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U.S.-Japan cooperative medical sciences program: 22nd International Conference on Emerging Infectious Diseases in the Pacific Rim

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ABSTRACT

This review summarizes the presentations given at the 22nd International conference on Emerging Infectious Diseases in the Pacific Rim. The purpose of this annual meeting is to foster international collaborations and address important public health issues in the Asia-Pacific region. This meeting was held in Bangkok in February 2020 and focused on emerging virus infections. Unexpectedly, the SARS-CoV-2 pandemic was in the initial stages leading to a special session on COVID-19 in addition to talks on dengue, influenza, hepatitis, AIDS, Zika, chikungunya, rabies, cervical cancer and nasopharyngeal carcinoma.

1. Introduction

The U.S.-Japan Cooperative Medical Sciences Program (USJCMSP), established in 1965, fosters international biomedical research collaboration and addresses important public health issues in the Asia-Pacific region. The nearly annual convening of an *International Conference on Emerging Infectious Diseases in the Pacific Rim* (EID), which started in 1996, is a key element of the USJCMSP. Its purpose is to facilitate scientific interaction and identify research collaboration opportunities. The EID is usually held in the Asia-Pacific region to encourage regional

participation, and they often take up to a year to plan. In 2019, as the planned late-February 2020 date of the meeting approached, the organizers quickly responded to the emerging COVID-19 threat by planning one of the first international research conferences with a major focus on SARS-CoV-2.

The 22nd EID and associated meetings, with a planned focus on viral diseases, convened from February 24–27, 2020, in Bangkok, Thailand, with the Thai Ministry of Public Health (MOPH), Chulalongkorn University, and the Thai Red Cross serving as the local co-hosts. These local hosts made convening the EID Conference at a time of great uncertainty

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possible by assuring participant well-being and facilitating safe scientific interaction. Other conference organizers - the Japan Agency for Medical Research and Development (AMED), the U.S. NIH National Cancer Institute (NCI) and the National Institute of Allergy and Infectious Diseases (NIAID) also supported the conference and organized a Scientific Planning Committee, which included members drawn from government and academic institutions in Japan, Thailand, and the U.S.

Because the conference immediately preceded most international travel restrictions, it convened in person and within all national guidelines, advisories and policies. Extra precautions were taken to keep participants safe. Speakers who chose not to travel to Bangkok were accommodated with options to participate remotely. As the pandemic evolved even during the week of the EID, the organizers and local hosts provided timely updates and enhanced health screening, as needed. Now-familiar protocols to prevent SARS-CoV-2 transmission and acquisition were implemented aggressively which allowed the meeting to proceed with due caution. As one of the few large scientific conferences to convene in person during 2020, the meeting also modified its agenda as emerging COVID-19 data became available and in response to changes in speaker availability. This allowed immediate reporting on rapidly accruing COVID-19 experience, findings, and hypotheses.

The overall theme of the 22nd EID was: the pathogenesis, prevention, and treatment of viral diseases of importance in the region that have global health impact. The objectives of this conference included sharing current research findings and fostering existing and potential international research collaborations that engage investigators and institutions in the Asia-Pacific region and the U.S. The 22nd EID also provided informal networking opportunities and poster sessions to include and highlight research presented by young/early-career scientists from the U.S. and the region. The conference was timely in the inclusion of a special session on COVID-19 emphasizing experiences, lessons learned and potential opportunities for collaboration to address this newly emerging viral disease.

2. Shimao-Takeda Lectureship - Usa Thisyakorn, Chulalongkorn University, Thailand (Fig. 1)

Dr. Usa Thisyakorn addressed clinical, epidemiological and immunological aspects of dengue, the most important vector-borne viral disease in this region, from her outstanding career as a world leading

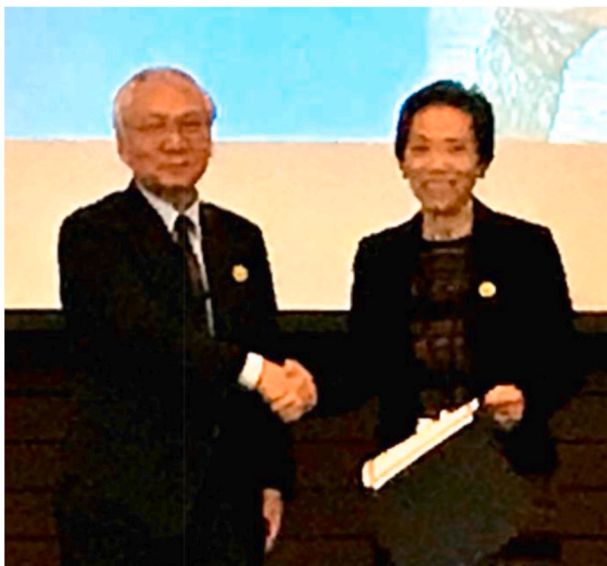


Fig. 1. Shimao-Takeda lecturer Usa Thisyakorn from Chulalongkorn University, Bangkok Thailand receiving a commemorative certificate from Ichiro Kurane, National Institute of Infectious Diseases, Japan.

pediatrician/scientist expert in this disease.

