Center for Asian Infectious Diseases

IMSUT Research Center for Infectious Diseases in China

中国における感染症研究拠点

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The Institute of Medical Science, University of Tokyo (IMSUT) has established Japan-China joint laboratories for research on emerging and re-emerging infectious diseases in Asia, in collaboration with the Chinese Academy of Sciences and Chinese Academy of Agricultural Sciences. In the laboratories, Japanese and Chinese scientists conduct research on the viral pathogenicity, the genetic variation of viruses in the field, the structure-function relationship of viral proteins, and so on.

BACKGROUND

Historically, China is a very important neighbor of Japan. Official diplomatic delegations were first sent from Japan during the Sui dynasty some 1400 years ago. Since late 20th century, geopolitical and economic interdependence between Japan and China has developed substantially and will deepen further in the future. China is an enormous country often symbolically referred to as the dragon. While China is developing and transforming rapidly in the coastal regions, its rural areas have been left far behind. With regard to infectious diseases, China is beset with problems ranging widely from those of a developing country to those of a dense urban environment. No one can discuss emerging and re-emerging infectious diseases without mentioning China. Severe acute respiratory syndrome (SARS) emerged in Guangdong and shocked the world in 2003. With Lake Qinghai as a reference point, avian influenza expanded westward in the Eurasian continent in 2005 and reached Africa in February 2006. The carrier rate of hepatitis viruses is very high and HIV infection is rapidly increasing.

Given these situations, academic collaboration on research in infectious diseases would be beneficial to both countries, facilitate mutual understanding, and help strengthen the stable long-term relationship between the two peoples. Establishing joint research laboratories in China is particularly important because this would allow Japanese scientists access to possible emerging pathogens and to have an opportunity to fight against possible emerging infections. Supported by a contract research fund from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) (*Japan-China Collaboration on Emerging and Re-emerging Infectious Diseases; MEXT Project Director: Aikichi Iwamoto*),

IMSUT established in 2006 two joint laboratories in Beijing in collaboration with the Institute of Biophysics and Institute of Microbiology, Chinese Academy of Sciences (IBPCAS and IMCAS, respectively); a collaborative research program with the Harbin Veterinary Research Institute (HVRI), the Chinese Academy of Agricultural Science; and IMSUT's project office in Beijing. The collaborating Chinese institutions are conducting highly advanced research on infections in their characteristic ways. This five-year project (fiscal 2005-2009) successfully ended in March 2010 (the academic activities are summarized in a brochure available from the Project Office at IMSUT or Beijing) and entered into the second term (fiscal 2010-2014) from April 2010.

Fiscal 2014 was the last year of the five-year second term of the project, so the annual workshop of *the China-Japan Joint Laboratory Workshop 2014: Pathogenesis, Gene Regulation and Signal Transduction* (organized by J. Inoue and Z. Matsuda) was held on 15 November at IBP with a somewhat festive mood for the 10th anniversary of the collaboration between China and Japan. PIs (Z. Matsuda and T. Ishida) of the joint laboratories presented at the meeting their summaries of five-year research, which were subjected to review by a committee (chaired by J. Inoue) including IMSUT professors. Their research consisted of molecular studies of HIV-1 and the molecular and epidemiological studies of HIV-1 and HBV using clinical specimens from the cohort of Chinese patients (in collaboration with Peking Union Medical College Hospital). During 2014, cooperation between Kumamoto University and the joint laboratory at IBPCAS continued as before; a group of IMSUT faculty members was conducting some studies to support the two joint laboratories in Beijing. The joint-lab seminars of the two laboratories were convened on 8 May 2014 in IBPCAS, 9 July 2014 in IMCAS, and 23 September 2014 in IBPCAS for discussion of ongoing studies between the laboratory members. Furthermore, in the second term, the collaborative research program with HVRI has contributed to revealing the molecular evolution of avian influenza virus in nature and its associated pathogenicity.

China contains hot spots for emerging and re-emerging infections, as exemplified by the high carrier rate of hepatitis virus, rapidly increasing HIV/AIDS, the occurrence of SARS, and epidemics of avian influenza. For various reasons, China is also at risk of new influenza pandemics. The outcome of the joint research conducted within this region should provide a useful basis for treating and preventing some of those diseases and for predicting their possible pandemics not only in China but for all of Asia.

