United States — Japan Cooperative Medical Sciences Program

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

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

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

Japan Agency for Medical Research and Development

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

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

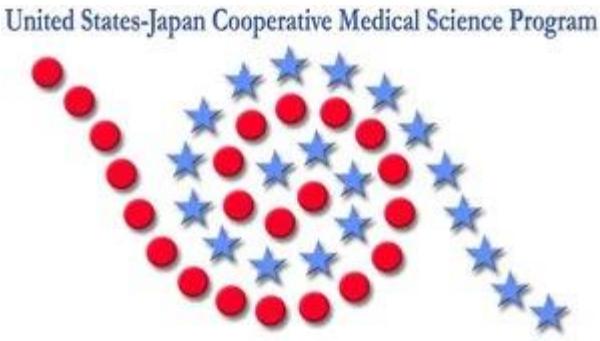
Ministry of Foreign Affairs (MOFA) of Japan

Korea Disease Control and Prevention Agency
National Institute of Health
National Institute of Infectious Diseases

International Vaccine Institute

National Institute of Allergy and Infectious Diseases

National Cancer Institute (NCI)



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

ABSTRACT E-BOOK

March 7 and 8: Viral Diseases Panel

[in order of presentation]

SESSION 1: Rabies and Measles

Kazunori Kimitsuki – Oita, Japan

Exploring Host Gene Expression Dynamics in Lymph Node Inoculated with Different Pathogenic Rabies Virus

Introduction: Rabies is a zoonotic disease caused by the Lyssavirus rabies virus (RABV) and has a case-fatality rate of almost 100%. One of the characteristics of the rabies is its long and unpredictable incubation period, during which RABV antigens and antibodies are undetectable. RABV is transmitted through the bite of a rabid animal and enters the Central Nervus System (CNS) via retrograde axonal transport. The mice peripherally infected with the 1088 strain (wt) of RABV were 100% lethal, while those infected with the mutant strain 1088N30 (N30) survived. Previous studies have found that in the early stages of infection, N30 has a higher virus-neutralizing antibody (VNA) and more immune cell infiltration in the CNS than wt. As CNS is an immune privilege, it is hypothesized that immune responses in peripheral tissue, such as lymph nodes, are important in causing pathogenicity. Therefore, this study aimed to compare the expression of host genes in the lymph nodes of mice infected with RABV of different virulence.

Methods: The virus strain used were 1088 (wt) and 1088N30 (N30). N30 had seven nucleotide substitutions, and the R196S mutation of the G protein led to an additional N-glycosylation. BALB/c mice were inoculated with 105 Focus Forming Units of 30 µL of these viruses in the right footpad, respectively. After the injection, the popliteal lymph nodes (PLN) at 3 and 5 days (3 dpi and 5 dpi) were harvested for transcriptome analysis and quantifying RABV gene expression. Gene Set Enrichment Analysis (GSEA) was used to identify the list of genes with variable expressions. Genes with differential expression were quantified by qPCR.

Results: The study found that the virus-neutralizing antibodies (VNA) in mice infected with the N30 strain of RABV was significantly higher at 3 and 5 dpi compared to mice infected with wt. The RABV gene expression in the lymph nodes was also higher in N30-infected mice. Gene expression analysis showed that there were more differentially expressed genes in N30-infected mice compared to wt-infected mice, with 1,036 genes in N30 and 744 genes in wt at 3 dpi, and 625 genes in N30 and 427 genes in wt at 5 dpi. 29.5% of the differentially expressed genes were common between wt and N30 at 3 dpi, while 49.6% were common at 5 dpi. GSEA revealed that at 3 dpi, genes related to innate immunity were more highly expressed in wt-infected mice, while genes related to cell division were more highly expressed in N30-infected mice. At 5 dpi, both groups showed higher expression of gene related to cell division, but N30-infected mice had increased

interferon gamma response related genes. On the other hand, wt-infected mice showed decreased expression of genes related to the inflammatory response. Real-time PCR showed significantly higher expression of immune genes Cxcl10, Irf1, and Cxcl11 in N30 than wt at 1 dpi.

Conclusions: Our study found differences in immune response in PLN after challenging RABV of different virulence. Specifically, immune-related gene expression decreases at 5 dpi and low expression of Cxcl10, Cxcl11, and Irf1 in wt at 1 dpi. These changes in expression may account for the reduced production of VNA and immune responses compared to N30.