Dengue is one disease entity with different clinical manifestations, often with unpredictable clinical evolutions and outcomes. The clinical spectrum of dengue disease ranges from mild illness to the life-threatening severe forms of the disease associated with plasma leakage, shock, severe bleeding and 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 indicates liver involvement during dengue infections.

Because specific antiviral medications are not available, the early recognition of dengue disease, bleeding tendency, and signs of circulatory collapse could reduce the mortality rate of dengue patients. Novel platforms utilizing innovative technologies for point of care dengue diagnostic and wearable patient monitors have the potential to revolutionize dengue surveillance, outbreak response, and management at population and individual levels. Until the present time, the pathogenesis of dengue disease is still unclear. Involved factors include interaction of dengue virus and host immune response as well as genetic diversity of dengue virus across geographic origins.

Many countries in Asia where *A.aegypti* and *A.albopictus* are widespread in both urban and rural areas have reported an epidemic shift of dengue from mainly affecting children to adolescents and adults. Dengue poses a heavy economic cost to health systems and societies and implementation of promising dengue prevention interventions could lessen these costs. Dr. Thisyakorn introduced the current dengue vaccine status, and concluded that sustainable vector control and effective dengue vaccines are keys to success for prevention and control of this disease.

3. La Montagne-Heilman Lectureship - Erica Ollmann Saphire, La Jolla Institute for immunology, U.S.A. (Fig. 2)

Dr. Saphire presented her watershed work on defining features of antibodies against Lassa virus. Lassa virus (LASV) is an Old-World arenavirus that causes Lassa fever (LF), an often-fatal viral hemorrhagic fever endemic in West Africa. Historically, researchers have found that it is hard to induce neutralizing antibodies to LASV, and in natural infections the T-cell response is thought to play a major role in protection. To understand this phenomenon, Dr. Saphire characterized antibodies in convalescent sera obtained through the Viral Immunotherapeutics Consortium (VIC).

The result was that the majority of the neutralizing antibodies recognized the surface glycoprotein (GPC) and clustered into a single competition group, termed GPC-B. Antibodies within this group bind only to prefusion GPC and the fully assembled GP1 (receptor binding)-GP2 (fusion machinery) complex. Comparison of the structure of three different neutralizing antibodies of varying potency—high (37.7H), moderate (25.5A), and low (18.5C)—in the GPC-B group identified three critical arginine residues (R36, R108, and R59) in the complementarity determining regions (CDR) of the antibody heavy chain. When the low potency antibody 18.5C was engineered by substituting with arginine found in the heavy chain CDR of high potency binders, this antibody not only had increased potency but also increased breadth for recognition of LASV strains.

Furthermore, two critical glycosylation sites in GPC were identified, N390 and N395. These sites interact with the CDR of the antibody light chain and when glycosylated hinder antibody binding. The germline antibody form of the low potency antibody 18.5C binds the non-glycosylated GPC but only weakly. Although the germ line form 18.5C antibody can be matured in vitro to a potent antibody by arginine substitution, its low binding affinity is too weak to overcome the hindrance of glycosylation at the N390 and N295 sites for affinity maturation.

The findings that glycans bordering the GPC-B epitope restrict binding by even mature antibodies and that immature antibody requires



Fig. 2. La Montagne-Heilman lecturer Erica Ollmann Saphire from the La Jolla Institute for Immunology, California USA receiving a commemorative certificate from Gray Handley, National Institute of Allergy and Infectious Diseases, USA.

the GPC trimer for recognition explain why LASV infection does not produce high antibody responses. The finding from this study can guide the design of effective vaccines against *Lassa virus*.

4. Special COVID-19 session and talks

As an example of the fluidity of the agenda and the pressing need to address the newly emerging COVID-19 pandemic in the region, three last minute presentations were arranged on the topic of the first evidence of the appearance of the disease in Thailand. Dr. Tanarak Plipat (Department of Disease Control, MOPH, Thailand) traced the timeline of the first known case of COVID-19 to appear outside China. He briefly described what emerged from the Chinese experience in the period of December 2019 through January 2020: an incubation period of 5 days, but up to 10 days; virus doubling time of 7.5 days; case fatality rate of 2.4%; 79,000 cases, 3,019 health workers recorded with COVID-19; whole genome sequenced. On January 8 the first case outside China was recognized in Thailand, and on January 13 the genome was shown to be identical to the prototype Wuhan strain. At the time of Dr. Plipat's presentation, 35 cases were identified in Thailand. Dr. Plipat attributed these accomplishments to excellent public health strategies from the top down, and experiences with the prior SARS and MERS incidents.

