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

| | T | | |
|---|--------------|----|---|
| Professor Jun-ichiro Inoue, Ph.D. 教授(兼務) 薬学博士 井 | Ľ. | 純一 | 郎 |
| Professor Yoshihiro Kawaoka, D.V.M., Ph.D. 教授(兼務) 獣医学博士 河 | 畄 | 義 | 裕 |
| Project Professor Mitsue Hayashi, Ph.D. 特任教授 法学博士 林 | | 光 | 江 |
| Project Professor Zene Matsuda, M.D., Ph.D., D.Sc. 特任教授 医学博士 松 | \mathbb{H} | 善 | 衛 |
| Project Associate Professor Takaomi Ishida, Ph.D. 特任准教授 博士(医学) 石 | \mathbb{H} | 尚 | 臣 |
| Project Associate Professor Seiya Yamayoshi, D.V.M., Ph.D. 特任准教授 博士(医学) 山 | 吉 | 誠 | 也 |
| Project Senior Assistant Professor Jin Gohda, Ph.D. 特任講師 博士(薬学) 合 | \mathbb{H} | | 仁 |
| Assistant Professor Mizuki Yamamoto, Ph.D. 助教(兼務) 博士(医学) 山 | 本 | 瑞 | 生 |
| Assistant Professor Akihisa Kato, Ph.D. 助教(兼務) 博士(医学) 加 | 藤 | 哲 | 久 |
| Assistant Professor Jun Arii, Ph.D. 助教(兼務) 博士(獣医学) 有 | 井 | 2 | 潤 |
| Assistant Professor Naoto Koyanagi, Ph.D. 助教(兼務) 博士(生命科学) 小 | 栁 | 直 | 人 |

Research Center for Asian Infectious Diseases has established three project joint 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.

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 Dengue virus (DENV) and Zika virus (ZIKV), in close collaboration with Z. Matsuda's group at IBPCAS (see below). For DENV and ZIKV, they developed a cell-based fusion assay for prME protein in a low pH-dependent manner, using Aedes albopictus cell line C6/36 cells expressing Renilla luciferase (RL)-based split reporter proteins, and optimized it for a 384-well format. Using these assays, they screened last year 1,017 FDA-approved drugs (ref. Annual Report 2016) for ZIKV inhibitors, obtaining a candidate compound. In addition, they screened this year 130,000 compounds from Drug Discovery Initiative, The University of Tokyo, to find lead compounds that could be developed to therapeutic drugs for ZIKV infection, obtaining several candidate compounds.

b. Joint Laboratory at IBPCAS

Z. Matsuda and his group at IBPCAS are conducting research on structure-function relationship

of the viral envelope proteins derived from HIV-1 and dengue virus (DENV) to develop peptide inhibitors of their membrane fusion. They have developed a prokaryotic system to screen the peptides that bind to the peptides derived from viral envelope proteins. They continued their structure-function relationship analysis of the distal portion of Cterminal heptad repeat (CHR) of the HIV-1 gp41 subunit. CHR is known for its importance in membrane fusion and CHR-derived peptide is in clinical use. In this study, they identified the critical region of CHR for cell-cell fusion. They are collaborating with J. Inoue's group to develop a new quantitative virus-cell infection assay of HIV-1. Their collaboration is ongoing also in the study of DENV envelope protein and screening of its potential inhibitors.

c. Joint Laboratory at IMCAS

The latently infected HIV-1 cannot be eradicated from the patient body with current combination anti-retroviral therapy (cART) alone. For a sterilizing cure of HIV-1 infected patients, the "Shock and Kill" strategy that involves the purge of HIV-1 from reservoir by re-activation under the cART has been proposed. For studying the mechanism of latent HIV-1 infection, T. Ishida and his group established model cell lines harboring latent HIV-1 provirus. With these model cell lines, they identified several potential activators of the latent HIV-1. They are also trying to identify the cellular factors involved in latent infection of HIV-1, using the same model cell lines.

d. Collaborative research program with HVRI

Since 2013, avian influenza A viruses of the H7N 9 subtype (H7N9) have caused sporadic infections in humans in China. In addition, in 2016, highly pathogenic avian influenza (HPAI) H7N9 viruses emerged raising concerns of a pandemic. In 2009, the novel influenza "pandemic (H1N1) 2009" emerged and spread rapidly throughout the world. In addition, since 2003, HPAI H5N1 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 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) Enhanced Replication of HPAI H7N9 virus in humans. To clarify the threat posed by the emergence of HPAI H7N9 virus infections among humans, Kawaoka's group characterized the viral polymerase complex. The PB2-482R, PB2-588V, and PA-479R mutations individually or additively enhanced viral polymerase activity, indicating that multiple replicationenhancing mutations in one isolate may contribute to virulence. These double substitutions could have an additive effect on virulence enhancement, suggesting that in mammalian hosts, these double-mutant viruses may be fitter than single-mutant viruses. Therefore, future H7N9 virus surveillance studies should take into consideration single markers and combinations of markers. (2) Effectiveness of whole, inactivated low pathogenic avian influenza (LPAI) H7N9 vaccine against antigenically distinct HPAI H7N9 virus. The recent emergence of HPAI H 7N9 variants poses a great risk to humans. Kawaoka's group showed that ferrets vaccinated with LPAI H7N9 virus vaccine do not develop severe symptoms after infection with an antigenically distinct HPAI H7N9 virus. These results demonstrate the protective benefits of this H7N9 vaccine.

