Research Center for Asian Infectious Diseases
アジア感染症研究拠点

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Research Center for Asian Infectious Diseases has established three project laboratories (one in Tokyo; two joint labs in Beijing) and a collaborative program (Harbin), supported by AMED, CAS, and CAAS. The center is conducting research on emerging and reemerging infections, aiming to translate its basic studies into practical use. And the project intends to train and educate young Japanese and Chinese scientists for the future generation.

BACKGROUND

China is an important neighbor of Japan, with geopolitical and economic interdependence. And it contains hot spots for emerging and reemerging infections, as exemplified by the occurrence of SARS coronavirus that shocked the world in 2003 and endemic avian influenza virus occasionally jumping from bird to human. The carrier rate of hepatitis viruses is very high and HIV infection is rapidly increasing. In the early 2000's the Institute of Medical Science, the University of Tokyo, (IMSUT) was looking for appropriate counterparts in China to strengthen the studies of emerging and reemerging infections.

IMSUT established three collaboration sites in fiscal 2005 in China, two in Beijing and one in Harbin, and had been conducting China-Japan research collaboration, for two 5-year terms (fiscal 2005-2010; 2010-2015), supported by the Ministry of Education, Culture, Sports, Science and Technology under the directorship of Aikichi Iwamoto, former project director. IMSUT thus set up a new sustainable system that allowed IMSUT scientists to work in China, along with Chinese scientists, focusing on the studies of emerging and reemerging infections. In 2015 Yasushi Kawaguchi succeeded A. Iwamoto as project director and launched the project *China-Japan Research Collaboration on Defense against Emerging and Reemerging Infections*, a new 5-year J-GRID program of Japan Agency for Medical Research and Development (AMED).

In 2005 IMSUT had founded two joint laboratories in collaboration with the Institute of Biophysics (IBP) and Institute of Microbiology (IM), which belong to the Chinese Academy of Sciences (CAS), a large national institution consisting of more than 100 research institutes all over China. IMSUT has dispatched Zene Matsuda and Takaomi Ishida to IBP and IM, respectively, as principal investigators (PIs). Along with their Japanese and Chinese staffs, these PIs are conducting basic and translational studies of HIV, MERS coronavirus, dengue virus and norovirus. In 2015 IMSUT has set up another project laboratory in Tokyo, whose studies complement those in Beijing. The activities of the three laboratories are under Jun-ichiro Inoue's direction. IMSUT is also conducting a joint research program

on avian influenza virus between Yoshihiro Kawaoka at IMSUT and Hualan Chen at the Harbin Veterinary Research Institute (HVRI) of Chinese Academy of Agricultural Sciences. The activities in Beijing and Harbin are supported by Mitsue Hayashi of the Beijing Project Office.

This project, making the most of the opportunity of collaboration with the highly advanced Chinese institution, aims to translate our basic studies into practical use in future. During the course of the collaboration the project intends to train and educate young Chinese and Japanese scientists for the future generation and hopes to contribute to the friendship between the two peoples.

PROJECT LABORATORIES AND PROGRAM

Y. Kawaguchi (Director of Research Center for Asian Infectious Diseases; Project Director) manages the Center and the AMED-supported Project, which includes the domestic and overseas laboratories and program. He coordinates their activities and decides the direction of research. He and his group conduct studies of molecular virology and immunology of herpes virus in the Research Center for Asian Infectious Diseases.

The project issued a press release August 23, 2016: J. Inoue, M. Yamamoto, Z. Matsuda and their project co-workers identified nafamostat as a potent inhibitor of MERS corona virus S-mediated membrane fusion; they found that nafamostat blocks the virus infection *in vitro*, suggesting it can be used for treatment of MERS. The paper was published August 22, 2016. For details (in Japanese), refer to URL (http://www.ims.u-tokyo.ac.jp/imsut/files/160822.pdf). For the abstract of the paper (No.9 of the publication list), refer to URL (http://aac.asm.org/content/early/2016/08/09/AAC.01043-16.abstract).