Dr. Weerawat Manosuthi (Bamrasnaradura Infectious Disease Institute (BIDI)/Thai MOPH) presented three clinical case experiences from these early incidents of COVID-19 infection in Thailand. The first case was a 61-year old Chinese female tourist who presented with mild symptoms. The second case was a 50-year old Thai male who also presented with mild symptoms. The third case was a 70-year old Thai male who presented at the emergency room with deterioration of consciousness and centralized weakness. Sequential chest x-rays showed infiltration, and the patient required mechanical ventilation. Some of the conclusions from those initial 17 cases of COVID-19 at the time were that most cases had mild disease with low-grade fever, that identification of co-infection with other pathogens should be part of the work-up (especially in patients with atypical presentation and elderly), and that most cases only need supportive care.

Dr. Supaporn Wacharapluesadee (Thai Red Cross) expanded further on that initial case of COVID-19 in Thailand. A pneumonia epidemic in Wuhan, China in December 2019 attracted global attention for its

virulence and unknown etiology. Thailand's proximity to China and the large number of Chinese tourists who visit the kingdom made it a likely point of spread beyond China's borders. Between January 4th and 8th, 2020, five visitors were suspected to have respiratory infections. Respiratory tract swabs and sputum samples were obtained from these individuals. Specimens were analyzed using PCR for influenza and coronavirus (CoV) detection. Repeat GenBank BLAST analysis after publication of SARS-CoV-2 nucleotide sequence showed 100% homology with the positive amplicons of the pan-CoV detection protocols. Close coordination between public health workers and supporting laboratories enabled rapid identification (within days) of infected patients. Family-wide PCR analysis was the key to this effort, and the very rapid results preceded the international publication of the novel coronavirus (SARS-CoV-2) nucleotide sequence on January 11, 2020 and confirmed the virus had already reached Thailand by the time when its identity was discovered.

Dr. Linfa Wang (Duke-National University Singapore) gave an important presentation on emerging bat-borne viruses in the last 25 years. Although there is no complete certainty that SARS-CoV-2 is a bat virus, phylogenetic studies suggest that there is a high probability that the virus originated from bats. Multiple zoonotic disease outbreaks caused by bat-borne viruses or probable bat viruses include Hendra virus infection in Australia (first detected in 1994), Nipah virus infection in Malaysia/Singapore (1998/9), SARS outbreak (2002/3), MERS outbreak (2012), large-scale Ebola virus disease outbreak (2014), and the COVID-19 pandemic (2019/20). The bat's innate defense and tolerance responses are better balanced in comparison to other mammals. They have high basal level defense systems (such as DNA damage repair, heat shock, membrane efflux pumps) that are switched on before encountering danger signals. However, they have evolved mechanisms to prevent overreaction (such as over inflammation) to viral infection and other cell stress signals. It is now well recognized that bats are a special group of mammals exceptionally fit as a natural reservoir for many different viruses. If changes are not made in how societies live, farm and eat, it is almost certain that such outbreaks will happen again in the not too distant future.

Dr. Denise Hsu (Military HIV Research Program (MHRP), Walter Reed Army Institute of Research) presented on the response to emerging infectious diseases by the Walter Reed Army Institute of Research (WRAIR). The global community has experienced growing waves of emerging infectious disease threats over the past 40 years. Response to these waves of threats requires organizations that have the competencies, mission, and resourcing to rapidly develop effective medical countermeasures. WRAIR is an intramural U.S. Government science and technology laboratory established in 1893. WRAIR has produced products to protect Service members and the global community against infectious disease threats over its 126-year history. WRAIR has its own set of state-of-the-art laboratories, animal model support, pilot bio-production facility, and clinical trials network to take products from basic science discovery through phase 3 trials and FDA licensure.

Dr. Jessica Manning (NIAID) described the first case of the COVID-19 confirmed in Cambodia on January 27, 2020. The cruise ship MS Westerdam was reportedly taking 1,455 passengers and 802 crew around Asia. Upon docking, in a span of nine days, Cambodia counted 9 new cases. The Pasteur Institute confirmed the identity of the coronavirus by RT-PCR within a few days and Dr. Jennifer Bell immediately sequenced the genome.

Dr. Ichiro Kurane (National Institute of Infectious Diseases, Japan) summarized Japan's national research and development (R&D) strategy for COVID-19 as of February 24, 2020. R&D has been comprehensively carried out by the National Institute of Infectious Diseases (NIID) on verification of new diagnostic tests and enhancement of testing capacity, by Japan AMED on research and development of diagnostic tests, therapeutic drugs and vaccines, by Ministry of Health, Labour and Welfare on clinical trials of existing drugs and dissemination of diagnostic kits, and by Ministry of Education, Science and Technology on COVID-19

related data collection in collaboration with Asian countries. This research has been funded by mobilizing the remaining governmental budget for fiscal year 2019 (April–March) and adjusting costs for the 2020 fiscal year.