LABORATORIES AND PROJECT OFFICE

a. Laboratory of Structural Virology and Immunology (LSVI), IBPCAS

We (Z. Matsuda's group in LSVI) have been studying the structure-function relationship of the viral envelope proteins to elucidate the mechanism of membrane fusion. The class I fusion protein such as HIV-1 envelope protein is known to form a quaternary structure called six helix bundle (6HB) during the membrane fusion process. To examine the relationship between the stability of the 6HB and efficiency of membrane fusion in HIV-1 envelope protein, we have constructed an assay system to measure the degree of the peptide-peptide interaction in bacteria. The system is based on a recovery of the activity of a pair of split luciferase attached to the peptides of interest derived from HIV-1 envelope protein. The strength of the association is evaluated by the value of the recovered luciferase activity. We are currently evaluating several mutant peptides in this system. We are also extending our analysis of the structure-function relationship to another class I envelope protein, the envelope protein of MERS corona virus.

b. Laboratory of Molecular Immunology and Molecular Microbiology (LMIMM), IMCAS

In LMIMM we (T. Ishida's research group) have been focusing on two research areas on HIV-1 infection: molecular epidemiology and cell biology. The former was done in collaboration with Professor T. Li of Peking Union Medical College Hospital. *Molecular epidemiology:* The co-receptor usage of HIV-1 derived from Chinese patients was examined by two methods, genotyping and phenotyping, to determine the current situation in China. Total 536 patients were analyzed. The genotype analysis showed that 65% of the CRF01_AE virus used CXCR4 (X4) as their co-receptor (232 patients were classified in CRF01_AE). The same samples showed only less than 20% of X4 usage in the phenotypic analysis. Such a discrepancy was, however, not detected in the subtype B viruses and CRF07/08_BC; both showed a very low prevalence of X4 usage (4%). Thus, it is important to be aware of the possible discrepancy in the treatment of HIV-1 infection. *Cell biology:* In 2013 we showed that the R5-tropic HIV-1 can infect osteoclasts and enhance their differentiation; in 2014 we showed that our findings hold true with the X4-tropic HIV-1. Also, we showed that both HIV-1s are able to up-regulate the osteoclast bone resorption activity. Taken together, our findings suggest that HIV-1 infection may be directly linked to the bone disorder observed in HIV-1 infected patients.

c. Collaborative research program with HVRI

In March 2013, several individuals were reported to be infected with an avian influenza A virus of the H7N9 subtype (A(H7N9)) in China. The sporadic infections with A(H7N9) have caused concern because of the appreciable case fatality rate associated with these infections, potential instances of human-to-human transmission, and the lack of pre-existing immunity among humans to viruses of this subtype. In 2009, the novel influenza "pandemic (H1N1) 2009 (pdmH1N1)" 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, Xiangang Kong) 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 and domestic poultry. Specifically, we (Y. Kawaoka's group) study type A influenza viruses from wild birds, waterfowl, poultry, swine, and horses, with an emphasis on viral pathogenicity in various hosts, viral evolution, and viral prevalence.

Our major findings this year include: (*1) Analyses of the mechanisms via which H5N1 viruses induce severe disease in humans.* We infected cynomologus macaques with six different H5N1 strains isolated from human patients and compare virus pathogenicity and host responses to infection. The results suggested that attenuated interferon-induced activation of innate immunity, apoptosis, and antigen presentation in the early phase of H5N1 virus infection lead to severe disease. *(2) Identification of amino acid changes in the PA protein that attenuate avian H5N1 viruses in mammals.* We reported that the PA proteins of several highly pathogenic avian H5N1 viruses have attenuating properties in mammalian cells and that the attenuating phenotype is conferred by strain-specific amino acid changes. Specifically, one amino acid change in PA induced strongly attenuating effects in vitro and in vivo. More importantly, the introduction of an attenuating residue commonly found in PA significantly increased the viral polymerase activity in mammalian cells as well as virus virulence and pathogenicity in mice. These findings demonstrate that the PA protein plays an important role in influenza virulence and pathogenicity.

d. IMSUT Project Office

The office (M. Hayashi) has been supporting the activities of the two joint laboratories in Beijing and one joint program in Harbin. It served as Secretariat for Steering Committee Meeting and has filed MOU and Minutes. It helped 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 has been presented and updated in Japanese on the website of the Project (http://www.rcaid.jp/).

Since the first three cases of critical human infection with H7N9 avian influenza virus were reported in China on March 31 2013, M. Hayashi has been collecting the relevant information in China and translated it into Japanese day by day. The H7N9 information in Japanese has been released to the public immediately on the project website.

IMPLEMENTATION OF COLLABORATION

The collaboration was implemented, being based on the renewed MOUs between IMSUT and the Chinese institutes. For the joint laboratories the implementation was controlled by the steering committee consisting of H. Kiyono, A. Iwamoto, S.J. Liu (L. Huang, until August 2013), and T. Xu. The collaborative program in Harbin was implemented by the steering committee consisting of H. Kiyono, Y. Kawaoka, X. Kong, and H. Chen.