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

- Sato R, Kato A, Chimura T, Saitoh S-I, Shibata T, Murakami Y, Fukui R, Liu K, Zhang Y, Arii J, Sun-Wada G-H, Wada Y, Ikenoue T, Barber G. N, Manabe T, Kawaguchi Y, Miyake K. Combating herpesvirus encephalitis by potentiating a TLR3-mTORC2 axis. Nat Immunol 19: 1071-1082, 2018.
- Arii J, Watanabe M, Maeda F, Tokai-Nishizumi N, Chihara T, Miura M, Maruzuru Y, Koyanagi N, Kato A, Kawaguchi Y. ESCRT-III mediates budding across the inner nuclear membrane and regulates its integrity. Nat Commun 9: 3379, 2018.
- Kato A, Oda S, Watanabe M, Oyama M, Kozuka-Hata H, Koyanagi N, Maruzuru Y, Arii J, Kawaguchi Y. Roles of the phosphorylation of herpes simplex virus 1 UL51 at a specific site in viral replication and pathogenicity. J Virol 92: e 01035-18, 2018.
- Koyanagi N, Kato A, Takeshima K, Maruzuru Y, Kozuka-Hata H, Oyama M, Arii J, Kawaguchi Y. Regulation of herpes simplex virus 2 protein kinase UL13 by phosphorylation and its role in viral pathogenesis. J Virol 92: e 00807-18, 2018.
- Maruzuru Y, Ichinohe T, Sato R, Miyake K, Okano T, Suzuki T, Koshiba T, Koyanagi N, Tsuda S, Watanabe M, Arii J, Kato A, Kawaguchi Y. Herpes simplex virus 1 VP22 inhibits AIM2-dependent inflammasome activation to enable efficient viral replication. Cell Host & Microbe 23: 254-265, 2018.
- 6. Kawaguchi Y, Mori Y, Kimura H. Human herpesviruses. Adv Exp Med Biol 1045, 2018.
- Kato A, Kawaguchi Y. Us3 protein kinase encoded by HSV: the precise function and mechanism on viral life cycle. Adv Exp Med Biol 1045:

45-62, 2018.

- 8. Arii J, Kawaguchi Y. The role of herpes simplex virus glycoproteins in mediating cell entry. Adv Exp Med Biol 1045: 3-21, 2018.
- Liu D, Wang H, Yamamoto M, Song J, Zhang R, Du Q, Kawaguchi Y, Inoue J, Matsuda Z. Sixhelix bundle completion in the distal C-terminal heptad repeat region of gp41 is required for efficient human immunodeficiency virus type 1 infection. Retrovirology 15: 27, 2018.
- Gohda J, Suzuki K, Liu K, Xie X, Takeuchi H, Inoue J, Kawaguchi Y, Ishida T. BI-2536 and BI-6727, dual Polo-like kinase/bromodomain inhibitors, effectively reactivate latent HIV-1. Scientific Reports 8: 3521, 2018.
- Hatta M, Zhong G, Chiba S, Lopes TJS, Neumann G, Kawaoka Y. Effectiveness of whole, inactivated, low pathogenicity influenza A(H7N9) vaccine against antigenically distinct, highly pathogenic H7N9 virus. Emerg Infect Dis 24: 1910-1913, 2018.
- Chiba S, Hill-Batorski L, Neumann G, Kawaoka Y. The cellular DExD/H-Box RNA helicase UAP 56 co-localizes with the influenza A virus NS1 protein. Front Microbiol 9: 2192, 2018.
- 13. Wu NC, Yamayoshi S, Ito M, Uraki R, Kawaoka Y, Wilson IA. Recurring and adaptable binding motifs in broadly neutralizing antibodies to influenza virus are encoded on the D3-9 segment of the Ig gene. Cell Host Microbe 24: 569-578, 2018.
- 14. Imai H, Dinis JM, Zhong G, Moncla LH, Lopes TJS, McBride R, Thompson AJ, Peng W, Le MTQ, Hanson A, Lauck M, Sakai-Tagawa Y, Yamada S, Eggenberger J, O'Connor DH, Suzuki Y, Hatta M, Paulson JC, Neumann G, Friedrich TC, Kawaoka Y. Diversity of influenza A(H5N1)

Viruses in infected humans, Northern Vietnam, 2004-2010. Emerg Infect Dis 24: 1128-1238, 2018.

