Frontier Research Initiative
フロンティア研究拠点

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The Frontier Research Initiative is managed under the theme adopted by the University of Tokyo, "Promotion of Independence for Young Investigators" (representative: President Hiroshi Komiyama), funded by "Program for Improvement of Research Environment for Young Researchers" from the Ministry of Education, Culture, Sports, Science and Technology (MEXT). In cooperation with the university-wide program steering committee, the office of program supervision established within the Institute of Medical Science supports the research and training of mainly two Frontier Research Team Leaders and four Frontier Researchers. Various professors from within the Institute serve as mentors, offering guidance and advice on research and training matters.

‹Susumu Nakae Group›

To understand the molecular mechanism for development of allergic diseases, we investigate the role of cytokines, Th17 cells and mast cells in the diseases using gene-deficient mice.

Caspase-1, caspase-8 and calpain are dispensable for IL-33 release by macrophages

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In addition to IL-1 and IL-18, IL-33 was recently identified as a member of the IL-1 cytokine family. Recombinant IL-33 (rIL-33) can promote production of Th2-type cytokines by Th 2 cells and mast cells in vitro. Administration of rIL-33 to mice results in increases in IgE secretion and eosinophilic inflammation. However, the precise immune-cell source of IL-33 remains unclear. Moreover, although recombinant pro-IL-33 is cleaved by recombinant caspase-1 *in vitro*, as are pro-IL-1β and pro-IL-18, the involvement of caspase-1 in pro-IL-33 cleavage remains controversial. Here, we show that mouse peritoneal macrophages, but not splenic dendritic cells, produced IL-33 upon stimulation with LPS. Likewise, mouse bone marrow cell-derived, cultured mast cells also produced a small but significant amount of IL-33 via FcεRI-crosslinking, but not in response to stimulation with LPS. To our surprise, IL-33 release was found even in

caspase-1-deficient, caspase-8 inhibitor-treated and calpain inhibitor-treated macrophages. These observations suggest that caspase-1-, caspase-8- and calpain-independent IL-33 production by macrophages and/or mast cells may contribute to the pathogenesis of Th2-type allergic inflammation.

‹Beate Heissig Group›

The major goal of our laboratory is to understand how stem cells are regulated and how we can apply this knowledge for stem cell-based regenerative medicine. Stem cells can differentiate into tissue-specific cells to repair damaged tissue, a process controlled in part by the microenvironment. Proteases, as part of the microenvironment act as processing enzymes that perform highly selective and limited cleavage of specific substrates including growth factors and their receptors, cell adhesion molecules, cytokines, chemokines, apoptotic ligands and angiogenic factors. Over the last year we focused on understanding the mechanism how the fibrinolytic pathway regulates myeloid-cell dependent neoangiogenesis during tissue regeneration.

Tissue type plasminogen activator regulates myeloid-cell dependent neoangiogenesis during tissue regeneration

Makiko Ohki, Yuichi Ohki, Makoto Ishihara, Chiemi Nishida, Yoshihiko Tashiro, Haruyo Akiyama, Hiromitsu Komiyama, Leif R. Lund[1], Atsumi Nitta[2], Kiyofumi Yamada[2], Zhenping Zhu[3], Hideoki Ogawa[4], Hideo Yagita[5], Ko Okumura[4], Hiromitsu Nakauchi, Zena Werb[6], Beate Heissig[7], Koichi Hattori: [1]Department of Cellular and Molecular Medicine, Faculty of Health Science, University of Copenhagen, [2]Department of Neuropsychopharmacology, Nagoya University Graduate School of Medicine, [3]ImClone Systems, NY, [4]Atopy (Allergy) Research Center, Juntendo University School of Medicine, [5]Department of Immunology, Juntendo University School of Medicine, [6]Department of Anatomy, University of California, [7]Department of Stem Cell Regulation, Institute of Medical Science, the University of Tokyo