5. Session 1: new approaches to vaccine development and clinical trials

Session 1 focused on new approaches to vaccine development and clinical trials. The session included six presentations that covered universal influenza virus vaccine strategy, dengue vaccine clinical trials, HIV vaccine trials, vector-targeted vaccine, broadly protective antibodies and vaccines, and coronaviruses.

Dr. Florian Krammer (Icahn School of Medicine at Mount Sinai, New York) contributed a remote (web-based/online) presentation on a universal influenza virus vaccine strategy based on the conserved stalk domain of the hemagglutinin (HA). Influenza virus infections remain a significant cause of morbidity and mortality worldwide. Current vaccines show efficacy against antigenically matched viruses by inducing strain-specific antibodies against the membrane-distal globular head domain of the viral HA but fail to protect against antigen-drifted and antigen-shifted strains. The Krammer lab developed a universal influenza virus vaccine based on the conserved stalk domain of group 1 and group 2 HAs. The vaccine was evaluated in mice and ferrets, and the strategy was further investigated in humans in a Phase I clinical trial. A universal influenza virus vaccine, which requires a single or only a few immunizations and can induce functional ADCC/ADCP HA stalk-specific antibodies and neuraminidase-inhibiting antibodies, would represent a major advance towards the control of influenza worldwide and would significantly enhance our pandemic preparedness.

Dr. Terapong Tantawichien (Chulalongkorn University) presented on dengue vaccine clinical trials. Dengue is endemic in over 140 countries and outbreaks occur in all age groups. Effective implementation of sustainable vector control and vaccines are keys to success for disease control. Three dengue vaccines have been tested in human clinical trials, and only a live-attenuated recombinant tetravalent dengue vaccine (CYD-TDV) employing the attenuated YF virus 17D strain as the replication backbone was licensed for use in 9 to 45-year old people in several endemic countries. However, CYD-TDV was associated with an excess risk of severe dengue observed from about three years after the first vaccine dose in the population who were seronegative before vaccination. Two live-attenuated dengue vaccines (TAK-003 and Butantan-DV) are under evaluation in phase 2 and 3 trials in Asia and Latin America.

Dr. Denise Hsu (MHRP, WRAIR, Armed Forces Research Institute of Medical Sciences) presented on HIV vaccine trials in Thailand. The RV144 phase III vaccine trial was an Army-led Thai HIV vaccine efficacy trial testing the “prime boost” combination of two vaccines, ALVAC® HIV vaccine (the prime) and AIDSVAX® B/E vaccine (the boost) in healthy volunteers, and showed partial efficacy against HIV infection. Subsequent analyses of immune correlates have shown that IgG antibodies to variable regions 1 and 2 of HIV-1 Env correlated inversely with the rate of HIV-1 infection, mediated via non-neutralizing antibodies. Ongoing cohort studies, such as RV305 and RV306, were developed to better understand high-risk groups suitable for efficient HIV-1 preventative vaccine efficacy trials.

Dr. Jessica Manning (NIAID) presented the first-in-human vector-targeted vaccine as a safe and viable option. The rationale to vaccinate against mosquito saliva rather than the pathogen itself is supported by animal studies that demonstrated that immunity against mosquito salivary proteins protects animals against mosquito-borne disease. AGS-v, a peptide vaccine derived from four *Anopheles gambiae* salivary proteins, was selected for a Phase 1 clinical trial. 49 healthy adults were enrolled and randomized to receive AGS-v with and without adjuvant (Montanide ISA 51) versus placebo to evaluate safety and durability of responses. AGS-v was well tolerated. Adjuvanted AGS-v produced a saliva

peptide-specific humoral response, although reactogenicity and immunogenicity to AGS-v were increased in the group who also received the adjuvant. These findings suggest that vector-targeted vaccine administration in humans is safe and can be a viable option for the growing burden of vector-borne disease.

Dr. Yoshimasa Takahashi (NIID, Japan) presented on broadly protective antibodies and vaccines. Several viruses, including influenza, escape from antibody surveillance through antigenic variation; however, the humoral immune responses counteract this viral strategy by inducing broadly protective antibodies. The Takahashi lab performed high-throughput screening of broadly protective HA IgG antibodies from humans to identify antibodies to serve as key targets for universal influenza vaccines. Multiple classes of broadly protective IgG antibodies were isolated. Animal studies demonstrated that immunization with post-translationally modified HA antigens elicits broadly protective IgG responses, which suggests the feasibility of utilizing modified HA antigens as vaccines.