SESSION 2: Hemorrhagic Viruses

Shuzo Urata – Nagasaki, Japan

The role of the NP as a Type I interferon antagonist in the Arenavirus replication

Arenavirus belongs to Bunyaviridae and includes several highly human pathogens, such as Lassa and Junin viruses. Arenaviruses contains two viral genomes (S and L segments) and each encodes two viral proteins, respectively. S segment encodes NP and GPC, a glycoprotein precursor, while L segment encodes matrix protein (Z) and RNA-dependent RNA polymerase (L).

Among these virus proteins, NP is known to be not only an essential component for the viral genome replication together with L, but also a type I Interferon antagonist. To antagonize the type I interferon, NP possesses two functions. The first one is binding directly to the components of the type I Interferon signaling pathways, TBK1 and IKKe, and the second one is the degradation of the viral RNA, which could activate type I Interferon signaling through the recognition by RIG-I Like Receptors.

In this study, mutant viruses of the Junin virus Candid#1 strain were rescued using a reverse genetics system, and the role of NP as a type I interferon antagonist on the replication was examined.

Sung Key Jang – South Korea

Overview of the Institute Pasteur Korea

Minato Hirano – Nagasaki, Japan

Analysis of host factors on viral replication cycle of Crimian-Congo hemorrhagic fever virus

Introduction: Crimean-Congo hemorrhagic fever virus (CCHFV) belongs to the family Nairoviridae, which has tri-segmented negative-sense RNAs as its genome. CCHFV is a tick-borne virus, typically transmitted by Hylaomma ssp. The fatality rate of CCHFV infection reaches 30% in humans, and there is no specific treatment available. Little is known about molecular details of CCHFV infection due to its difficulty in the research in bio-safety level (BSL) -4 containment. In this study, we tried to identify the host factors regulating viral replication to understand the virology of the virus and contribute to the development of specific viral treatments.

Methods: Proteins interacting with nucleoprotein (NP) of CCHFV were screened with Alpha Screen assay of a human protein library synthesized in wheat cell-free system. HEK293T cells were transfected with plasmids expressing the host factors identified in the screening or siRNAs for them. Viral replication in the cells was analyzed by using the minigenome system of CCHFV. Briefly, the cells expressing NP and L proteins were transfected with a reporter RNA coding for secretory NanoLuciferase (secNluc), and luciferase activity was measured as a surrogate of viral replication.

Results and Conclusions: Several host factors were identified to interact with CCHFV NP and some of them affected viral replication. Two of the host factors, ZFP36L1 and L2, belong to the same family of RNA-binding proteins regulating the RNA degradation pathway. Overexpression of these proteins significantly decreased replication of the minigenome, indicating that viral RNAs can be a target of these host factors. Mutagenesis of C3H RNA-binding motifs of the ZFP36L1 dampened the inhibitory activity of the minigenome replication, but, the mutations of the ZFP36L2 did not. This indicated that different mechanisms may be involved in the inhibition of genome replication. These studies will contribute to elucidating the molecular details of CCHFV infection and will help to develop the therapeutic target.

Choi Young Ki – KVRI, South Korea

Severe Fever with Thrombocytopenia Syndrome Virus: Prevalence and its age-dependent pathogenesis

SESSION 3: Enteric Viruses

Kei Haga – National Institute of Infectios Diseases, Japan

Neonatal Fc receptor works as a classical human astrovirus receptor

Classical Human Astrovirus (HAstV) is known to gastroenteritis in infants, elderlies, and immunocompromised individuals. HAstV is a nonenveloped and a positive single strand RNA virus, consisting of eight serotypes.

In this study, we established highly susceptible clone to HAstV from Caco2 cell line, derived from human colorectal adenocarcinoma. Using this cell line, a genome wide CRISPR-Cas9 screening was applied to identify host factors involved in HAstV susceptibility.

Our results revealed that knockout of FCGRT or B2M remarkably inhibited HAstV susceptibility in Caco2 cells. These molecules are components of the Neonatal Fc receptor (FcRn), which plays a role in regulating IgG and serum albumin turnover. We also showed that exogenous FcRn expression increased susceptibility of non- or low-permissive cell lines to HAstV, and that FcRn bound directly to the HAstV spike protein. These findings suggest FcRn works as an HAstV receptor, shedding light on the mechanism of HAstV entry.