Ischemia of the heart, brain and limbs is a leading cause of morbidity and mortality worldwide. Treatment with tissue type plasminogen activator (tPA) can dissolve blood clots and can ameliorate the clinical outcome in ischemic diseases. But the underlying mechanism by which tPA improves ischemic tissue regeneration is not well understood. Bone marrow (BM)-derived myeloid cells facilitate angiogenesis during tissue regeneration. Here we report that a serpin-resistant form of tPA by activating the extracellular proteases matrix metalloproteinase-9 and plasmin expands the myeloid cell pool and mobilizes CD45+CD11b+ pro-angiogenic, myeloid cells, a process dependent on vascular endothelial growth factor-A (VEGF-A) and Kit ligand signaling. tPA improves the incorporation of CD 11b+ cells into ischemic tissues, and increases expression of neoangiogenesis-related genes including VEGF-A. Remarkably, transplantation of BM-derived tPA-mobilized CD11b+ cells and VEGFR-1+ cells, but not carrier-mobilized cells or CD11b− cells, accelerates neovascularization and ischemic tissue regeneration. Inhibition of VEGF-signaling suppresses tPA-induced neovascularization in a model of hindlimb ischemia. Thus, tPA mobilizes CD11b+ cells from the BM and increases systemic and local (cellular) VEGF-A, which can locally promote angiogenesis during ischemic recovery. tPA might be useful to induce therapeutic revascularization in the growing field of regenerative medicine.

‹Riu Yamashita Group›

New features of DBTSS (DataBase of Transcription Start Sites) and promoter analysis based on the huge number of sequences

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We have constructed the DataBase of Transcription Start Sites (DBTSS: http://dbtss.hgc.jp/) to support the analysis of transcription regulation network. DBTSS contains the information of accurate transcription start sites (TSSs) based on experimentally determined 5′-end clones. In this year, we have updated the database, and now DBTSS has 328 million new short sequences generated by massively sequencing the 5′ end of oligo-cap selected cDNAs in humans and mice. The sequences were collected from 31 different cell types or culture conditions: therefore, these data provides the dynamical change of transcription start sites in different cell types and conditions.

Using DBTSS data, we observed several biological new aspects. For example, we characterized 140 million transcriptional start sites (TSSs)

in 12 human cell types. Despite the large number of TSS clusters (TSCs), the TSCs with significant expression levels were rare. We also observed significant characteristics difference in major TSCs: namely, highly ordered nucleosome structures, strong RNA polymerase II binding signals, more frequent translation. These results indicate that our database provides a powerful solution to discriminate TSCs having clear biological significance from the other possible noise level transcriptions.

‹Katsuyoshi Yamamoto Group›

Regulation of the yeast MAPK pathways by nutrition-dependent switching of the binding modes between the Opy2 membrane anchor and the Ste11- Ste50 complex through phosphorylation

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Stress-responsive MAPK pathways are essential for eukaryotic cells to adapt to various environmental changes. In yeast, activation of three functionally distinct MAPK pathways (the mating pathway, the filamentous growth (FG) pathway, and the osmoregulatory HOG pathway) shares the essential Ste11 MAPKKK. Nonetheless, they are differentially activated by distinct stimuli. In all these pathways, Ste11 is phosphorylated and activated by the Ste20 kinase. Because active Ste20 is anchored onto the plasma membrane via membrane-bound Cdc42, localization of Ste11 to the membrane is crucial for its activation. In the mating pathway, the scaffolding protein Ste5 directly recruits Ste11 to the membrane. In the FG and HOG pathways, in contrast, the membrane protein Opy2 indirectly recruits Ste11 to the membrane via Ste50, which binds both Ste11 and Opy2. To study the role of Ste50-Opy2 binding in the FG and HOG pathways, we identified the binding sites for Ste50 in Opy2. Opy2 has three separate binding sites (=A, B, and D) for Ste50 in its cytoplasmic region. Whereas the B site binds Ste50 only when phosphorylated under high glucose conditions, the A and D sites bind Ste50 only when glucose is low. The C site does not bind Ste50, but negatively regulates Ste50 binding to the B site by suppressing its phosphorylation. Analyses of mutants of the Ste50 binding sites showed that the three binding sites play different roles in the regulation of the FG and HOG pathways.