Dr. Susan Baker (Loyola University, Chicago) closed the session with a presentation on how coronaviruses evade host sensors by trimming their RNA. Coronaviruses (CoVs) are positive-sense RNA viruses that can emerge from endemic reservoirs and infect zoonotically, causing significant morbidity and mortality. CoVs encode an endoribonuclease (EndoU) that facilitates evasion of host pattern recognition receptor MDA5, but the target of EndoU activity was not known. The Baker lab found that EndoU cleaves the 5'-Poly-Uridines from Negative-sense RNA, termed PUN RNA, which is the product of polyA-templated RNA synthesis. With additional experiments, it was shown that coronavirus PUN RNA is a novel MDA5-dependent pathogen-associated molecular pattern (PAMP) and a mechanism was established for EndoU activity to cleave and limit the activation from this novel PAMP. Because EndoU activity is highly conserved in all CoVs, inhibiting this activity is a potential therapeutic intervention for existing and emerging CoV infections.

6. Session 2: new diagnostics and therapeutic trials (including antivirals)

Session two focused on new diagnostics and therapeutic approaches to viral diseases. The session included four presentations that addressed the development of antivirals against HIV, HBV and norovirus infections, as well as molecular diagnostics using CRISPR/Cas technology.

Dr. Kiat Ruxrungtham (Chulalongkorn University, Bangkok) reviewed the development of anti-HIV treatment regimens in Thailand from the late 1980's to the present, emphasizing its impact in the dramatic decline of AIDS-related mortality and improved life expectancy. Current combined antiretroviral therapies (cART) formulated as well-tolerated, single pill regimens have dramatically improved adherence to life-long therapy. Newly approved HIV integrase inhibitors and long-acting antiretrovirals provide even more effective coverage for both treatment and prevention of HIV infections and provide the opportunity to meet the world-wide goal of ending the AIDS epidemic by 2030.

Dr. Sheikh Mohammad Fazle Akbar (Ehime University, Japan) discussed the development of a new therapeutic vaccine to treat chronic hepatitis B virus (HBV) infections. The vaccine, NASVAC, which contains HBV surface and core antigens, can be administered nasally or by inoculation. The vaccine has been in clinical trials since 2007, and newly adjuvanted formulations have proven more efficacious, providing the potential to replace nucleoside analogs for treatment of chronic HBV patients.

Dr. Kazuhiko Katayama (Kitasato University, Japan) presented on the development of therapeutics against human norovirus (HuNoV) infection, targeting cellular fucosyltransferase-2 (FUT2). Experiments using FUT2 knock-out cell lines demonstrated that fucosylation is an essential step for norovirus infection of human intestinal epithelium. Based on this finding, a FUT inhibitor was identified that reduces norovirus infection, paving the way for the development of potential new

therapeutics for norovirus-mediated gastroenteritis.

Dr. James Broughton (Mammoth Biosciences, USA) presented on the development of rapid and sensitive molecular diagnostics based on CRISPR/Cas enzymes. The research team has established a diagnostics platform that can detect virtually any genetic sequence of interest by applying the natural diversity of CRISPR. The method aims to produce programmable CRISPR-powered diagnostic tests that have the accuracy in an ultra-portable, low-cost format. Dr. Broughton reported that a rapid diagnostic test for 2019-SARS-CoV2 was being developed using this technology.

7. Session 3: transmission and pathogenesis of viral infections

Session three focused on the transmission and pathogenesis of viral infections, including vectors and neurological complications. This session comprised of four major presentations on HIV/SIV, Zika virus, hepatitis C virus, and rabies. Dr. Thomas Hope (Northwestern University, USA) discussed the use of a variety of imaging tools as applied to tissue slices to study SIV/HIV transmission, dissemination, reservoirs, and prevention. In particular non-invasive bioluminescent imaging (NIBLI) of HIV-1 infection dynamics allows real-time monitoring of viral spread and the localization of infected cell populations in living animals. Full-length replication-competent GFP and Nanoluciferase (Nluc) expression allows direct monitoring of longitudinal viral spread at whole-animal resolution via bioluminescence imaging. For correlative PET imaging, three probes were used: labeled antibodies to detect kinetics and distribution, labeled HIV/SIV for monitoring viral particle distribution, and labeled Fab to detect viral envelop protein. Dr. Hope emphasized the importance of understanding pathogenesis on the basis of anatomy and physiology for successful development of vaccines and therapeutics.

