in cccDNA-producing cells without direct effect on viral core DNA replication. In addition, transcomplementation of LIG1/3 in the corresponding knock-out or knock-down cells was able to restore cccDNA formation. Furthermore, LIG4, a component in non-homologous end joining DNA repair apparatus, was found to be responsible for cccDNA formation from the viral double stranded linear (dsl) DNA, but not rcDNA.

Conclusion: We demonstrate that hepadnaviruses utilize the whole spectrum of host DNA ligases for cccDNA formation, which sheds light on a coherent molecular pathway of cccDNA biosynthesis, as well as the development of novel antiviral strategies for treatment of hepatitis B.

Outcome of chronically hepatitis C virus-infected patients treated with direct antiviral agents

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Background: Direct-acting antiviral (DAA) against hepatitis C virus (HCV) has a higher sustained virological response (SVR) rate with shorter treatment duration and less adverse events. We treated chronically HCV-infected patients with DAA and characterized their treatment outcome. Patient sera and PBMCs were collected for analyses of immune parameters.

Methods and Results: HCV-infected patients were treated with a daily combination of HCV NS5B polymerase inhibitor sofosbuvir (400 mg)/NS5A inhibitor ledipasvir (90 mg) for 12 weeks with or without achievement of rapid virological response (RVR). All patients in the treatment groups were infected with HCV GT1b, achieved SVR12 and were defined as HCV RNA negativity at week 12 after the end of treatment. Blood was collected from patients upon obtaining written informed consent for preparation of sera and PBMCs by Ficoll-Paque PREMIUM density gradient separation technique.

Conclusions: Our study provided important post-treatment information concerning on RVR and non-RVR patients after treatment. Further examinations of the differences between RVR and non-RVR patients using immunological parameters are currently in progress.

Possible role of DOCK11 in HBV persistence

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Introduction: Recent HBV polymerase inhibitors efficiently inhibit the virus replication, however, HBV is persistently present during the treatment, and replication of HBV is often activated after cessation of the treatment. Understanding of the mechanism of viral persistence would be important for the development of a new drug against HBV.

Methods: We established a cell line (KM) from a transplanted HCC tumor originated in HBV infected patient liver. The level of HBV DNA and cccDNA in the cell line was gradually decreased but maintained during passage. HBV core antigen was immunohistochemically detected in fewer than 0.1% of the cells. To understand the mechanism of HBV persistence in the cells, we applied single-cell transcriptome analysis to identify HBV persistence-related genes in the cells.

Results: By annotating the expression data from single cells, we found only one cell expressing HBV mRNA in 3,000 cells of a KM cell line. When transcripts of the HBV mRNA expressing cell were compared with those of other 2,999cells, we found HBV mRNA expressing cell specific transcripts including DOCK11. It has been reported that DOCK11 is an exchange factor for the Rho GTPases Rac and Cdc42, and overexpression of DOCK11 activates Cdc42. In primary human hepatocytes infected with HBV, knockdown of DOCK11 decreased the amount of both HBV DNA and cccDNA to below the detection limit.

Conclusions: Inhibition of DOCK11 may become a new therapeutic strategy against HBV.

Synchronized macrophage-derived chemokine and IL-21 elevation indicates HBsAg loss in acute hepatitis B

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INTRODUCTION: Early kinetics of innate immune responses after the primary HBV exposure, including incubation phase, remains largely unknown. We aimed to clarify the dynamics of immune responses leading to HBV clearance, by enrolling subjects observed throughout the course of primary HBV infection including the window period.

METHODS: We performed longitudinal and/or cross-sectional analyses of 40 cytokines/chemokines and IFNs in the sera of three different groups of subjects with primary HBV infection: 1) One blood donor who repetitively donated blood to the Blood Center during 15 weeks of window period (HBsAg-/HBVDNA-negative) and subsequent 4 weeks of HBVDNA-positive, 2) Six chimpanzees inoculated with HBV, 3) Patients with 23 acute hepatitis (AH) (self-limited) and 14 chronic hepatitis (CH) with hepatic flare (persistent infection). To identify the source of chemokines, we cultured HBV-infected Huh7 cells with macrophages and plasmacytoid dendritic cells (pDCs).

RESULTS: In a blood donor, CX3CL1 and CXCL13 were elevated prior to the donor becoming HBV DNA-positive. In patients with self-limiting hepatitis, CX3CL1, CXCL9, CXCL10, CXCL11, CXCL13, and IL-21 were elevated at the acute phase of hepatitis with subsequent decline of HBV DNA, the levels of which were higher than that of chronic hepatitis. Interestingly, IL-21 levels were higher in resolving acute hepatitis patients than in non-resolvers. Similar synchronized elevation of CXCL9, CXCL10, CXCL11, CXCL13, and IL-21 in hepatitis was observed in some chimpanzees, whose peripheral follicular helper T cells were concomitantly increased. *In vitro*, macrophages produced CXCL9, CXCL10, CXCL11 and CXCL13 in response to HBV with the aid of pDC-derived IFN-alfa.

CONCLUSIONS: Elevation of serum CX3CL1, CXCL9, CXCL10, CXCL11, CXCL13 and IL-21 is an indicator of HBsAg clearance in patients with acute hepatitis. Active interplay between macrophages and pDCs underlies such chemokine responses against HBV.

HBcrAg as a surrogate marker for cccDNA

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Hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) is a key to viral persistence in chronic hepatitis B infection. HBV DNA is a surrogate marker for intrahepatic cccDNA in natural course. Although, under nucleos(t)ide analog (NUC) therapy, the levels of serum HBV DNA is rapidly decreased and lost the correlation with levels of cccDNA.

A sensitive chemiluminescence enzyme immunoassay for total antigen including complex via pre-treatment (iTACT CLEIA) specific for hepatitis B virus core antigen (HBcAg), p22cr antigen and hepatitis B e antigen (HBeAg) was developed. We designated the precore/core gene products as hepatitis B virus (HBV) core-related antigens (HBcrAg). In order to detect HBcrAg even in anti-HBc/e antibody-positive specimens.

Of the 30 patients with undetectable serum HBV DNA (<300 copies/ml) under the NUCs treatment, the levels of HBcrAg is still well correlated with the levels of cccDNA (r=0.578, p=0.001). A total of 126 chronic hepatitis B patients who discontinued NUC therapy were recruited retrospectively to assess the factors associated with hepatitis recurrence after discontinuation of NUCs. higher levels of both hepatitis B surface (HBsAg) and HBcrAg at the time of discontinuation were significantly associated with relapse by multivariate analysis. A total of 95 patients with chronic hepatitis B being treated with NUCs were enrolled to the prospective cohort study searched for factors associated with a response to NUC/peg-interferon (NUC/peg-IFN) sequential therapy. Twenty-six patients (27%) were judged to be responders at 48 weeks after the completion of peg-IFN. Analysis of baseline factors revealed that lower hepatitis B surface antigen (HBsAg) of < 3.1 log IU/ml and lower HB core-related antigen (HBcrAg) of < 3.9 log U/ml were significant indicators of a treatment response.

The HBcrAg assay is useful for monitoring the antiviral effects of NUCs. And it could be one of the good predictor for successful cessation of NUC or NUC/Peg-IFN sequential therapy.

HBV cccDNA formation by a host repair factor, FEN1

Kouichi Kitamura, Kousho Wakae, Lusheng Que, Md Mohiuddin, and Masamichi Muramatsu

Introduction: Covalently closed circular DNA (cccDNA) is an essential viral intermediate of the hepatitis B virus (HBV). cccDNA is produced from its precursor, relaxed circular (RC) DNA. RC-DNA has some unique structures that are absent in cccDNA. The P protein and a short RNA oligomer are covalently attached to the 5' ends of the minus and plus strands of RC-DNA, respectively. In addition, approximately 11 nt terminal redundancy (r) is added to the 5' end of the minus-strand DNA, which may potentially form a flap structure with minus and plus strands of RC-DNA. Theoretically, these RC-DNA-specific structures should be removed either before or during cccDNA formation. Flap endonuclease 1 (FEN1) is a host endonuclease that removes 5' overhanging DNA generated during long-patch repair process and lagging-strand DNA synthesis. Therefore, FEN1 is a potential candidate for removing the potential flap structure in RC-DNA. In this study, we investigated the requirement of FEN1 for cccDNA formation.

Methods and Results: We used in vitro cell culture models that produce nuclear cccDNA. First, we took loss-of-function approaches to assess FEN1 involvement in the formation of cccDNA. FEN1-specific inhibitor treatment, siRNA- and shRNA-mediated knockdowns, and CRISPR-based genome editing of FEN1 resulted in a reduction of cccDNA levels without decreasing RC-DNA levels. Restoring wild-type FEN1 expression rescued the reduction of cccDNA levels observed in FEN1-knock down cell lines, but this did not occur with mutant FEN1s. Chromatin immunoprecipitation (ChIP) revealed the binding of FEN1 with HBV DNA. Furthermore, immunoprecipitated wild-type FEN1 protein but not mutant FEN1s cleaved a model substrate mimicking the HBV DNA flap structure in vitro.

Conclusion: These results demonstrate that FEN1 is involved in cccDNA formation at least in culture models. The role of FEN1 in HBV infection will be discussed.

Role of Kupffer cells in innate immunity against HCV infection and the viral evasion strategy mediated by the viral particles divergence

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The innate immune cells such as dendritic cells and macrophages are believed to serve as the central component of the frontline defense against viral infection. These cell types have a great capacity to engulf, digest and sense the Pathogen Associated Molecular Pattern (PAMP) via host encoding Pattern Recognition Receptors (PRR). The PAMP-PRR interaction in the innate immune cells results in the robust induction of antiviral and inflammatory cytokines.

Kupffer cells (KCs) are tissue-resident macrophage of the liver and constitute up to 90% of total body macrophage. KCs are localized in the liver sinusoid. Therefore, it has been presumed that KCs expose to the high concentration of circulating HCV particle. However, the significance of Kupffer cells in innate antiviral immunity has been largely unexplored. Thus, this study is designed to develop our understanding of the KC's innate immune response to HCV particle.