Publications

1. Wang H, Li X, Nakane S, Liu S, Ishikawa H, Iwamoto A, Matsuda Z. Co-expression of foreign proteins tethered to HIV-1 envelope glycoprotein on the cell surface by introducing an intervening second membrane-spanning domain. PLoS ONE 9(5): e96790, 2014.
2. Nakayama-Hosoya K, Ishida T, Youngblood B, Nakamura H, Hosoya N, Koga M, Koibuchi T, Iwamoto A, Kawana-Tachikawa A. Epigenetic repression of interleukin 2 expression in senescent CD4+ T cells during chronic HIV type 1 infection. J Infect Dis 211: 28-39, 2015.
3. Li Y, Gu L, Han Y, Xie J, Wang H, Lv W, Song X, Li Y, Iwamoto A, Ishida T, Li T. HIV-1 subtype B/B' and baseline drug resistance mutation are associated with virologic failure: a multicenter cohort study in China. J Acquir Immune Defic Syndr, in press.
4. Gu L, Kawana-Tachikawa A, Shiino T, Nakamura H, Koga M, Kikuchi T, Adach E, Koibuchi T, Ishida T, Gao GF, Matsushita M, Sugiura W, Iwamoto A, Hosoya N. Development and customization of a color-coded microbeads-based assay for drug resistance in HIV-1 re-

verse transcriptase. PLos ONE 9: e09823, 2014.
5. Sakai-Tagawa Y, Ozawa M, Yamada S, Uchida Y, Saito T, Takahashi K, Sugaya N, Tashiro M, Kawaoka Y. Detection sensitivity of influenza rapid diagnostic tests. Microbiol Immunol 58: 600-606, 2014.
6. Fan S, Hatta M, Kim JH, Halfmann P, Imai M, Macken CA, Le MQ, Nguyen T, Neumann G, Kawaoka Y. Novel residues in avian influenza virus PB2 protein affect virulence in mammalian hosts. Nat Commun 5: 5021, 2014.
7. Fan S, Hatta M, Kim JH, Le MQ, Neumann G, Kawaoka Y. Amino Acid Changes in the Influenza A Virus PA Protein That Attenuate Avian H5N1 Viruses in Mammals. J Virol 88: 13737-13746, 2014.
8. Watanabe T, Zhong G, Russell CA, Nakajima N, Hatta M, Hanson A, McBride R, Burke DF, Takahashi K, Fukuyama S, Tomita Y, Maher A, Watanabe S, Imai M, Neumann G, Hasegawa H, Paulson JC, Smith DJ, Kawaoka Y. Circulating avian influenza viruses closely related to the 1918 virus have pandemic potential. Cell Host & Microbe 15: 692-705, 2014.
9. Muramoto Y, Shoemaker JE, Le MQ, Itoh Y, Tamura D, Sakai-Tagawa Y, Imai H, Uraki R, Takano R, Kawakami E, Ito M, Okamoto K, Ishigaki H, Mimuro H, Sasakawa C, Matsuoka Y, Noda T, Fukuyama S, Ogasawara K, Kitano H, Kawaoka Y. Disease severity is associated with differential gene expression at the early and late phases of infection in nonhuman primates infected with different H5N1 highly pathogenic avian influenza viruses. J Virol 88: 8981-8997, 2014.
10. Itoh Y, Yoshida R, Shichinohe S, Higuchi M, Ishigaki H, Nakayama M, Pham VL, Ishida H, Kitano M, Arikata M, Kitagawa N, Mitsuishi Y, Ogasawara K, Tsuchiya H, Hiono T, Okamatsu M, Sakoda Y, Kida H, Ito M, Quynh Mai L, Kawaoka Y, Miyamoto H, Ishijima M, Igarashi M, Suzuki Y, Takada A. Protective efficacy of passive immunization with monoclonal antibodies in animal models of H5N1 highly pathogenic avian influenza virus infection. PLoS Pathog. 10: e1004192, 2014.
11. Uraki R, Das SC, Hatta M, Kiso M, Iwatsuki-Horimoto K, Ozawa M, Coban C, Ishii KJ, Kawaoka Y. Hemozoin as a novel adjuvant for inactivated whole virion influenza vaccine. Vaccine 32: 5295-5300, 2014.
12. Liu Z, Kato A, Shindo K, Noda T, Sagara H, Kawaoka Y, Arii J, Kawaguchi Y. Herpes simplex virus 1 UL47 interacts with viral nuclear egress factors UL31, UL34 and Us3, and regulates viral nuclear egress. J Virol 88: 4657-4667, 2014.
13. Neumann G, Macken CA, Kawaoka Y. Identification of amino acid changes that may have been critical for the genesis of A(H7N9) influenza viruses. J Virol 88: 4877-4896, 2014.
14. Yamayoshi S, Yamada S, Fukuyama S, Murakami S, Zhao D, Uraki R, Watanabe T, Tomita Y, Macken C, Neumann G, Kawaoka Y. Virulence-affecting amino acid changes in the PA protein of H7N9 influenza A viruses. J Virol 88: 3127-3134, 2014.