Dr. Padet Siriyasatien (Chulalongkorn University, Thailand), discussed his epic comprehensive survey of the diversity of Zika virus (ZIKV). ZIKV infections were first confirmed in Thailand in 1954 among indigenous residents. Several cases of ZIKV infection were reported in travelers returning from Thailand in 2014, and reported cases have been increasing in Thailand since 2015 and 2016., The vectors of ZIKV have not been determined. Dr. Siriyasatien investigated the molecular epidemiology and genetic diversity of ZIKV from mosquitoes collected in different geographic regions. Non-structural protein (NS5) gene of ZIKV was amplified by PCR, and sequenced. A total of 1026 mosquito samples (females, males, larvae) were collected from active ZIKV patients' houses in 31 provinces. ZIKV was detected in 79 samples (7.7%) in 15 provinces, including *Aedes aegypti*, *Culex quinquefasciatus*, and *Armigeres subalbatus*; no ZIKV was detected in *Aedes albopictus*. The genome sequences were classified into two clades: the sequences previously detected in Thailand, and those in the Americas. Detection of ZIKV in male and larval mosquitoes suggests that vertical transmission of ZIKV occurs in these mosquito species, which was demonstrated in laboratory *Cx. quinquefasciatus*. The study provides deep insight into the molecular epidemiology of ZIKV in Thailand. The data could also be used for the development of more effective prevention and control strategies of ZIKV in Thailand.

Dr. Andrea Cox (Johns Hopkins University, USA) presented lessons learned from the recently concluded human phase I and II hepatitis C virus (HCV) vaccine trials. Even after the introduction of highly efficacious antivirals, 54 out of 91 countries with available data on cure showed more new HCV cases than cures in 2016, dropping the net HCV infection rate of 69.6 million to 69.3 million (0.7%). Therefore, a safe and effective vaccine to prevent chronic HCV infection is essential to reduce transmission. The vaccines used for the human trial were a recombinant chimpanzee adenovirus-3 vector vaccine carrying HCV NS3-NS5B polyprotein, with a genetically inactivated NS5B polymerase (ChAd3-NSmut) prime followed by a recombinant modified vaccinia Ankara virus (MVA-NSmut) boost, both encoding HCV nonstructural proteins. Vaccine recipients induced robust T cell responses in healthy

volunteers. The safety and efficacy of this vaccine were tested in a randomized, multicenter, double-blind, placebo-controlled efficacy trial in 548 HCV uninfected adults (age 18 to 45 old) at-risk for HCV infection due to active injection drug use. The primary outcome assessed was progression to chronic HCV infection at 6 months. Secondary outcomes included HCV RNA change from incident infection and peak HCV RNA. Although there were no differences in the 6-month chronic infection rate between vaccine and placebo arms, significant differences were seen in HCV RNA geometric mean peak (GMP) between vaccine and placebo groups: GMP HCV RNA was 193,795 IU/L and 1,078,092 IU/L, respectively at 1 month. Dr. Cox concluded that detailed characterization of immune responses that lead to GMP HCV RNA blunting in this trial would likely enhance the design of vaccines encoding T cell antigens.

Dr. Thiravat Hemachudha (Chulalongkorn University, Thailand) focused his presentation on the neuropathogenesis, prophylaxis and treatment aspects of rabies. It is known that dogs with rabies can show the furious or paralytic form. There are greater amounts of virus in the nerve cell bodies in the furious form than in paralytic form. The virus spreads from nerve cell to nerve cell via connections between cell processes. The amount of rabies viral protein within nerve cell processes was measured in rabid dogs shortly after developing illness. The amount of viral protein in cell processes decreased in the brain compared with spinal cord, as did the amount of viral protein in cell bodies and the percentage of positive cell bodies. There was a delay in cell process involvement; early virus replication is in the cell body and later moves to the processes. Greater amounts of viral protein were detected in cell processes in dogs with the furious form compared to the paralytic form. These findings suggest that intracellular transport of the virus may be slower in paralytic rabies, resulting in slower viral spread in the brain. There is a need to develop diagnostic tools appropriate for use in countries with appreciable rabies cases. These include methods for viral isolation and for antigen detection with ELISA tools, dRIT, RIDT, and the gold standard FAT technique. Molecular diagnostic tools such as RTPCR, real-time PCR, or other techniques such as NASBA or LAMP should also be made available more widely for diagnosis of human cases in rabies-endemic countries.

8. Session 4: biology, natural history and prevention of oncogenic infections and their associated cancers

Session four focused on the biology, natural history and prevention of oncogenic infections and their associated cancers. The session included two lectures on human papillomavirus and two lectures on Epstein-Barr virus associated nasopharyngeal carcinoma.

The plenary and Cancer Panel lectures on human papillomavirus (HPV) centered on prevention of HPV-associated cancers. The biological basis for the high efficacy and effectiveness of HPV vaccines, apparently even after a single dose, was discussed by Dr. John Schiller (National Cancer Institute, USA). Contributing immunologic factors include the potent activation of B cells by multimeric engagement of their B cell receptors by the dense repetitively displayed epitopes on the virus-like particles that comprise the vaccine. Contributing virologic factors include the unique HPV infection mechanism of target tissues that makes the virions exceptionally susceptible to infection inhibition by low levels of antibodies. A central question for the future was whether the consistently strong and durable antibody responses observed against the HPV VLPs can be translated into virus-like display vaccines against other infectious diseases.