Craig Cameron – University of North Carolina

Development of therapeutics to treat infection by RNA viruses

With each viral outbreak, it becomes clearer that we have yet to master the ability to predict accurately newly emerging or reemerging viruses. One mission of our laboratory has been the creation of fundamental knowledge on the chemistry and biology of the viral RNA-dependent RNA polymerase (RdRp) to facilitate development of broad-spectrum antiviral therapeutics and RdRp-mechanism based strategies for viral attenuation and vaccine development. Our goal is to enable a rapid response to a viral outbreak independent of etiology. This lecture will describe our recent studies exploiting a high-throughput, magnetic-tweezers assay for polymerase elongation and a high-throughput, microfluidics-based assay to monitor viral infection dynamics in single cells. These studies reveal unexpected mechanisms of action for antiviral therapeutics and provide insight into the virus lifecycle masked by population-level studies. In addition, we make the case for single-cell analysis enabling the observation of between-individual variation in outcomes of infection, a capability essential to applying principles of personalized medicine to antiviral therapy.

Sakura Kobayashi – National institute of Infectious Diseases, Japan

Do nutrients also help norovirus to infect in the small intestine?

Ondrej Mach – World Health Organization, Switzerland

The last hurdles on the path to poliovirus eradication and new tools to achieve it

Jesse Eramus – HDT Bio

Enabling high dose RNA vaccination for development of a multivalent enterovirus vaccine

Enterovirus D68 (EV-D68) has emerged as a significant public health concern, associated with severe respiratory illnesses and neurological complications such as acute flaccid myelitis (AFM). Recent outbreaks of EV-D68 infections have highlighted the virus's potential for widespread impact, particularly among children. Despite its public health significance, no vaccine for the prevention of AFM is available for use in humans.

The unprecedented development of first-generation mRNA/lipid nanoparticle (LNP) vaccines has marked a significant milestone in our response to global health emergencies. However, the established tradeoff between safety and immunogenicity of this technology remains a critical hurdle to overcome. Studies have consistently shown a positive correlation between systemic reactogenicity—the tendency to produce common, often mild side effects and, in rare instances, severe adverse events—and immunogenicity, suggesting that diminishing one may inadvertently impair the other. As we pivot towards utilizing mRNA vaccines in prophylactic scenarios within non-crisis conditions, such as for the prevention of AFM in healthy children, enhancing safety and tolerability is essential for improving vaccine uptake and utilization towards improving public health.

We have developed a distinct technology from mRNA/LNP that uses a cationic nanocarrier called LION™ that has been optimized for the delivery of self-amplifying replicon RNA (repRNA). By studying repRNA/LION mechanisms of action, we have found that we can decouple the link between reactogenicity and immunogenicity by limiting systemic biodistribution with localized delivery mediated by cationic nanocarriers such as LION. Here, we describe the application of our clinical-stage repRNA/LION platform to develop vaccines against the 6 major subclades of EV-D68 to enable multivalent vaccination.

SESSION 4: Arboviruses

Sonja Best – National Institute of Allergy and Infectious Disease

Beyond retroviruses: new effector functions for the antiviral restriction factor, TRIM5

TRIpartite Motif (TRIM) proteins belong to a large protein family, many of which are inducible by type I interferon and serve to suppress virus infection through direct interactions with viral proteins. Primate TRIM5α is a consequential inhibitor that suppresses lentivirus replication (e.g HIV-1) in a highly host species- and virus species-specific fashion to limit cross-species transmission of these viruses. Importantly, the antiviral effects of TRIM5α have been thought to function exclusively in the context of lentivirus infection. Our research interests center on the flaviviruses that include significant pathogens that have emerged into human populations from primates (e.g. dengue virus, Zika virus, yellow fever virus) prompting us to determine whether TRIM5α could also function to inhibit flavivirus replication. Surprisingly, this work has revealed a new function for TRIM5α as a potent restriction factor for replication of specific flaviviruses. The mechanisms of restriction, flavivirus escape, and the implications of TRIM5a as an early barrier to flavivirus replication will be discussed.

Dimitri Lavillette – Pastuer

Zika virus NS5 forms nuclear-body structures via conjugation to small ubiquitin-like modifier on a lineage-specific lysine to modulate virus pathogenicity

Shintaro Kobayashi – Hokkaido

West Nile virus capsid protein induces nuclear membrane loss and promotes viral replication

Richard Kuhn – Purdue University

Structural and biochemical studies of flaviviruses in complex with antibodies and attachment factors

Moi Meng Ling – University of Tokyo

Longitudinal analysis of Dengue virus-1 (DENV-1) infection induced cross-neutralization and antibody-dependent enhancement activity across genotypes levels and T-cell epitopes

SESSION 5: Bat Immunology & Zoonotic Viruses

Anne Rimion – University of California Los Angelas (virtual)