HCV particles in circulation distribute in a wide range of buoyant density. The divergence in the virion density is belied to result from both the pleomorphism of the viral particle and the interaction with lipoproteins. It has been known that the divergence in the viral particle densitty is associated with differential infectivity to hepatocytes. However, the significance of the virion diversity has never been studied in the context of innate immunity. Consequently, we have assessed the immunogenicity of HCV particle in each density fractions using freshly isolated murine Kupffer cells. Our result revealed that KCs provoked substantial innate immune activation when co-cultured with low-density viral particles. In contrast, KCs exposed to the high-density viral particle failed to induce the expression of antiviral and inflammatory cytokines. Our follow up investigation suggested that the buoyant fraction of the high-density particle also intersects with the buoyant fraction of the exosome. Lastly, our protein mass spectrometry analysis suggested that the poor immunogenicity of high buoyant density fraction is mediated by the HCV non-structural (NS) proteins embedded in the exosomes.

In summary, our study results suggested that HCV particle produced from infected hepatocytes contains both immunogenic and non-immunogenic viral particle. In addition, our result suggested that exosome containing viral NS proteins serves as immune evasion program of HCV that escape from the activation of KCs.

Immune related cytokine and chemokine changes after DAA treatment of HCV patients

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Introduction: Hepatitis C virus (HCV) often causes chronic persistent infection, and has become an increasingly important factor in the etiology of cirrhosis and hepatocellular carcinoma (HCC). Direct-acting antivirals (DAAs) can clear HCV RNA, but face issues such as altered immune status, reinfection and carcinogenesis.

Methods: To investigate the immune status following DAA treatment, serum cytokine and chemokine analysis and complement ELISA were performed for comparison among pre-treatment, post-treatment, and spontaneously recovered HCV-infected subjects.

Results: IFN-α2 and IFN-γ were significantly downregulated in sera post-treatment as compared to pre-treatment. T-helper 1 cell response associated CCL3 (macrophage inflammatory protein-1α; MIP-1α), CCL4 (MIP-1β), IL-12P70, interferon-γ inducible protein-10 (CXCL10) and GM-CSF were significantly downregulated in sera post-treatment as compared to pre-treatment. IFN-α2, IFN-γ and IL-12P70 were higher especially in rapid virological response (RVR) patient sera as compared to non-RVR patients. These results suggest that the Th1 associated immune response remains active, at least partly, in chronic HCV-infected patients. This may be related to the high level of CXCL10. On the other hand, IL-7 and IL-15 as memory cell markers were significantly up-regulated in HCV-infected patient sera, but reduced in sustained virological response at week 12 (SVR12), suggesting a lack of memory T cell function.

Conclusions: DAAs significantly clear HCV RNA with a corresponding reduction in many immunoregulatory cytokines. RVR patients, in general, displayed an enhanced level of cytokine expression compared to that seen in non-RVR patients.

Modeling HBV infection, pathogenesis and therapy in mice

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Introduction: Over 250 million individuals currently are chronically infected with hepatitis B virus (HBV), and many patients with chronic HBV infection (CHB) develop serious liver diseases including liver fibrosis, cirrhosis, and hepatocellular carcinoma. There is no reliable cure strategy for CHB, although an effective HBV preventive vaccine has been available for decades. A major hurdle to elucidating mechanisms underlying the CHB diseases, and to developing effective therapies, is the lack of small animal models that reproduce human-like infection with HBV.

Methods: In recent years, we have developed novel HBV infection mouse models including improved humanized mice that can co-engraft human livers and human immune cells (Hu-HEP/HSC mice) and AAV8-mediated HBV persistence in immunocompetent mice (AAV8-HBV1.3 mice).

Results and Conclusions: Using Hu-HEP/HSC mice, we have discovered that 1) Hu-HEP/HSC mice support HBV persistent infection, associated with immunopathogenesis and human-specific liver fibrosis; 2) HBV infection in Hu-HEP/HSC mice leads to HBV-specific T cell tolerance in the liver; 3) HBV infection induces M2/MDSC-like pathogenic macrophages (pMAC) in the liver of Hu-HEP/HSC mice and of CHB patients; and 4) we have identified adjuvants that effectively stimulate vaccine-induced human T cell response in humanized mice. In AAV8-HBV1.3 mice with HBV persistence and immune tolerance, we have discovered that 1) AAV8-HBV1.3 in adult/newborn mice both leads to HBV persistence and immune tolerance in a dose-dependent fashion; 2) HBsAg levels determine relative immune tolerance and anti-HBs mAb that efficiently depletes HBs in vivo can break immune tolerance; 3) preS1 is present at much lower levels in HBV+ hosts, which shows low/no immune tolerance to preS1-based vaccines; and 4) vaccination with preS1-based antigens lowers tolerance to HBsAg in HBV carrier mice, thus allows effective vaccination with current HBs vaccines. Our findings will advance the field of HBV research on HBV infection and immune responses, and shed light on developing novel treatment for viral liver diseases and functional cure of CHB.

HBV prevalence in Vietnam

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Introduction: Vietnam is one of the countries with the highest mortality from hepatocellular carcinoma (HCC), which is mostly attributed to hepatitis B virus (HBV) infections. For planning preventive strategies against HBV infections, we investigated prevalence of HBV infections and infectious root among adults living in Binh Thuan, a southern coastal area of Vietnam.

Methods: Our study consisted of two sero-epidemiological surveys. "Prevalence survey" is aimed to clear the prevalence of HBV infections and their risk factors. The sample size of the subject was calculated based on anticipated rate of hepatitis B surface antigen (HBsAg). 509 adults, comprised of 230 men (45.2%) and 279 women (54.8%) were randomly sampled using a multistage method. Average age was 40.8 ± 1.1 years (ranged 20–81).

"Confirmation and family-tree surveys" were conducted to examine persistent HBV infection and intra-familial HBV transmission, respectively. 48 residents and four families among carriers that we detected in the prevalence study were participle the detailed genetic analysis. The route of infection and genomic characteristics related to HCC were studied in HBV spread.

Results: As for 509 adults for prevalence study, prevalence of HBsAg, hepatitis B surface antibody and hepatitis B core antibody were 15.3%, 60.3% and 71.7%, respectively. Most HBV DNA positive sera were classified as genotype B (75.3%) and C (11.7%). Of HBsAg positive subjects, 96.7% were persistently infected and one acutely HBV infected person was identified.

As for confirmation and family tree survey, The HBV genotype was B4 in 91.7% and C1 in 8.3% of the 48 cases. The intra-family's HBV sequence homology was high at 96.8-99.4%. However, it was also high at 99.4-99.8% among residents of the same age and sex as family members.

In addition, full genome analysis was performed in 21 cases. The HBV genotype B4 isolates were found not only to be recombinants of genotype C, which results in a high cancer risk, but also to have other risk of HCC, pre-S deletions, the G1613A mutation, and X region insertions corresponding to the promoter.

Conclusion: In Binh Thuan, prevalences of HBV infections are high, HBV genotype B is predominant, and horizontal HBV transmission may still occur. Therefore, raising the coverage of a universal HBV vaccination program may be an effective liver cancer control in Vietnam.

These genomic characters were suggested to be one of the factors to explain the high HCC mortality rate in Vietnam.

Abstracts of Papers Presented at the

50th Joint Working Conference on Viral Diseases

US-Japan Cooperative Medical Science Program

January 10-11, 2018
The Coli Hotel
Shenzhen
China

History of Viral Diseases Panel Meetings

I.	1967	Honolulu, HI, USA	December 6-8
II.	1968	Tokyo, JAPAN	December 2-4
III.	1969	Bethesda, MD, USA	August 4-6
IV.	1970	Tokyo, JAPAN	October 12-14
V.	1971	Honolulu, HI, USA	September 22-24
VI.	1972	Sapporo, JAPAN	September 20-22
VII.	1973	Bethesda, MD, USA	July 23-25
VIII.	1974	Tokyo, JAPAN	August 6-8
IX.	1975	San Antonio, TX, USA	October 14-16
X.	1976	Sendai, JAPAN	August 25-27
XI.	1977	Bethesda, MD, USA	July 11-13
XII.	1978	Osaka, JAPAN	October 3-5
XIII.	1979	Atlanta, GA, USA	October 1-3
XIV.	1980	Oiso, JAPAN	November 17-19
XV.	1981	Bethesda, MD, USA	November 9-11
XVI.	1982	Tokyo, JAPAN	July 19-21
XVII.	1983	La Jolla, CA, USA	September 8-10
XVIII.	1984	Osio, JAPAN	September 9-11
XIX.	1985	Bethesda, MD, USA	October 28-30
XX.	1986	Nagasaki, JAPAN	November 18-20
XXI.	1987	Monterey, CA, USA	December 2-4
XXII.	1988	Tokyo, JAPAN	July 18-20
XXIII.	1989	Annapolis, MD, USA	August 21-23
XXIV.	1990	Sendai, JAPAN	September 27-29
XXV.	1991	Charlottesville, VA, USA	September 23-25
XXVI.	1992	Nara, JAPAN	September 8-10
XXVII.	1993	San Diego, CA, USA	August 30 – September 1
XXVIII.	1994	Tokyo, JAPAN	August 1-3
XXIX.	1995	Santa Fe, NM, USA	August 28-30
XXX.	1996	Sapporo, JAPAN	July 30 – August 1
XXXI.	1997	Atlanta, GA, USA	July 8-10
XXXII.	1998	Kyoto, JAPAN	September 2-4
XXXIII.	1999	Chevy Chase, MD, USA	June 28 – July 1
XXXIV.	2000	Inuyama City, JAPAN	July 20-22
XXXV.	2001	Honolulu, HI, USA	August 6-9
XXXVI.	2002	Matsumoto, JAPAN	July 16-18
XXXVII.	2003	Houston, TX, USA	July 18-20
XXXVIII.	2004	Kyoto, JAPAN	December 7-10
XXXIX.	2005	Palo Alto, CA, USA	July 29-30
XL.	2006	Sendai, JAPAN	July 24-26
XLI.	2007	Baltimore, MD, USA	July 23-25
XLII.	2008	Nagasaki, JAPAN	May 26-28
XLIII.	2009	Philadelphia, PA, USA	July 20-23
XLIV.	2010	Sapporo, JAPAN	June 28-30
XLV.	2011	Palo Alto, California, USA	June 20-22