Dr. Julia Brotherton (VCS Foundation, Australia) summarized the remarkable population effectiveness of Australia's HPV vaccination program. Infection and disease manifestations, e.g. genital warts and cervical precancers, caused by the four HPV types targeted by Gardasil (the vaccine in general use in Australia until recently) have dramatically decreased in vaccinated subjects, to the point of virtual disappearance in some instances. In addition, major decreases in infection and disease in

unvaccinated females and males (who were not widely vaccinated until recently) in the vaccinated age cohorts provided evidence that strong herd immunity is being generated by the vaccination program. The central question raised by her presentations is how to achieve similar levels of vaccination coverage in the target age groups (9–14 years) in other countries, particularly lower income ones with high cancer burdens, and thereby achieve similar reductions in HPV infection and diseases.

Two lectures in the cancer session focused on efforts to accelerate the diagnosis of Epstein-Barr virus (EBV) associated nasopharyngeal carcinoma (NPC). NPC is typically diagnosed at late stages resulting in high mortality. Results from studies that have evaluated EBV-related risk prediction markers have shown the promise of EBV-based screening in high-risk regions or among high-risk subgroups for early detection of NPC at a stage when treatments are highly effective and often curative.

Dr. Allan Hildesheim (National Cancer Institute, USA) summarized results from studies that have evaluated risk prediction models based on anti-EBV antibodies. Risk prediction models based on anti-EBV VCA and EBNA1 IgA antibody testing demonstrated good performance in a large, ongoing screening trial in Guangdong, China. Use of a broader panel of IgA and IgG antibodies covering EBV proteins involved in viral latency, switch from latency to lytic replication, and lytic productive infection was also shown to be highly accurate for NPC prediction in three independent studies conducted in Taiwan.

Dr. Ann King (Chinese University of Hong Kong) presented remotely results from a study that evaluated screening for EBV plasma DNA (liquid biopsy) in Hong Kong, where referral of screen-positive individuals to endoscopy and MRI was highly sensitive and specific for NPC and led to considerable down-staging of cases compared to the historical NPC stage distribution in Hong Kong. Dr. King also presented results from studies in Hong Kong and Guangdong, China, which highlighted the limitations of endoscopy alone for the diagnosis of NPC and the potential benefit of MRI to maximize detection of early-stage disease.

9. Session 5: microbiome, virome, and viral pathogenesis

Session five focused on the microbiome and virome in viral pathogenesis. The session included talks on the microbiome in relation to HIV and chikungunya virus infections and on the role of defective RNA virus genomes in pathogenesis.

Dr. Supriya Mehta (University of Illinois, Chicago, USA) spoke on the impact of host genetic determinants of the vaginal microbiome and bacterial vaginosis (BV) in Kenyan women. She noted that BV is associated with higher risk to sexually transmitted diseases, including HIV and HSV, as well as miscarriage and low birth weight. In a large single nucleotide polymorphism (SNP) study, the team identified toll-like receptor (TLR) and Interleukin signaling pathways and other immune regulatory loci associated with BV, suggesting a link between immune regulation of inflammation and BV. Dr. Mehta indicated that both genetic and environmental factors influence the risk for BV, and that application of live therapeutic bacteria may impact vaginal health and disease risk.

Dr. Kenneth Stapleford (New York University School of Medicine, USA) spoke on the impact of the mouse microbiome on chikungunya virus (CHIKV) infection and pathogenesis. He showed that germ free mice had higher viral titers than wildtype mice and this could be recapitulated by treating wild type mice with antibiotics. Moreover, he showed that the mouse microbiota was responsible for inflammation induced by CHIKV variants that had mutations in the E1 glycoprotein. Finally, he showed that even though inflammation was reduced in germ free mice, viral titers remained unchanged suggesting that the host microbiome is impacting inflammation independent of virus infection. Taken together, Dr. Stapleford highlighted the role of the mouse microbiome at distal sites of infection such as the footpad and future studies are underway to understand the mechanisms behind this.

Dr. Carolina López-Zalaquett (University of Pennsylvania, USA) presented her work on the role of defective genomic RNAs (gRNA) in virus infections. Defective viral genomes (DVG) are formed during genome replication and include point mutations, deletions and copy-back transcription, all of which result in non-infectious gRNA. DVGs can impact viral pathogenesis by interfering with viral replication, stimulating innate immune responses, or enhancing viral persistence. During Sendai virus infection, for example, Dr. Lopez showed that infected cells exhibit heterogeneity in the content of viral genomes in the infected population. Cells enriched in DVGs expressed interferons and other pro-inflammatory molecules and appeared to induce a pro-survival program, suggesting a mechanism for persistent viral infection. This work has provided new appreciation for DVG diversity and the role of DVGs in defining the clinical outcome of infections.