Studies of MPOX

Daved Fremont – Washington University, USA

Poxvirus sabotage of T-cell co-stimulation

Orthopoxviruses dedicate a significant fraction of their genome to undermining host immune defenses. Of particular interest is the poxvirus immune evasion (PIE) domain-containing family of proteins, which are typically dispensable for viral entry and replication. Diverse functions have been ascribed to PIE proteins, including the sequestration of proinflammatory chemokines and cytokines and the sabotage of major histocompatibility complex (MHC) class I antigen presentation. In this talk we will discuss the poxvirus encoded M2 protein, which we found can specifically bind B7.1 (CD80) and B7.2 (CD86), cell surface proteins expressed mainly by professional antigen-presenting cells that serve as ligands of the T cell costimulatory receptor CD28 and inhibitory receptor CTLA4. Functionally, we found that M2 blocks CD28-mediated T cell activation in vitro and in a mouse model of cowpox infection.

Michelle Baker – Centers for Disease Control and Prevention, Australia

Antiviral immunity in the Australian black flying fox

Bats have been identified as the natural reservoir to an increasing number of emerging and re-emerging zoonotic viruses including the henipaviruses, Nipah and Hendra virus – both members (family Paramyxoviridae). Both Hendra and Nipah are associated with severe neurological and respiratory disease and high mortality rates in humans. However, similar to the response of bats to other viruses, no clinical signs of disease are observed in bats during natural or experimental infections. The long co-evolutionary history of bats with viruses may have shaped the immune system of bats and resulted in unique adaptations for the control of viral replication. To understand how bats coexist with viruses we have developed the Australian black flying fox, the natural reservoir of Hendra virus as a model species for studying virus-host interactions. Experimental infection of cells lines and live bats with Hendra virus has revealed differences in the kinetics of viral infection and the innate and adaptive immune response of the flying fox that may play a role in its ability to coexist with viruses in the absence of disease.

Cara Brook – University of Chicago, USA

Understanding persistence of potentially zoonotic, bat-borne henipaviruses in Madagascar

Bats host viruses that cause higher case fatality rates upon spillover to humans than those derived from any other mammal, including Ebola and Marburg filoviruses, Hendra and Nipah henipaviruses, and SARS and MERS coronaviruses. We use a combination of evolutionary theory and tissue cell culture to demonstrate how bats' unique physiologies, evolved to mitigate the metabolic damage incurred from powered flight, may select for the evolution of viral traits that cause virulence following cross-species emergence. We then carry these insights into the field to interrogate the transmission mechanisms by which wild fruit bat populations in Madagascar maintain novel, likely virulent henipaviruses with a demonstrated capacity to infect human cells. Ultimately, we aim to understand the natural circulation of zoonotic viruses in wild bat hosts to identify intervention opportunities for mitigating zoonotic risk.

Ayato Takada – Hokkaido, Japan

Niemann-Pick C1 heterogeneity and filovirus tropism to bat cells

Introduction: Bats are suspected to be natural hosts of filoviruses, including Ebola virus (EBOV) and Marburg virus (MARV). Interestingly, previous studies have suggested that these viruses have different tropisms depending on the bat species. However, the molecular mechanisms for the differences are poorly understood.

Methods: Bat-derived cell lines were tested for their susceptibility to EBOV, MARV, and Lloviu virus (LLOV), using non-replicating vesicular stomatitis Indiana viruses (VSIV) pseudotyped with the envelope glycoprotein of these filoviruses and infectious EBOV and MARV. Amino acid sequences of Niemann-Pick C1 (NPC1) protein, one of the cellular receptors interacting with the filovirus glycoprotein (GP), were determined for various bat cell lines. Mutant NPC1 and GP genes were produced by site-directed mutagenesis. Vero E6 cells stably expressing exogenous NPC1 molecules were generated using an NPC1-knockout cell line and a retrovirus vector.

Results: We found that bat-derived cell lines FBKT1, ZFBK13-76E, and SuBK12-08 might have comparatively higher susceptibility to EBOV, MARV, and LLOV, respectively. In these cell lines, unique amino acid sequences were found in loop 1 and loop 2 structures of the NPC1 protein, which interact with the receptor binding domain of GP. Using VSIVs pseudotyped with GPs of EBOV, MARV, and LLOV, it was also found that these unique amino acid residues in NPC1, as well as a few amino acid differences among GPs of these filoviruses, were crucial for the differential susceptibility to filoviruses.

Conclusion: Taken together, we demonstrated that GP-NPC1 engagement is one of the genetic determinants of the host-range restriction of filoviruses in bat species, implying a molecular basis underlying the filovirus tropism.