XLVI.	2012	Beppu, JAPAN	June 19-20
XLVII.	2013	Singapore	March 12-13
XLVIII.	2015	Taipei, Taiwan	January 28-29
XLIX.	2016	Bethesda, MD, USA	January 13-14

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Schedule of Events

Wednesday, 10 January 2018

7:30-8:15 Registration

8:15-8:30 **WELCOME REMARKS**

Session I **Hemorrhagic Viruses** (15 min. talks + 5 min. Q&A)

Moderators: Shigeru Morikawa & Jae Jung

8:30-8:50 Yoshimi Tsuda - The role of viral replication in macrophages for pathogenesis of Ebola virus infection

8:50-9:10 George Gao - Patriotic Health Campaign: China's experience in disease control

9:10-9:30 Roger Hewson - Lassa fever virus and other arena viruses

9:30-9:50 Mi-Fang Liang - Molecular epidemiology of Severe Fever with Thrombocytopenia Syndrome

9:50-10:10 Jae Jung – Severe Fever with Thrombocytopenia Syndrome virus non-structural protein -mediated immunopathogenesis

10:10-10:40 **Break/Poster viewing**

10:40-11:00 Shigeru Morikawa - SFTS in animals; a risk of direct infection from animals to human in Japan

11:00-11:20 Shuzo Urata - Identification and characterization of novel compounds for Severe Fever with Thrombocytopenia Syndrome (SFTS) virus

11:20-11:40 Kumiko Yoshimatsu - Relationship between hantavirus infection and Chronic Kidney Disease by unknown etiology (CKDu) in Sri Lanka

SESSION II **Enteric Viruses**

Moderators: Kazuhiko Katayama & Harry Greenberg

11:40-12:00 Akira Fujimoto - Towards development of a human norovirus culture system

12:00-12:20 Kazuhiko Katayama - Study of norovirus infection mechanism.

12:20-12:40 Mary Estes - Cultivation of human noroviruses: Success and new challenges

12:40-1:40 **LUNCH**

1:40-2:00 Harry Greenberg - STAG2 deficiency induces interferon responses via the cGAS-STING pathway and restricts virus infection

2:00-2:20 Takeshi Kobayashi - A plasmid-based reverse genetics system for rotavirus

2:20-2:40 Raul Andino - RNA virus evolution: mechanisms and consequences for infection

2:40-3:00 Hiroyuki Shimizu - Understanding of the pathogenesis of enterovirus 71 infection based on the identification of the receptors

3:00-3:30 **Break/Poster viewing**

SESSION III Arboviruses

Moderators: Akira Nishizono & Diane Griffin

3:30-3:50 Pei-Yong Shi - An evolutionary mutation that enhances Zika virus evasion of host interferon induction

3:50-4:10 Usa Thisyakorn - Dengue: Pitfalls in diagnosis and management

4:10-4:30 Scott Weaver - Rapid and durable Alphavirus immunity after a single dose of vaccines based on the insect-specific Eilat Virus

4:30-4:50 Diane Griffin - Role of nsP3 in alphavirus replication and pathogenesis

4:50-5:10 Richard Kuhn - Structural studies of flavivirus antibody complexes

5:10-5:30 Gong Cheng - Story of arboviral lifecycle: Acquisition mechanism from hosts to mosquitoes

Thursday, 11 January 2018

SESSION III Arboviruses - continued

Moderators: Koichi Morita & Raul Andino

9:00-9:20 Anna Durbin - Use of a controlled human infection model in Dengue vaccine development

9:20-9:40 Chang-Kwong Lim - Animal model of Zika fever

9:40-10:00 Albert Ko - Emergence of congenital Zika syndrome

10:00-10:20 Alan Rothman - Pre-Infection cytokine responses *in vitro* correlate with clinical outcome in Dengue

10:20-11:00 **Break/Poster Viewing**

SESSION IV Rabies Virus

Moderators: Koichi Morita & Raul Andino

11:00-11:20 Kentaro Yamada - iRFP720 is optimal for *in viv*o fluorescent imaging of rabies virus infection

11:20-11:40 Akira Nishizono - Possible antiviral therapy for rabies.

FLASH TALKS (10 min. talks + 5 min. Q&A)

Moderators: Jiro Arikawa & Richard Kuhn

11:40-11:55 Eun-Sil Park - Analysis of entry mechanisms of Bas-Congo virus using a pseudotype VSV

11:55-12:10 Jessica Manning - Safety and Immunogenicity of AGS-v, a mosquito saliva Peptide vaccine: a Randomized, double-blind, placebo-controlled phase 1 Trial

12:10-12:25 Nam-Joon Cho - A Broad-spectrum antiviral peptide for combating emerging viral pathogens

12:25-12:30 Wrap-up - Jiro Arikawa & Richard Kuhn

George Gao is planning a tour of BGI in the afternoon 1:30

Oral Presentation Abstracts

RNA virus evolution: mechanisms and consequences for infection

Raul Andino

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RNA virus population dynamics is central to the establishment of a successful infection even before adaptive immunity takes effect. Mutation and recombination are central to population diversity. While a high mutation rate fuels adaptation, it also generates deleterious mutations. RNA recombination between different genomes resolves this paradox, alleviating the effect of clonal interference and purging deleterious mutations. Rapid adaptation to diverse intrahost environments is key to virus spread within the infected individual. Diversity enables spread of virus between host tissues through constant optimization of the proportion of variants with distinct functions, which together cooperate to circumvent tissue specific challenges. We propose that the genetic composition of an RNA virus population is a virulence determinant allowing dynamic regulation of viral functions required to overcome barriers to infection.

Story of Arboviral Lifecycle：Acquisition Mechanism From Hosts to Mosquitoes

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Introduction: The arbovirus life cycle involves viral transfer between a vertebrate host and an arthropod vector, and acquisition virus from an infected mammalian host by a vector is an essential step in this process. However, little information is available regarding the common mechanisms underlying the viral acquisition by mosquitoes.

Results: We first reveal that the hematophagous nature of mosquitoes contributes to arboviral acquisition after a blood meal, which suppresses antiviral innate immunity by activating the GABAergic pathway. The ingestion of blood by mosquitoes resulted in robust GABA (gama-aminobutyric acid) production from glutamic acid derived from blood protein digestion, thereby enhancing arbovirus replication in mosquitoes through activation of the GABAergic system. In the second part, we report that flavivirus nonstructural protein-1 (NS1), which is abundantly secreted into the serum of an infected host, plays a critical role in flavivirus acquisition by mosquitoes. The presence of dengue virus (DENV) NS1 in the blood of infected interferon alpha and gamma receptor-deficient mice (AG6) facilitated virus acquisition by their native mosquito vectors because the protein enabled the virus to overcome the immune barrier of the mosquito midgut.

Conclusions: Our study demonstrates that arboviruses utilize multiple materials produced during their vertebrate phases to enhance their acquisition by vectors, therefore facilitating arbovirus survival in nature.

Use of a Controlled Human Infection Model in Dengue Vaccine Development

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Dengue virus (DENV) has become the most important arbovirus worldwide with estimates of up to 400 dengue infections occurring annually. Neutralizing antibody, the accepted immunologic endpoint chosen to move candidate DENV vaccines forward, was not predictive of protection against DENV illness in two efficacy trials of the recently licensed live attenuated dengue vaccine Dengvaxia®. The vaccine was found to have significantly lower protection in those subjects who were dengue-naïve at the time of first vaccination and it induced an excess number of hospitalized and severe cases of dengue in the third year of the study in children younger than 9. These results have have heightened the need to define correlates of protection and risk for dengue. A dengue human infection model (DHIM) would be useful in down-selecting candidate vaccines prior to testing in endemic areas as well as identifying putative correlates of protection. We have developed a DHIM for both DENV-2 and DENV-3. All 42 subjects vaccinated with either the TV003 (21) or TV005 (21) live attenuated dengue vaccine were protected against viremia and rash when challenged with DENV-2. In contrast, 100% of 41 controls were viremic (mean peak titer 2.3 $\log_{10}$ PFU/mL) for multiple days and 80% - 100% developed a rash. To evaluate the role of heterotypic immunity in protection against dengue, we administered a trivalent vaccine formulation consisting of the DENV-1, DENV-3, and DENV-4 vaccine candidate viruses and challenged subjects 6 months later with DENV-2. We are also evaluated the evolution of the immune response to sequential DENV infection by infecting subjects with the DENV-1 vaccine candidate virus and then administering the DENV-2 challenge virus 9 months later. In addition, we recently developed a challenge model for DENV-3. The clinical, virologic, and immunologic results from these studies will be discussed.

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Cultivation of Human Norovirus: Success and New Challenges

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Introduction: Human norovirus (HuNoV) is the leading cause of gastroenteritis worldwide. Although these viruses were discovered 48 years ago, the major barrier to understanding HuNoVs has been the lack of a robust and reproducible *in vitro* cultivation system.

Methods: We have established and used intestinal stem cell-derived human intestinal enteroid (HIE) cultures to cultivate human noroviruses ex vivo.

Results: HIEs are produced from stem cells obtained from crypts of human intestinal tissue, and they are self-organizing, 3-dimensional, physiologically active, multicellular cultures that recapitulate the structure of the intestinal epithelium. We have successfully cultivated several different HuNoVs (including GII.4 and GII.3 strains) by optimizing HIE culture conditions to mimic the environment of the intestinal milieu. Different virus strains require distinct cultivation conditions. These cultures do not support replication of inactivated virus, and virus replication is biologically relevant as it only occurs in differentiated cultures from individuals that express the needed histoblood group antigens. Replication of GII.4 viruses has been highly reproducible over one year and has been confirmed in other laboratories. Conditions to achieve replication of all strains continue to be optimized.