a. Project Laboratory at IMSUT

J. Inoue and his group at IMSUT are trying to find small molecular weight compounds that inhibit the membrane fusion caused by emerging viruses such as HIV-1, MERS coronavirus (MERS-CoV) and Dengue virus (DENV), in close collaboration with Matsuda's group at IBPCAS (see below). For MERS-CoV, they developed a cell-based fusion assay for MERS S protein in a TMPRSS2-dependent manner using cell lines expressing *Renilla* luciferase (RL)-based split reporter proteins and optimized for a 384-well format. Nafamostat, a serine protease inhibitor, was identified as a potent inhibitor of MERS S-mediated membrane fusion in a screening of about 1000 drugs approved for use by the US Food and Drug Administration. Nafamostat also blocked MERS-CoV infection *in vitro*. For HIV, they also developed cell-based fusion assays for HXB2-env (CXCR4-tropic) and JRFL-env (CCR5-tropic). Using these assays, they tried to find specific inhibitors for HXB2-env (CXCR4-tropic) in a screening of 9600 compounds from Drug Discovery Initiative, The University of Tokyo, and found a candidate compound. For DENV, they are establishing the cell fusion assay for a 384-well format.

b. Joint Laboratory at IBPCAS

In close collaboration with J. Inoue's group, Z. Matsuda and his group at IBPCAS have established cell-cell fusion assay systems based on DSP for HIV-1, MERS-CoV and Flaviviruses including DENV. The application of the DSP assay to MERS-CoV led to the identification of nafamostat, a clinically available serine protease inhibitor, as a potential drug for the treatment of MERS. In IBP, they are also conducting research on structure-function relationship of the viral envelope proteins derived from these viruses to develop peptide inhibitors of membrane fusion.

c. Joint Laboratory at IMCAS

T. Ishida and his group are studying the mechanism of HIV-1 latent infection and established model cell lines harboring the HIV-1 provirus. Using these cell lines, they have started screening for potential activators of the latently infected HIV-1. Such an activator can be applied to the "Shock and Kill" strategy for purging and eradicating HIV-1 from the latently infected cells.

d. Collaborative research program with HVRI

Since 2013, avian influenza A virus of the H7N9 subtype (A(H7N9)) have caused sporadic infections in humans in China. In 2009, the novel influenza "pandemic (H1N1) 2009" emerged and spread rapidly throughout the world. In addition, since 2003, highly pathogenic avian H5N1 influenza viruses have continued to cause unprecedented global outbreaks with high case fatality rates in humans. For these reasons, HVRI (Director, Zhigao Bu) has been conducting collaborative research on influenza virus isolates from all over Asia.

HVRI focuses on avian influenza viruses (AIVs) that are circulating in Chinese wild waterfowl, domestic poultry, and swine. Specifically, Y. Kawaoka and his group study type A influenza viruses from wild birds, waterfowl, poultry, and swine, with an emphasis on viral pathogenicity in various hosts, viral evolution, and viral prevalence.

Their major findings this year include: *(1) Selection of antigenically advanced variants of seasonal influenza viruses*. To establish experimental approaches to support the current vaccine strain selection process, they selected antigenic variants from human H1N1 and H3N2 virus libraries possessing random

mutations at the antigenic sites of hemagglutinin protein by incubating them incubating with human and/or ferret convalescent sera to human H1N1 and H3N2 viruses. They also selected antigenic escape variants from human viruses treated with convalescent sera and from mice that had been previously immunized against human influenza viruses. Their pilot studies with past influenza viruses identified escape mutants that were antigenically similar to variants that emerged in nature, establishing the feasibility of their approach. Their studies with contemporary human influenza viruses identified escape mutants before they caused an epidemic in 2014-2015. This approach may aid in the prediction of potential antigenic escape variants and the selection of future vaccine candidates before they become widespread in nature. *(2) Risk assessment of recent H5N1 viruses*. Y. Kawaoka's group tested nine naturally occurring Egyptian H5N1 viruses (isolated in 2014-2015) in ferrets and found that three of them transmitted via respiratory droplets, causing a fatal infection in one of the exposed animals. All isolates were sensitive to neuraminidase inhibitors. However, these viruses were not transmitted via respiratory droplets in three additional transmission experiments in ferrets. Currently, they do not know if the efficiency of transmission is very low or if subtle differences in experimental parameters contributed to these inconsistent results. Nonetheless, their findings heighten concern regarding the pandemic potential of recent Egyptian H5N1 influenza viruses.

IMSUT PROJECT OFFICE

The office (M. Hayashi) supports the activities of the two joint laboratories in Beijing and one joint research program in Harbin. It serves as Secretariat for Steering Committee Meeting and files MOU and Minutes. It helps scientists visiting the joint laboratories and program for collaborative research. It has been gathering the information about emerging infections in China from the Chinese mass media and official announcements, and the gathered information (in Japanese) has been presented and updated on the website of the Project (http://www.rcaid.jp/).

Publications

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