10. Flash talks

Since 2016, the USJCMSP Collaborative Awards initiative has funded collaborative infectious disease and immunology research among scientists in Japan, the Asia-Pacific region, and the United States. The awards have prioritized funding early-stage and female investigators and have enabled investigators to acquire additional research funding. Recipients for the USJCMSP Collaborative Awards Initiative presented their research in flash talk and poster sessions at the EID conference.

Several USJCMSP collaborative awards presentations highlighted important research on evaluating antibody-dependent enhancement (ADE) and modifying neutralizing antibodies for the purposes of vaccine development and cure for significant viral pathogens, such as HIV, dengue, and other flaviviruses. These approaches are critical to the development of new vaccines to emerging viral pathogens, like SARS-CoV-2. In the midst of a pandemic, scientists are well-equipped to exploit their expertise of other viral pathogens to characterize antibody responses and enhancement to vaccine and cure strategies.

The problem of latent HIV reservoirs has impeded development of a cure for HIV. To address this problem, Dr. Li and Dr. Huang collaborated to modify pharmacophores on gnidimacrin derivatives and to evaluate these compounds for their effects on HIV latency activation and replication. Building on findings on structure-activity relationships like the essential roles of the free C-5 and C-20 hydroxy in gnidimacrin compounds, further research is better positioned to develop gnidimacrin-like compounds that can inhibit HIV replication and latency activation in sight of an HIV cure.

Dr. Yamamoto's collaborative work advanced knowledge towards an HIV vaccine, evaluating broadly neutralizing antibodies (bNAbs) against CRF01_A/E envelope glycoproteins. Through their research, they established new effector cell lines with high affinity to CD16 for antibody-dependent cell-mediated cytotoxicity (ADCC) assays using natural killer cell lines. The researchers were able to identify effective and ineffective bNAbs against CRF01_A/E envelope glycoproteins and developed Fc mutants of selected bNAbs.

Among flaviviruses, there are three phylogenetic groups of E proteins. Dr. Takahashi and Dr. Sadun characterized neutralizing and antibody-dependent enhancement (ADE) activities of memory B-cell derived antibodies as the basis to search for cross-reactive neutralizing antibodies against flaviviruses to develop a vaccine. One identified novel IgG antibody did not show ADE activity, but conferred flavivirus protection in mice. Future research should characterize the epitope structures for this novel antibody.

Dr. Moi and Dr. Le built on the knowledge of immune responses to dengue infection with implications for improved vaccine development. They developed an ADE assay, determined the levels of ADE titers following the 2017 dengue-1 outbreak in Vietnam, and performed a B-cell receptor repertoire sequencing analysis for those with primary and secondary dengue infection. The scientists found that 50% of individuals with secondary dengue infection had high ADE titers, suggesting increased diversification of the immune response to dengue virus

infection.

Dr. Yoshimatsu-Morimatsu presented collaborative work on chronic kidney disease of unknown origin (CKDu). With increased rates of CKDu in Sri Lanka with almost 50% seropositive for hantavirus, they searched for possible reservoirs for this virus in *Rattus rattus*, *Bandicota* spp., and *Mus booduga*. Using indirect immunofluorescence for antibody detection in serum and RT-PCR for viral RNA on lung samples of rodent lung tissues, they identified a novel hantavirus, Lanka virus, with high viral copies and antibody titers in *Mus booduga*, which has typical characteristics for a virus reservoir. These findings prompt future work to focus on infectivity of the virus to humans and CKDu patients and potentially CKDu progression due to hantavirus infection.

11. Conclusion

Closing comments by organizers noted the outstanding breadth, depth, and scientific standard of presentations, the timeliness of the topics, and the remarkable value of the conference in the midst of what had come to be recognized during the meeting as a major pandemic.

Dr. Patrick Brennan (former Chairman of the USA Delegation of the USJCMSP) pointed out how impressed he had been by how Thai colleagues had reported on identifying their first cases of COVID-19 and dealing with them through diagnosis, contact tracing, clinical care, genetic, virologic and viral evolution research. He noted that he found equally profound an array of presentations made by scientists from Thailand, Cambodia, Japan and other participants. He concluded by noting that many of the presentations demonstrated the value of the historic USJCMSP and the importance of convening EID Conferences where findings of such importance can be shared.

In his concluding remarks Mr. Gray Handley (Associate Director for International Research Affairs, NIAID, NIH) thanked and honored all the individuals who had contributed to the success of the meeting and its safe convening. He also expressed the USJCMSP's appreciation for all the hard work done by the local hosts from Chulalongkorn University, the Thai Red Cross and the Ministry of Public Health, Thailand. He noted that the outstanding program was only possible through the dedicated efforts of the USJCMSP Japanese and USA Delegations, the EID Scientific Planning Committee and the Logistics Planning Committee. He

closed by thanking all the presenters and participants noting that their flexibility and willingness to share research findings quickly, particularly on COVID-19 work that is underway, made the meeting not only successful but historic.

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