Conclusions: This novel non-transformed human intestinal culture replication system allows the assessment of methods to prevent and treat HuNoV infections as well as methods to inactivate virus with practical applications for food safety and public health management of these infections.

Towards development of a human norovirus culture system

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Introduction: Human norovirus (HuNoV) is the leading cause of gastroenteritis worldwide. Since several reports suggest the association between secretor genotype (fucosyltransferase 2; FUT2) status and NoV susceptibility in clinical research, but relevance of their findings is not well understood at the cellular level. Recently, method to cultivate HuNoV in human intestinal organoid (HIO) has been reported as an efficient in vitro viral culture system.

Methods: Secretor status of the HIO was determined by sequencing the coding exon of the FUT2 gene. FUT2 of HIO was inhibited by treatment with fucosyltransferase inhibitor (2F-Peracetyl-Fucose) or knockout of FUT2 gene by CRISPR/Cas9 system. Then, HIOs were induced to differentiate by changing culture conditions and subsequently inoculated with a stool suspension of HuNoV (GII.4 Sydney 2012 variant strain), and newly synthesized HuNoV in cell-culture supernatant was quantified by qRT-PCR.

Results: Number of HuNoV genomes was increased (at most 100–1000 times) at 6 days post-infection (dpi) in untreated HIO. On the other hand, the HIO cells treatment with fucosyltransferase inhibitor significantly reduced HuNoV. Furthermore, HuNoV replication was intensively abrogated in vitro when the FUT2 gene was knockout.

Conclusions: In agreement with a previous report, secretor status was associated with of HuNoV (GII.4) infection also in HIO cell culture system. These results increase our understanding of the factors explaining secretor status in HuNoV lifecycle in vitro and show us the necessity to develop the new highly effective drugs targeting the FUT2.

Patriotic Health Campaign: China's experience for infectious disease control

George F Gao

China CDC and Chinese Academy of Sciences

This year, China celebrated its 65th-year anniversary of the Patriotic Health Campaign. As evidenced in its successful disease control in China in the last several decades, this campaign has been playing a very important role for China, which would be a good model for the developing countries in the world. This talk will discuss the key elements of the campaign. Government strong commitment is the key for this campaign and public understanding (education) of the disease control is another key element. A good example for individual disease control is malaria. Through vector (mosquitos) control and people's active involvement, malaria epidemic declined rapidly in China in the last decades. We believe this disease control model can be applied in Africa, esp., in the eve of establishment of Africa CDC. China should play a key role in the aid of disease control in Africa, which will benefit for the whole world.

STAG2 deficiency induces interferon responses via the cGAS-STING pathway and restricts virus infection.

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Introduction and Methods: Cohesin is a multi-subunit nuclear protein complex that coordinate sister chromatid separation during cell division. A high frequency of somatic mutations in genes encoding the core cohesin subunits has been observed in multiple cancer types. Here, we used a genome-wide CRISPR-Cas9 screening approach to identify host dependency factors of rotavirus (RV) infection and replication as well as novel innate immune regulators.

Results: Here we demonstrate that the loss of *STAG2*, an important component of the cohesin complex, confers significant resistance to RV replication in cell culture and human intestinal enteroids. Mechanistically, *STAG2* deficiency leads to spontaneous genomic DNA damage and robust interferon (IFN) expression via the cGAS-STING cytosolic DNA sensing pathway. The resultant activation of JAK-STAT signaling and IFN-stimulated gene (ISG) expression broadly protects against sevderal viral infections including RVs.

Conclusions: These findings highlight a previously undocumented role for the cohesin complex in regulating IFN homeostasis. In addition, we identify an underappreciated interaction between RNA viruses and the DNA damage pathway and a potential new therapeutic avenue for manipulating the innate immunity.

Role of nsP3 in alphavirus replication and pathogenesis

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Alphaviruses encode four nonstructural proteins that are translated as a polyprotein upon delivery of genomic RNA to produce the replicase complex, induce spherule formation and produce genomic and subgenomic RNAs. The function of nsP3 has been enigmatic. It has 3 domains: a highly conserved N-terminal macrodomain, a middle linker domain and an unstructured, variable, Ser/Thr phosphorylated C-terminal domain. The C-terminal hypervariable domain interacts with many host proteins throughout the replication cycle. Macrodomains are highly conserved protein folds that are present in the nsPs of all alphaviruses and coronaviruses, but the function is unclear. Macrodomains bind ADP-ribose conjugated to proteins. ADP-ribosylation is accomplished by a family of ADP-ribosyltransferases known as poly(ADP-ribose) polymerases (PARPs), of which several are regulated during viral infection. Viral macrodomains also have hydrolase activity and can remove ADP-ribose from modified host proteins. Mutation of the nsP3 macrodomain of CHIKV or SINV to decrease ADP-ribose binding or hydrolase activity impairs virus replication *in vitro* and decreases neurovirulence in mice.

Lassa fever virus and other arena viruses

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The Arenaviruses have the dubious distinction of containing among their members one of greatest proportions of hazard group 4 viruses of any recognised taxonomic family. These viruses cause persistent asymptomatic infections in their rodent reservoirs, however occasional zoonotic spill over results in severe viral haemorrhagic fever (VHF) and high mortality. Transmission to humans is thought to occur via contact with infected rodent excreta, through for example contaminated food and inhalation of aerosols from dried urine or faeces. Human to human transmission also occurs. In sub-Sharan West Africa, Lass virus has been identified as a VHF since 1969 and is endemic over much of rural Nigeria, the countries of Mano River Union, including Ghana, Togo and Benin. From a global health security perspective it is of significant international interest since it the most commonly imported VHF into non-endemic countries. In nearly every imported circumstance, the cryptic nature of Lassa fever and related difficulties in diagnosis, places an enormous upheaval and demand on clinical, laboratory and public health resources of the recipient country. Given our current understanding of LF including the ever developing international connections across the world, attention should be focused on other killer arenaviruses which have similar incubation times and could similarly result in overburdening public health systems.

SFTSV nonstructural protein NSs-mediated immunopathogenesis

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SFTS virus listed in the World Health Organization Prioritized Pathogens is an emerging Phlebovirus in the *Phenuiviridae* family. The virus was discovered in China in 2010 and has since spread to other countries in East Asia. SFTS has fatality rates ranging from 12% to as high as 30% in some areas and induces immunopathogenic disease with a characteristic thrombocytopenia that is remarkably similar to viral hemorrhagic fevers. Infected ticks are the major source of human SFTSV infection, however, animal-to-human and human-to-human transmissions by direct contact have been reported. Due to the lack of therapies and vaccines against SFTSV infection, there is a pressing need to understand the pathogenesis of SFTSV to develop effective vaccines and antiviral agents. We and others have shown that the virally encoded nonstructural protein (NSs) blocks type-I interferon (IFN) induction and is thought to facilitate disease progression. I will discuss that SFTSV NSs plays an essential role in viral immunopathogenesis. Specifically, SFTSV NSs targeted the TPL2 kinase pathway to robustly induce expression of immune suppressive genes, specifically IL-10 cytokine. Combined use of viral reverse genetics, a kinase inhibitor, and *Tpl2*$^{-/-}$ or *IL-10*$^{-/-}$ mice showed that SFTSV NSs targets the TPL2 signalling pathway to induce expression of immune suppressive genes including IL-10 as means to dampen the host defense and promote viral pathogenesis. Thus, TPL2 signalling pathway is a potential therapeutic target to treat SFTSV-infected patients.

Study of norovirus infection mechanism

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Human norovirus (HuNoV) is major cause of epidemic nonbacterial gastroenteritis worldwide and often cause an outbreak, but there is no efficient treatment and vaccine at present. Murine norovirus (MNV) has been the only species of noroviruses which can be grown in culture cells and thus most commonly has been used for studies as a surrogate of HuNoV. A low resolution cryo-electron microscopy (cryo-EM) structure of the whole particle of the MNV type 1 (MNV-1) and the high resolution crystallographic structure of the protruding (P) domain have been reported that the MNV has the unique structure in Caliciviridae where the P domain is lifted off the shell (S) domain by 16 Å and rotated by 40° in a clockwise fashion and forms new interactions with adjacent P domains at the P1 subdomain. However, the absence of the atomic structure of the whole capsid has hampered further studies.

Here we present the structure of virus-like-particles (VLPs) of MNV type S7 (MNV-S7) by single-particle cryo-EM at 3.7-Å resolutions and build the atomic model, in addition to the cryo-EM maps of MNV-S7 and MNV-1 virions at 5.7-Å and 5.1-Å resolutions, respectively. The Cryo-EM map permitted to build the atomic model of the MNV-S7 capsid. The capsid structure of MNV-S7 was compared with that of Murine norovirus type 1 (MNV-1) previously reported. Although the difference of the amino acids is only 6%, the conformation of the capsid is obviously different. CD300lf, a receptor for norovirus internalization into cells recently discovered, can be more easily access to the binding site of the P domain in MNV-S7 than that in MNV-1. The structure of the P domain of the norovirus particle affects the binding with the receptor and may control infectivity.

A plasmid-based reverse genetics system for rotavirus

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Rotavirus (RV), a non-enveloped icosahedral virus containing eleven gene segments of double-stranded RNA, is the leading cause of severe, acute diarrhea among infants and young children worldwide. The understanding of the molecular mechanisms underlying RV replication and pathogenesis has been hampered by the lack of an entirely plasmid-based reverse genetics (RG) system. RG systems are definitive and essential for studying the functions of viral genes by generating mutant viruses. Recently, we developed a novel RG system which enabled recovery of recombinant RVs entirely from cloned cDNAs. This new strategy requires co-expression of a fusion-associated small transmembrane protein (FAST) that accelerates cell-to-cell fusion and vaccinia virus (VV) capping enzyme.

Full-length 11 RV cDNAs were generated from viral dsRNA genome-extracted RV strain SA11 virions. Viral cDNAs were flanked by a T7 promoter and an antigenomic hepatitis delta virus ribozyme. To generate recombinant RVs, BHK-T7 cells stably expressing T7 RNA polymerase were co-transfected with 11 RV cDNAs along with expression plasmids encoding the FAST and VV capping enzyme.