- 15. Ueki H, Wang IH, Fukuyama S, Katsura H, da Silva Lopes TJ, Neumann G, Kawaoka Y. In vivo imaging of the pathophysiological changes and neutrophil dynamics in influenza virus-infected mouse lungs. Proc Natl Acad Sci USA 115: E6622-E6629, 2018.
- 16. Oishi K, Yamayoshi S, Kozuka-Hata H, Oyama M, Kawaoka Y. N-terminal acetylation by NatB is required for the shutoff activity of influenza A virus PA-X. Cell Rep 24(4): 851-860, 2018.
- Furusawa Y, Yamada S, Kawaoka Y. Host factor nucleoporin 93 is involved in the nuclear export of influenza virus RNA. Front Microbiol 9: 1675, 2018.
- 18. Iwatsuki-Horimoto K, Shi J, Wang X, Sakai-Tagawa Y, Ito M, Murakami K, da Silva Lopes TJ, Nakaishi K, Yamayoshi S, Watabe S, Chen H, Kawaoka Y. Development of an influenza rapid diagnostic kit specific for the H7 subtype. Front Microbiol 9: 1346, 2018.
- Sammaibashi S, Yamayoshi S, Kawaoka Y. Strain-specific contribution of eukaryotic elongation factor 1 gamma to the translation of influenza A virus proteins. Front Microbiol 9: 1446, 2018.
- 20. Iwatsuki-Horimoto K, Nakajima N, Kiso M, Takahashi K, Ito M, Inoue T, Horiuchi M, Okahara N, Sasaki E, Hasegawa H, Kawaoka Y. The marmoset as an animal model of influenza: infection with A(H1N1)pdm09 and highly pathogenic A(H5N1) viruses via the conventional or tracheal spray route. Front Microbiol 9: 844, 2018.
- Matsui K, Ozawa M, Kiso M, Yamashita M, Maekawa T, Kubota M, Sugano S, Kawaoka Y. Stimulation of alpha2-adrenergic receptors impairs influenza virus infection. Sci Rep 8: 4631, 2018.
- 22. Soni P, Yasuhara A, Takenaga T, Iwatsuki-Horimoto K, Uraki R, Ito M, Sasaki T, Ikuta K, Yamayoshi S, Kawaoka Y. Evaluation of the fusion partner cell line SPYMEG for obtaining human monoclonal antibodies against influenza B virus. J Vet Med Sci 80: 1020-1024, 2018.
- 23. Maemura T, Fukuyama S, Sugita Y, Lopes TJS, Nakao T, Noda T, Kawaoka Y. Lung-derived exosomal miR-483-3p regulates the innate im-

mune response to influenza virus infection. J Infect Dis 217: 1372-1382, 2018.

- 24. Yamayoshi S, Kiso M, Yasuhara A, Ito M, Shu Y, Kawaoka Y. Identification of amino acids that enhance replication in mammalian hosts in the PB2 and PA proteins of a highly pathogenic H7 N9 influenza virus isolated from a human. Emerg Infect Dis 24: 746-750, 2018.
- 25. Watanabe T, Iwatsuki-Horimoto K, Kiso M, Nakajima N, Takahashi K, Lops TJS, Ito M, Fukuyama S, Hasegawa H, Kawaoka Y. Experimental infection of Cynomolgus Macaques with highly pathogenic H5N1 influenza virus through the aerosol route. Sci Rep 8: 4801, 2018.
- 26. Kiso M, Lopes TJS, Yamayoshi S, Ito M, Yamashita M, Nakajima N, Hasegawa H, Neumann G, Kawaoka Y. Combination therapy with neuraminidase and polymerase inhibitors in nude mice infected with influenza virus. J Infect Dis 217: 887-896, 2018.
- 27. Iwatsuki-Horimoto K, Nakajima N, Ichiko Y, Sakai-Tagawa Y, Noda T, Hasegawa H, Kawaoka Y. Syrian hamster as an animal model for the study of human influenza virus infection. J Virol 92: e01693-17, 2018.
- 28. Zhong G, Le MQ, Lopes TJS, Halfmann P, Hatta M, Fan S, Neumann G, Kawaoka Y. Mutations in the PA protein of avian H5N1 influenza viruses affect polymerase activity and mouse virulence. J Virol 92: e01557-17, 2018.
- 29. Yamayoshi S, Ito M, Uraki R, Sasaki T, Ikuta K, Kawaoka Y. Human protective monoclonal antibodies against the HA stem of group 2 HAs derived from an H3N2 virus-infected human. J Infect 76: 177-185, 2018.
- 30. Oishi K, Yamayoshi S, Kawaoka Y. Identification of novel amino acid residues of influenza virus PA-X that are important for PA-X shutoff activity by using yeast. Virology 516: 71-75, 2018.
- 31. Hatta M, Zhong G, Gao Y, Nakajima N, Fan S, Chiba S, Deering KM, Ito M, Imai M, Kiso M, Nakatsu S, Lopes TJ, Thompson AJ, McBride R, Suarez DL, Macken CA, Sugita S, Neumann G, Hasegawa H, Paulson JC, Toohey-Kurth KL, Kawaoka Y. Characterization of a feline influenza A(H7N2) virus. Emerg Infect Dis 24: 75-86, 2018.