Using this technique, we generated a recombinant virus lacking the C-terminal region of NSP1 and used the recombinant virus to investigate the function of this protein in combating the innate immune response. Furthermore, by insertion of the exogenous fragment into the NSP1 ORF, we recovered a recombinant virus that expresses a NanoLuc luciferase. This technology is expected to promote research in the field of RV biology and advance the development of new RV vaccines.

Structural Studies of Flavivirus Antibody Complexes

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Introduction: Zika virus, a member of the flavivirus genus of positive RNA strand viruses, emerged in 2015-2016 in the Americas with rapid spread and substantial impact. We solved the structure of this flavivirus virion to near atomic resolution using cryo-electron microscopy (cryo-EM) and this revealed the similarity with previous flavivirus structures such as dengue virus and West Nile virus. We have been studying the binding of antibodies to the virion to understand: 1) what are critical epitopes; 2) to understand what sites are recognized by the human immune system; 3) how do neutralizing antibodies function; and 4) general properties of flavivirus neutralizing antibodies.

Methods: These studies have employed monoclonal Fab fragments that recognize either the E protein, which is the dominant immunogen for flavivirus immunity, or the prM protein, which is found in immature or partially particles. Purified Fabs were prepared and were complexed with highly purified virus particles, cryo-frozen, imaged using a FEI Titan Krios with a K2 DED, and reconstructions performed. The image reconstructions were compared with native virus structures and interpreted.

Results: Several neutralizing antibody Fab – virus complexes from Zika and dengue were analyzed by cryo-EM. The cryo-EM analyses provide three-dimensional high resolution images of these complexes. In some cases, crystal structures of the Fab were determined and fitted into the cryo-EM density. These different antibody – virus complexes demonstrate different modes of binding and thus different mechanisms of virus neutralization.

Conclusions: The ability to compare virus particles and functional antibodies from multiple members of the flavivirus genus in terms of structure and function serves as a powerful strategy to discern common processes and distinct features that contribute to virus biology and disease.

Molecular epidemiology of Severe Fever with Thrombocytopenia Syndrome

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Introduction: Severe Fever with Thrombocytopenia Syndrome (SFTS), caused by SFTS virus (SFTSV) in genus *Phlebovirus* of the family *Phenuiviridae*, is a newly discovered highly pathogenic infectious disease. Here we analyzed SFTS surveillance data and SFTSV full-length genome sequences to understanding of SFTS epidemiological characteristics and molecular evolution.

Methods: SFTS data in Mainland China between 2010 and 2016 were obtained from the China disease prevention and control information system. The cases analyzed included suspected cases, laboratory confirmed cases and clinical diagnosis cases. SPSS 18.0 software was used to analyze epidemiological characteristics. Whole genome sequencing of 72 SFTSV samples was carried out by high-throughput sequencing. Bioinformatics analysis was performed on the newly obtained sequences and the data sets of all the SFTSV full-length genome sequences in the ViPR database. BEAST software was used to analyze the origin of SFTSV in time and space.

Results: From 2010 to 2016, a total of 10917 SFTS cases, including 484 deaths, were reported in Mainland China with the average annual incidence rate of 0.12/lakh and case fatality rate of 4.4%. SFTS cases increased from 107 (2010) to 2949 (2016), but the annual fatality rate decreased from 14% (2010) to 2.5% (2016). The top 7 provinces were Henan, Shandong, Hubei, Anhui, Liaoning, Zhejiang, and Jiangsu, which accounted for more than 99% of the total cases. The period from April to October was the epidemic season. Reported cases were mainly clustered in age group of 40-79 years. Farmers were under the highest risk. SFTSV samples used in evolutionary analysis distributed in China (443), South Korea (23), and Japan (64). Six genotypes of SFTSV were clearly divided. Genotype F was the dominant epidemic genotype of Japan, South Korea and Zhejiang province of China, genotype A was mainly epidemic in Henan, Hubei, and Anhui, genotype B was main in Shandong and Liaoning, and the genotypes are most abundant in Jiangsu and Anhui provinces. A large number of reassortant and recombinant strains of SFTSV were found. We also found a number of genotype-specific mutation sites, and proposed that two mutations (T501S and P662S) in Glycoprotein of genotype F might be associated with the high pathogenicity of genotype F. Many co-mutation sites and their corresponding branches in the phylogenetic tree were identified as well. It was found that SFTSV originated in the early 18th century, and the most likely origin was from Zhejiang province (about 50% probability). Besides, genotype F is the most primitive genotype.

Conclusions: The number of SFTS cases increased annually and the endemic areas expanded in China. Bioinformatics analysis clearly divided the six genotypes of SFTSV, and different location showed different dominant types. Reassortment or recombinant strains existed. The genotype-specific mutations may act a molecular marker for diagnosis or pathogenic analysis.

SFTS in animals; a risk of direct infection from animals to human in Japan

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Severe fever with thrombocytopenia syndrome (SFTS) is an emerging viral disease, with fatality rate ranging from 2 to 30%, first identified in China in 2009, then found in Japan and South Korea in 2013. SFTS virus (SFTSV), a member of the *Phlebovirus* genus in the family *Bunyaviridae*, is transmitted by tick. In Japan, more than 250 laboratory-confirmed SFTS cases with fatality rate of some 20% were reported since 2013. All the patients were reported in the western region of Japan. In Japan, wild Japanese deer and other animals have been shown to have SFTSV antibodies. To understand the significance of SFTSV antibody positive rate in wild deer, serological surveillance of SFTSV in wild deer was conducted in the SFTS endemic and non-endemic region. Statistical analysis was performed if there is positive correlation between antibody positive rate in wild deer and number of SFTS patients in the 20 prefectures in Japan. SFTSV antibody positive deer were found in 17 out of 20 prefectures in Japan and SFTS patients were reported in 10 out of 20 prefectures between 2015 and 2016. Pearson's positive correlation with correlation coefficient of some 0.7 was observed between SFTSV antibody positive rate in wild deer and number of SFTS patients in the prefectures so far tested. SFTSV is widely distributed in Japan, however, antibody prevalence in wild deer has been shown to be significantly high in the endemic region. SFTSV is maintained in an animal-tick interface, and wild deer are considered to play a crucial role in the interface. Antibody prevalence in wild deer is shown to be correlated with number of SFTS patients in the prefacture, thus it is important to conduct serosurveillance in animals to evaluate risk of SFTS in the region. Variety of animals were known to be susceptible for the virus infection, while they were considered to be subclinically infected. However, recently, several animals, cats, dog and cheetah were shown to be contracted with lethal SFTS. Close contact with diseased animals resulted in direct infection of SFTS to humans, indicating there is a risk of direct infection of SFTS virus from animals to human.

Possible antiviral therapy for rabies

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Rabies is a zoonotic disease caused by the rabies virus (RABV), which is usually transmitted by animal bites and causes lethal encephalitis. The case fatality rate for rabies remains almost 100%, and there is still no means of cure for this disease after the onset of symptoms although the discovery of antiviral drugs against RABV has been attempted for a long time. Favipiravir (6-fluoro-3-hydroxy-2-pyrazinecarboxamide) has been shown to be active against a broad range of RNA viruses and been expected to be a therapeutic drug for rabies (influenza virus, Ebola virus, SFTSV). We recently reported that favipiravir effectively also inhibited RABV replication *in vitro* and improved survival of RABV-inoculated mice when the administration was initiated immediately after inoculation, as it turned out that favipiravir can be used as the purpose of post-exposure prophylaxis (PEP). However, the administration of favipiravir after the onset of symptoms could not increase the survivability in rabid mice. A recombinant virus expressing a luciferase is widely used for a high-throughput screening (HTS) assay for antiviral drug discovery. Therefore, we have generated luciferase-expressing recombinant RABVs (the street strain 1088) and established the HTS system on 96-well plate format. Since several reports showed the synergistic effect by combinational use of favipiravir and another drug, we attempt to discover anti-RABV drugs/compounds using the HTS system in the presence or absence of favipiravir. Furthermore, to evaluate efficacy of anti-RABV drugs precisely in a mouse model, we are attempting to establish *in vivo* bioluminescence imaging of RABV infection, which seems to be more sensitive method than the fluorescence imaging that we have established recently.

Pre-infection cytokine responses in vitro correlate with clinical outcome in Dengue

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Introduction: Dengue virus (DENV) infection can be asymptomatic or result in mild or severe illness. Cohort studies demonstrate that previous exposure to DENV has a major influence on the outcome of a subsequent DENV exposure, which can either be beneficial (more asymptomatic infections) or detrimental (more symptomatic and severe infections). The identification of immunological mechanisms and correlates of these effects is important for understanding disease pathogenesis and development and evaluation of vaccines.

Methods: In a longitudinal cohort study of Thai schoolchildren, we collected blood samples annually and conducted active surveillance for acute febrile illnesses. From this cohort, we selected 22 children who had a laboratory-confirmed symptomatic dengue illness and 29 children who had a subclinical DENV infection based on serologic testing. Cryopreserved peripheral blood mononuclear cells collected prior to the incident DENV infection were stimulated in vitro with DENV and cytokine responses were measured by multiplex bead-based immunoassay and flow cytometry.

Results: We detected significant differences in cytokine responses in vitro based on both the clinical outcome of infection and the DENV type used for stimulation. Secretion of six cytokines, including IL-12, RANTES, MIP-1α, and frequencies of IFN-γ- and IL-2- producing CD4+ T cells were significantly higher in PBMC of children with subclinical DENV infections, particularly in response to stimulation with DENV types 2, 3, and 4. In contrast, secretion of IL-6, IL-15, and MCP-1 was significantly higher in PBMC of children with symptomatic dengue illnesses, particularly in response to stimulation with DENV type 1.

Conclusions: Our data identify both positive and negative associations between pre-infection DENV-specific immune responses and the clinical outcome of subsequent DENV infections. These data should be useful to guide mechanistic studies and may be useful for evaluation of candidate vaccines.

An evolutionary mutation that enhances Zika virus evasion of host interferon induction

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Introduction: Until 2007, Zika virus (ZIKV) had silently circulated in many parts of Africa and Asia without causing detected severe disease or large outbreaks, with fewer than 20 documented human infections. After 2007, ZIKV started to cause explosive outbreaks with devastating sever diseases such as congenital malformations and Guillain-Barré syndrome. A number of potential mechanisms could account for the recent increase in ZIKV epidemics, including the acquisition of genetic changes that enhance its ability to infect in humans and mosquitos.

Methods: Using reverse genetic system and animal models of ZIKV, we investigate the functions of mutations that have occurred in the recent epidemic strains of ZIKV.

Results: The Asian lineage of Zika virus (ZIKV), responsible for the recent epidemics, has fixed a mutation in the NS1 gene after 2012 that enhances mosquito infection (Liu et al., Nature. 2017. doi: 10.1038/nature22365). We found that the same mutation confers NS1 to inhibit interferon-β induction. This mutation enables NS1 binding to TBK1 and reduces TBK1 phosphorylation. Engineering the mutation into a pre-epidemic ZIKV strain debilitated the virus for interferon-β induction; reversing the mutation in an epidemic ZIKV strain invigorated the virus for interferon-β induction; these mutational effects were lost in IRF3-knockout cells. Additionally, ZIKV NS2A, NS2B, NS4A, NS4B, and NS5 could also suppress interferon-β production through targeting distinct components of the RIG-I pathway; however, for these proteins, no antagonistic difference was observed among various ZIKV strains.

Conclusions: Our results support the mechanism that ZIKV has accumulated mutation(s) that increases the ability to evade immune response and potentiates infection and epidemics.

Understanding of the pathogenesis of enterovirus 71 infection based on the identification of the receptors

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Introduction: Hand, foot, and, mouth disease (HFMD) is a common febrile disease mainly in infants and children. The major causative agents of HFMD are coxsackievirus A6, A16, and enterovirus 71 (EV71). Large HFMD outbreaks, including cases with severe neurological diseases, mainly due to EV71, have been recently reported especially in the Asia-Pacific region, including in Malaysia, Taiwan, China, Cambodia, and Vietnam. EV71 infections causes a diverse range of neurological diseases and resulted in thousands of deaths in young children, posing a serious threat to public health in the region. A number of cell-surface molecules have been identified to be involved in the early stage of EV71 infection. By using different approaches, our group and Dr Koike and colleagues in Japan have identified two human transmembrane proteins, P-selectin glycoprotein ligand-1 (PSGL-1) and scavenger receptor class B, member 2 (SCARB2), respectively. SCARB2 is expressed on a broad variety of tissues, however, PSGL-1 is primarily expressed on leukocytes. An amino acid residue 145 of the capsid protein VP1 (VP1-145) defined PSGL-1-binding (PB) and PSGL-1-nonbinding (non-PB) phenotypes of EV71 and has been identified as a potential neurovirulence determinant in humans and experimental mouse models.

Methods: To elucidate the *in vivo* involvement of VP1-145 in PSGL-1-depentent replication and pathogenesis, we investigated viral replication, genetic stability, and the pathogenicity of PB and non-PB strains of EV71 in a cynomolgus monkey model. Cynomolgus monkeys were intravenously inoculated with cDNA-derived PB and non-PB strains of EV71, EV71-02363-EG and EV71-02363-KE strains, respectively.

Results: Mild neurological symptoms, transient lymphocytopenia, and inflammatory cytokine responses, were found predominantly in the 02363-KE-inoculated monkeys. Histopathological analysis of CNS tissues revealed that 02363-KE induced neuropathogenesis more efficiently than that induced by 02363-EG. After inoculation with 02363-EG, almost all EV71 variants detected in clinical samples, possessed a G to E substitution at VP1-145, suggesting a strong *in vivo* selection of VP1-145E variants and CNS spread presumably in a PSGL-1-independent manner. EV71 variants with VP1-145G were identified only in PBMCs from two PB-inoculated monkeys.

Conclusions: VP1-145E non-PB variants are mainly responsible for the development of viremia and neuropathogenesis in a non-human primate model, suggesting the involvement of amino acid polymorphism at VP1-145 in cell-specific viral replication, *in vivo* fitness, and pathogenesis in EV71-infected individuals. I will discuss the involvement of VP1-145 in viral replication and pathogenesis in EV71 infection, and reliable animal models to be established in the future.

Dengue: Pitfalls in diagnosis and management

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Dengue is a mosquito-borne viral disease, which is currently an important and rapidly growing health problem across the globe. Four closely related dengue serotypes cause the disease with different clinical manifestations often with unpredictable clinical evolutions and outcomes. The disease ranges from asymptomatic infection to undifferentiated fever, dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). Specific antiviral medications are not available for dengue and successful treatment, which is mainly supportive, depends on early recognition of the disease and careful monitoring for shock. In 2009, the World Health Organization (WHO) developed a severity-based revised dengue classification for medical interventions, which is used in most countries. Laboratory diagnosis includes virus isolation, serology, and detection of dengue ribonucleic acid. Since dengue poses a heavy economic cost to health systems and societies, the potential economic benefits are associated with promising dengue preventive interventions such as dengue vaccines and vector control innovations.

The role of viral replication in macrophages for pathogenesis of Ebola virus infection

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Introduction: Cells of the mononuclear phagocyte system (MPS) such as macrophages and dendritic cells are early targets for Ebola virus (EBOV) infection. However, the pathobiological significance of EBOV replication and host responses in these cells is not fully understood. The cellular tropism of viruses can be engineered through the insertion of target sequences for cell-specific microRNA (miRt) in the viral genome. We constructed the recombinant EBOV that demonstrates reduced replication capacity in MPS cells, and examined viral virulence in mouse.

Methods: We generated the rEBOV possessing the target sequence of microRNA that is expressed predominantly in the MPS cells within the 3'untranslated region of the EBOV L gene. Viral replication of rEBOV-miRt in human hepatoma cell line (Huh7) and human macrophage derived cell line (THP-1) was evaluated. We also generated mouse-adapted EBOV with miRt 8

Results: Compared with parental rEBOV and rEBOV with reverse control sequence (rEBOV-miRr), rEBOV-miRt showed reduced viral replication capacity in THP-1 cells despite both viruses having similar growth kinetics in Huh7 cells, suggesting that rEBOV-miRt replication was attenuated specifically in macrophages. Mice infected with 100 LD50 of parental rMAEBOV and rMAEBOV-miRr succumbed to a disease by 7 days post-infection. In contrast, two of twelve mice infected with rMAEBOV-miRt succumbed to a disease, but others survived without severe clinical symptoms. In addition, rMAEBOV-miRt showed reduced viral replication in peritoneal macrophages at early time point, and subsequent viral replication in target tissues was also suppressed. Thus, rMAEBOV-miRt showed attenuated virulence in mice compared with the parental rMAEBOV.

Conclusions: Our results suggest that viral replication capacity in macrophages is an important factor for lethal outcome associated with EBOV disease. Our study demonstrated direct involvement of the MPS cells in the pathogenesis of EBOV infection in vivo.

Identification and characterization of novel anti-Sever fever with thrombocytopenia syndrome (SFTS) virus compounds

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Background and purpose: Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by SFTS Phlebovirus (SFTSV), which has a high mortality rate. Currently, no licensed vaccines or therapeutic agents have been approved for treating SFTSV infected patients. Our aim is to identify effective compounds which can be used to treat SFTSV infected patients.

Materials and Methods: To identify effective anti-SFTSV compounds which could use in clinic, FDA-approved compound library (Enzo life science) was used to examine their anti-SFTSV effect. For the screening, SW13 cells were infected with SFTSV (YG-1) and replaced the media including the compounds at 90 min post infection (p.i). Cells were fixed and stained with anti-SFTSV N antibody at 48 h p.i.. DMSO was used as a control and SFTSV N positive cells were counted. Cell viability upon compounds' treatment was also examined using Cell-Titer Glo (Promega). Compounds which showed more than 70% reduction of SFTSV N positive cells and less than 30% cell toxicity were selected. Titration of Armstrong strain of Lymphocytic Choriomeningitis virus (LCMV) was performed using anti-LCMV NP antibody (VL-4). Titration of Hazara virus (HAZV) and Vesicular Stomatitis Indiana virus (VSV) was performed with plaque assay.

Results and Discussion: Six of approximately 650 compounds from library, which belong to L-type calcium channel blocker, exhibited strong anti-SFTSV effects. We further examined to rule out the target of the calcium channel blocker, Manidipine, on SFTSV replication and propagation and found that it targets replication/transcription step of SFTSV. We also found that this anti-viral effect by Manidipine is not restricted only to SFTSV, but also to other negative strand RNA viruses, including HAZV (*Nairoviridae*), LCMV (A*renaviridae*), and VSV (*Rhabdoviridae*). The target of Manidipine against these viruses is also the replication step in the cell. Since calcium channel blockers have been used as a medication to decrease blood pressure in patients with hypertension in clinic, it might be possible to apply these calcium channel blockers to treat SFTS patients. These results enhance our understanding of the SFTSV replication mechanism and may contribute to the development of novel therapies for SFTSV infection.

Rapid and Durable Alphavirus Immunity After a Single dose of Vaccines Based on the Insect-specific Eilat Virus

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Introduction: Alphaviruses including chikungunya, Venezuelan and eastern equine encephalitis are important human and livestock pathogens based on their frequent emergence to cause epidemics/epizootics and their potential use as biological weapons. Chikungunya in particular has emerged repeatedly and spread nearly worldwide from Africa and Asia since 2004 to cause extensive outbreaks of severe and chronic arthralgia. No vaccines or specific therapeutics are licensed to prevent or treat any alphavirus infections.

Methods: We capitalized on the recently characterized Eilat alphavirus, an insect-specific virus discovered in mosquitoes, which is fundamentally defective for replication in vertebrates, to develop a chimeric platform for vaccine development. The structural polyprotein genes of CHIKV, VEEV and EEEV were used to replace those of EILV in cDNA clones, and the chimeric viruses rescued were tested for safety, immunogenicity and efficacy in protection against alphavirus infections using murine and nonhuman primate models.

Results: The resulting EILV/CHIKV, EILV/VEEV and EILV/EEEV chimeras rescued in mosquito cell cultures retained the host restriction of EILV, yet were structurally identical to the pathogenic vaccine target alphaviruses. Safety was demonstrated by lack of replication in several vertebrate cells and in the brains of infant mice, the most permissive vertebrate environment for most alphaviruses. Single doses of the chimeric vaccine strains protected completely against viremia and disease in fatal mouse models and in cynomolgus macaques, with a rapid induction of neutralizing antibodies that persisted in mice and protected for at least 9 months.

Conclusions: These EILV-based chimeric vaccine candidates appear to offer the unusual combination of single-dose, rapid and long lasting protective immunity despite being completely replication-defective for vertebrates, a strong indication of safety. They can be produced efficiently in mosquito cell cultures at low cost, offering a promising alternative to more costly and challenging-to-manufacture alternative platforms. The EILV-based chimeras are also excellent, safe viral antigens for ELISA based on their authentic antigenic structures, and as indicated by the use of EILV/CHIKV in commercial IgM and IgG kits.

iRFP720 is optimal for *in vivo* fluorescent imaging of rabies virus infection

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Background: *In vivo* imaging is a noninvasive method that enables real-time monitoring of viral infection dynamics in a small animal, which allows a better understanding of viral pathogenesis. Rabies virus (RABV) causes lethal encephalitis after a long and variable incubation period. It is not fully understood where and how RABV persists in a host during the incubation period, which makes it a good example to study using *in vivo* imaging. Currently, *in vivo* bioluminescence imaging of virus infection is widely used, but fluorescence imaging (FLI) is not used because of severe autofluorescence. Far-red and near-infrared fluorescent probes are known to be suitable for *in vivo* FLI because of less tissue autofluorescence and higher tissue transparency. Recently, several far-red and near-infrared fluorescent proteins (FPs) have been developed that have been shown to be useful for whole-body fluorescence imaging but not evaluated in the imaging of viral infection. In this study, to identify the optimal FP for *in vivo* FLI of virus infection, we performed a comparative study using Katushka2S (Ka2S), E2-Crimson (E2Cr), iRFP670, and iRFP720.

Methods: The FP-expressing RABVs (the strain 1088), 1088/Ka2S, 1088/E2Cr, 1088/iRFP670, and 1088/iRFP720, were generated. Nude mice were inoculated intracerebrally (i.c.) or intramuscularly (i.m.) with each virus and then subjected to *in vivo* imaging analysis using the Lumazone imaging system with filter sets, 607/697 (607/36 nm for excitation; 697/75 nm for emission), 655/732 (655/40 nm; 732/68 nm), and 710/785 (710/40 nm; 785/62 nm).

Results: The comparative analysis using i.c.-inoculated mice revealed that 1088/iRFP720 showed the highest performance in live imaging. The signal from the 1088/iRFP720 infection demonstrated the best signal-to-noise ratio (16.27) using the 710/785 filter set at 8 days post-inoculation. In 1088/iRFP720 infection, the signal from brain began to be detectable in two of four mice at day 4 and was clearly detected in all of the mice after day 5. For the other infections, the signal began to be detectable later than that for the 1088/iRFP720 infection. Furthermore, the viral infection dynamics could be traced in nude mice inoculated i.m. with 1088/iRFP720. In an inoculated mouse, a weak fluorescence signal was detectable from the spinal cord on day 6 before the mouse began to lose weight and was clearly detectable at day 7. At that time, a spot of signal was detected from the brain, and the mouse started losing weight. The signals from the brain and spinal cord continued to increase until day 12. In addition, *ex vivo* imaging of 1088/iRFP720-inoculated mice was also performed. Notably, a strong signal was detected from the terminals of the spinal cord, the signal was stronger on the right side than on the left, and we could detect signals from the sciatic and femoral nerves of the right hind limb. These observations were considered to reflect the route of virus inoculation.

Conclusion: We consider that using the near-infrared FP iRFP720, the most red-shifted FP available, with a filter set for >700 nm is useful for *in vivo* FLI of not only RABV infection but also other virus infections.

Relationship between hantavirus infection and Chronic Kidney Disease by unknown etiology (CKDu) in Sri Lanka

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Introduction: Chronic kidney disease (CKD) is recognized as a major public health problem worldwide. Generally, diabetes, hypertension and glomerulonephritis are known as main risk factors of CKD. However, high prevalence of CKD cases without known risk factors has been identified in the dry zone areas of Sri Lanka since 1990s. This new form of disease is called as chronic kidney disease of unknown etiology (CKDu). Recently, we reported high seroprevalence to hantavirus among CKDu patients in Girandrukotte, Sri Lanka where CKDu is endemic. This result suggests that hantavirus infection may be a risk for progression of CKDu. To clarify the hypothesis, we are conducting the following studies.

Methods: Groups of CKDu patient and healthy control sera from endemic and non-endemic area of Sri Lanka were examined for their seroprevalence to hantavirus by indirect immune fluorescent assay (IFA) and enzyme linked immunosorbent assay (ELISA). To estimate hantavirus species infected to CKDu patients, serotyping analyses by truncated N protein-based ELISA and neutralization assay were carried out. Statistical analysis of gender difference in seroprevalence was performed. To identify reservoir rodent and virus, rodents were collected from Girandrukotte and examined their serum antibody and viral genome.

Results:

1. Quite high seroprevalence (42-48%) were observed from groups of CKDu patients in CKDu endemic area, Girandrukotte, Medawachchiya, and Willgamua. High seroprevalence (13.5-18%) were also obtained from healthy control groups in endemic area. On the other hand, seroprevalence of non-endemic area, Matale district showed lower seroprevalence, 3.5%.
2. Seroprevalence in male was higher than that in female among both CKDu patients and healthy controls.
3. Seropositive individuals derived from both CKDu patients and healthy control groups were considered to be infected with Thailand type hantavirus or antigenically related hantavirus.
4. In Girandrukotte, seropositive black rats were found. However viral genome has not been identified yet.

Conclusion: In this study, the extensive serological surveillance of hantavirus among CKDu patients was carried out. This observation strongly suggests that the hantavirus infection is a risk for progression of CKDu. In addition, high seroprevalence in male who engaged paddy farming suggested that they suffered Thailand type orthohantavirus from black rats in rice field.

In spite of high seroprevalence, typical hantaviral diseases such as hemorrhagic fever with renal syndrome or hantavirus pulmonary syndrome have not been reported in Sri Lanka. Therefore, Sri Lankan Thailand virus is low pathogenic to human in acute phase. However, it might be related to deadly chronic kidney disease. To clarify the relationship between hantavirus infection and CKDu, further investigation in human and rats is necessary.

Poster Abstracts

Molecular epidemiology and factors related to the severity of hand foot mouth disease in northern Thailand in 2016: A prospective cohort study

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The study aimed to determine the molecular epidemiology and factors related to hand foot mouth disease (HFMD) in northern Thailand. Subjects who had been diagnosed in HFMD from 1 January 2016 to 31 December 2016 were asked to enroll the study. The subjects were collected from 41 hospitals from northern Thailand. Throat swab and validated questionnaire were used as research tool. Specimens were tested for enterovirus and genomes had been identified to those positive for enterovirus. Logistic regression was used to detect the association on severity of HFMD at alpha 0.05.

Results: total of 8,261 cases were analyzed in the study. 56.0% were males, 96.1% were aged less than 6 years, 97.4% were out-patient department (OPD) cases, 75.5% were reported in raining season, and 43.2% were from Chiang Mai Province. The number of HFMD cases had statistical significant correlations with temperature, air pressure, relative humidity, and rainfall amount. Averagely, 216 baht and 3,678 baht per case per visit had to be expended for medical cost in OPD and IPD cases respectively. Most of the cases had been reported in the border areas; Thai-Myanmar, and Thai-Lao. 58.9% were positive on enterovirus. Among these, 48.9% were E71.

Thailand health care system should provide a concrete schedule for taking care of HFMD patients during raining season, and should develop an effective preventive and control program for HFMD particularly among children less than 6 years.

A Broad-Spectrum Antiviral Peptide for Combating Emerging Viral Pathogens

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Viral infections are a leading cause of global morbidity and mortality that urgently need effective therapeutic strategies. While there have been important advances in antiviral drug development over the past few decades, there remain major challenges associated with the large number of emerging and re-emerging viruses as well as with the rise of drug-resistant virus strains. Developing broad-spectrum antiviral strategies that work against multiple viruses is a high priority to counter emerging viral threats. One promising strategy involves utilizing antiviral agents that target the lipid membrane surrounding a wide range of enveloped viruses such as Zika (ZIKV), Dengue (DENV), and Ebola. Unlike other antiviral targets, the lipid envelope is derived from host cell membranes and there is a high barrier to the emergence of drug-resistant virus strains. In this talk, I will present ongoing work to develop a membrane-active peptide that exhibits broad-spectrum antiviral activity against medically important viruses by selectively destabilizing high-curvature viral membranes. By utilizing biophysical assays, we have characterized the mechanism of action of drug candidates down to the single-virus particle level with real-time measurement readouts. Based on these characterization efforts, we have identified a lead peptide drug candidate that exhibits potent, *in vitro* antiviral activity against ZIKV and DENV (all four serotypes) at nanomolar concentrations whereas it is nontoxic to mammalian cells at 1000-fold higher concentrations. The therapeutic efficacy of the peptide was also evaluated in a lethal ZIKV mouse model and treatment started three days after infection. Therapeutic administration of the peptide not only significantly reduced mortality, clinical symptoms, viremia, and inflammation, but also prevented neurodegeneration and brain damage. Furthermore, in a humanized mouse model of DENV infection, peptide treatment reduced viremia levels *in vivo* to nearly undetectable levels. Other arboviruses as well as filoviruses have also proven to be susceptible to this targeting strategy. Looking forward, our findings support that selective targeting of viral membranes holds great potential for combing emerging viral threats, including ZIKV and beyond.

Epidemiological survey of hantavirus infection in small mammals in Okinawa, Japan

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Variety of hantaviruses are maintained by small mammal species such as rodents, shrews, moles, and bats in the world. Some of the viruses are the etiological agents of hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. Okinawa has a unique fauna and flora but there is little information about hantavirus infection in wild animals. Therefore, we carried out epidemiological surveys for hantavirus infection in small mammals in Okinawa. In 2015 to 2017, small mammals such as *Suncus murinus* (136), *Rattus rattus* (20), *Rattus norvegicus* (1), and *Mus musclus* (30), were captured. Sera from the animals were tested by ELISA, IFA, and neutralization test for anti-hantavirus antibodies. Anti-hantavirus antibodies were detected in 29 *S. murinus* (Asian house shrew) by ELISA. In these ELISA positive sera, 22 were also positive by IFA. Seventeen *S. murinus* sera were confirmed to be positive to Thottapalayam olthohantavirus (one of the shrew-associated hantaviruses) by ELISA, IFA, and neutralization test. No positive cases were detected in rodents (*R. rattus*, *R. norvegicus*, and *M. musclus*). The results suggest that the Asian house shrews in Okinawa maintain hantavirus probably Thottapalayam virus or closely related virus.

Safety and Immunogenicity of AGS-v, a Mosquito Saliva Peptide Vaccine: a Randomized, Double-blind, Placebo-controlled Phase 1 Trial

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Introduction: Mosquito-borne diseases continue to cause significant morbidity and mortality worldwide despite on-going control efforts. A new approach to disease prevention focuses on the arthropod salivary-mediated transmission of pathogens. Parasites and viruses carried within mosquito saliva appear to initiate or enhance severity of host infection by taking advantage of saliva-human host interactions. This leads to alteration of the cutaneous environment and modulation of the host's innate and adaptive immune responses, thereby providing a rationale for creating vaccines against mosquito salivary proteins rather than the pathogens contained within the saliva. AGS-v is a vaccine composed of four salivary peptides that are common across a number of arthropods.

Methods: We enrolled and randomized 47 healthy adult participants to receive the AGS-v vaccine with and without adjuvant (Montanide ISA 51) versus placebo. Vaccinations occurred at Day 0 and Day 21 followed by a clean mosquito feeding at Day 42. Primary objectives are: 1) to assess safety via incidence of adverse events and 2) to evaluate humoral and cellular immunity by respectively measuring total AGS-v specific immunoglobulins and Th1-associated cytokine release after incubation of peripheral blood mononuclear cells (PBMCs) with AGS-v antigens. Secondary objectives are post-mosquito feeding measures of AGS-v specific immunoglobulins and Th1-related cytokine release, mosquito survival and fecundity, as well as the effects of saliva-coated Zika virus on cytokine production by PBMCs from immunized individuals.

Results and Conclusion:
The data blind will still be in place at the time of the conference. However, the concept of this first-in-human study along with blinded vaccine reaction photos can be presented.

Analysis of entry mechanisms of Bas-Congo virus using a pseudotype VSV

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Objectives: A novel rhabdovirus, Bas-Congo virus (BASV), was identified from viral hemorrhagic fever patients in Bas-Congo province of Democratic Republic of the Congo in 2009. Two out of 3 patients in the outbreak died. BASV was not isolated but 98.2% of the genome sequence was determined. To develop antiviral strategies to BASV infection, it is important to understand virus-host cell interactions, especially focused on BASV cell entry into the host cells. In the present study, a pseudotype vesicular stomatitis virus (VSV) bearing BASV-G protein was employed to analyze the entry of the BASV into the host cells.

Methods: Pseudotype VSV bearing BASV-G protein (BASVpv) was constructed using BASV-G expression plasmid (pCAG-BASV-G) and either VSV-ΔG-GFP or VSV-ΔG- Luc. Infectivity of BASpv on a variety of mammalian cells were compared. To examine the pH-dependent endocytosis of the BASV, the effect of lysosomotropic agents, ammonium chloride and bafilomycin A_1 on BASVpv entry was examined. To examine the involvement of cholesterol transporter protein (Niemann-Pick C1; NPC1) in BASVpv infection, the effect of cholesterol transport inhibitor, imipramine and U18666A, was also examined.

Results: A majority of cell lines so far tested were susceptible to BASVpv infection. The infection of BASVpv to Huh7 cells was inhibited by anti-BASV-G rabbit serum in a dose-dependent manner. Cell fusion upon transfection of pCAG-BASV-G was initiated by a transient treatment of the cells with a low pH buffer. BASVpv infection was inhibited by ammonium chloride, bafilomycin A_1, imipramine and U18666A, respectively, in a dose-dependent manner.

Conclusion: BASVpv infected various mammalian cells and its infection was mediated by BASV-G protein. As for the other rhabdoviruses, low-pH-induced membrane fusion in the endosome by BASV-G is crucial for the viral entry to the cells. In addition, the cholesterol transporter protein is thought to be necessary for the entry of BASV. The result is considered to be valuable for the development of antiviral strategies against BASV.

A neutralization assay for multiple flaviviruses using single-round infectious particles

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Introduction: The diseases caused by the genus Flavivirus including dengue virus (DENV) and zika virus (ZIKV), have a serious impact on public health around the world. Due to serological cross-reactivity between flaviviruses, current IgM/G ELISA assays cannot reliably distinguish between different flavivirus infections. Therefore, there is a need for specific flavivirus diagnostic tests. In this study, we developed a rapid and safer neutralization assay using single-round infectious particles (SRIPs) derived from representative flaviviruses, and performed neutralization test of sera from monovalent flavivirus antigen-immunized mice.

Methods: SRIPs were generated by transfection of 293T cells with a prME-encoding plasmid from DENV, ZIKV, Japanese encephalitis virus (JEV), West Nile virus (WNV), yellow fever virus (YFV), Usutu virus (USUV) and tick-borne encephalitis virus (TBEV), along with a plasmid carrying DENV-1 replicon containing the luciferase gene.
The infection of SRIPs was evaluated by inoculating the SRIPs into Vero cells, followed by measurement of luciferase activity. Mice were immunized with each prME-expressing plasmid into femoral muscle followed by in vivo electroporation 3 times. Immunized sera were subjected to neutralization assays for several flaviviruses.

Results: SRIPs derived from DENV1-4, ZIKV, JEV, WNV, YFV, USUV and TBEV were generated. Luciferase activity on SRIPs-infected cells was well correlated with each viral load. The monovalent immunized mice showed the highest neutralization titers against their homologous flavivirus, but marginal cross-neutralization against heterologous flaviviruses were also observed**.**

Conclusions: These results indicate that our high-throughput SRIP-based neutralization assay for multiple flaviviruses is a rapid and safer diagnostic method to determine the cause of primary flavivirus infection. The assay also may contribute to evaluation of vaccine efficacy as well as assist in the surveillance of flaviviruses.

Conclusions

DS-1-like G1P[8] strains that have emerged in Thailand and Japan were assumed to have originated from a common ancestor. Moreover, the great genomic diversity among the DS-1-like G1P[8] strains seemed to have been generated through reassortment involving human and animal strains. The emergence of DS-1-like intergenogroup reassortant strains having G3P[8] and G2P[8] genotypes in Thailand may imply the constant circulation of DS-1-like G1P[8] strains and the occurrence of reassortment involving them, at least in Asia. Because DS-1-like G1P[8] strains have successfully spread in broad locations in Asia, continued surveillance of DS-1-like G1P[8] strains and related reassortant ones is required.

Identification of amino acid residue important for fusion of the glycoprotein of severe fever with thrombocytopenia syndrome virus

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Severe fever with thrombocytopenia syndrome (SFTS) is an infectious disease with a high fatality rate caused by SFTS virus (SFTSV). Because little is known about the nature of SFTSV, basic studies are required for the developments of a vaccine and effective therapies. We previously demonstrated that characteristics of the glycoprotein (GP) including membrane fusion activity and entry mechanisms of SFTSV. In this study, we determined the amino acid residue, which is important in membrane fusion of SFTSV-GP. Syncytium formations were observed in Huh7 cells expressed with each of the GPs of YG1 and other strains under low pH conditions. In contrast, no or only weak syncytium formations were observed in Huh7 cells expressed with the GP of HB29 strain under the same condition. Several residues of the amino acid sequences of GPs were different between YG1 and HB29 strains. A variety of chimeric GPs between the 2 strains were expressed and their fusion activities were analyzed to identify amino acid residue(s) responsible for fusion activity of the GP. Syncytium formation activity under the low pH conditions in Huh7 cells expressing each mutant GP was measured by a cell-fusion reporter assay. Membrane fusion was induced upon expression of HB29 GP with a substitution of arginine to serine at an amino acid residue 962 (R962S) was expressed, while that was not upon expression of YG1 GP with a substitution of serine to arginine at the residue 962 (S962R). Infectivity of pseudotyped vesicular stomatitis virus (VSVpv) bearing R962S-GP (R962Spv) was significantly higher than that of VSVpv bearing other mutant GPs. Furthermore, an infectivity of R962Spv was less neutralized by anti-GP monoclonal antibody. In conclusion, serine at the residue 962 in the SFTSV GP was critical for membrane fusion and viral infectivity. Furthermore, the region including the 962S was partially involved in the recognition of a neutralizing antibody.

Zika virus disease expansion rate: the analysis from the first detection in Thailand

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The outbreak of Zika virus infection is an important public health problem. The worldwide expansion of disease becomes a big health issue. There is still no specific report assessing the expansion rate of disease in affected county. Here, the author studied on Zika virus disease expansion rate based on the analysis from the first detection in Thailand until present (September 2017). The GIS based technique was used for disease mapping then further calculation of the disease expansion rate was done. Finally, mathematically modeling technique was used to formulation the equation representing the expansion distance, affected area and outbreak acceleration. From mathematical modeling, the function for distance expansion can be $S = {}_{o}\int^{T} VdT$ or 5.06T. The calculated area of expansion can be $25.6036T^2$. For the acceleration of disease expansion, the equation can be represented as dV/dT. According to the present analysis, there is a linear progression of Zika disease expansion. Therefore, there is no acceleration of disease expansion in the studied setting.

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