‹Kazumasa Yokoyama Group›

Analysis of the NYAP family-mediated signaling pathway in neurons

Kazumasa Yokoyama[1,2], Tadashi Yamamoto[2]: [1]Frontier Research Initiative, [2]Division of Oncology, Institute of Medical Science, University of Tokyo, Japan

The Src family protein tyrosine kinases are implicated in various neural functions such as neuronal development, myelination, and synaptic plasticity. To analyze roles of Src family kinases, we have been trying to identify binding partners and substrates of the kinases in the brain. To identify substrates of Src family tyrosine kinases in the brain, we performed solid-phase phosphorylation screening and identified a novel protein family, which we termed NYAP. We demonstrated that the NYAP family regulates both upstream and downstream of the phosphoinositide 3-kinase (PI3K) signaling in developing neurons. Upon stimulation of Contactin family membrane proteins, NYAP family proteins were tyrosine phosphorylated by Fyn, thereby providing the major binding sites for PI 3K in neurons. Disruptions of NYAP family members decreased Rho, Rac, PI3K and Akt activity, and affected neuronal morphology and nurturing behaviors. Furthermore, NYAP family proteins associated with the NCKAP1/CYFIP1/WAVE1 complex which is an essential link between Rac1 activation and actin polymerization. Rac1 is activated by PI3K-produced PIP3. Interestingly, we found that WAVE1 is associated with PI3K p85 in the developing brain, while this association is not observed at all in non-neuronal tissues. More importantly, this association in the brain completely depends on the NYAP family: that is, WAVE1 is not associated with PI3K p85 in the brain of NYAP family triple knockout mice. Thus, the roles of the NYAP family are 1) activation of PI3K, and 2) recruitment of effector proteins which are activated downstream of PI3K such as WAVE1 in developing neurons. Thus, we proposed that the NYAP family is the central scaffold of PI3K, tightening a molecular link between cell surface Contactin family proteins and neuronal morphogenesis through Fyn, PI3K, and WAVE1.

Many proteins containing phospho-Tyr-x-x-Met (YxxM) motifs have been reported to bind with and activate PI3K p85, but their relative contributions to PI3K activation have not been studied. In this study, we revealed that the NYAP family accounts for almost all of PI3K p 85-binding phosphoproteins in the brain. This means that previously reported YxxM proteins have relatively small contributions to PI3K activation in spatially- and temporally-restricted

situations in neurons. Previous models of PI3K activation and function, therefore, would be refined to take into account contributions of NYAP family proteins in neurons.

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Global COE Program of University of Tokyo

Center of Education and Research for Advanced Genome-Based Medicine: For personalized medicine and the control of worldwide infectious diseases Unit of Disease Control Genome Medicine

ゲノム情報に基づく先端医療の教育研究拠点 オーダーメイド医療の実現と感染症克服を目指して 疾患制御ゲノム医学ユニット

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Our major goal is to establish personalized medicine for patients with infectious diseases or cancers, especially in the gastrointestinal and hepatic fields, based on human or pathogenic microbe genome information.

1. Not HBx but fusion HBx translated from HBV integrant is associated with the development and progression of hepatocellular carcinoma

Ryosuke Muroyama, Norie Kowatari, Wenwen Li, Naoya Kato

Hepatitis B virus (HBV) is a major risk factor of hepatocellular carcinoma (HCC), and HBV X protein (HBx) has been suggested to playing an important role in hepatocarcinogenesis. However, HBV asymptomatic carriers expressing a large amount of HBx rarely develop HCC. In this study, we identified fusion HBx (3'-trancated HBx+human peptides) translated from HBV integrant in human hepatoma cell line, and investigated its role in hepatocarcinogenesis. First, we determined full-length fusion mRNA from HBV integrant in Hep3B cells, which had been established from HBV-related HCC. We could identify full-length fusion mRNA from HBV integrant in Hep3B cells, which was length of 3725 bp containing 1877 bp human sequences at 3' end. From fusion mRNA, fusion HBx (3'trancated HBx+human peptides) was supposed to be translated. Second, we silenced the expression of fusion HBx, which was

supposed to be translated from fusion mRNA, by siRNA in Hep3B cells, and established stable knock down cell clones (KD cells). We confirmed nearly 90% reduction of fusion mRNA expression in KD cells using real-time PCR and Northern blot analysis, and fusion HBx was disappeared in KD cells by immunofluorescence. Third, using KD cells, we examined the effect of fusion HBx on cell growth and invasion ability by MTT assay and matrigel invasion assay. KD cells demonstrated significant reduction in cell proliferation and invasion ability. Fourth, we constructed the plasmids expressing wild HBx and fusion HBx, and compared anchorage-independent growth ability and transactivation ability of NFκB, AP-1, Wnt/β-catenin, and androgen receptor pathway by soft agar assay and luciferase assay, respectively. Although fusion HBx had significantly decreased transactivation ability compared to wild HBx, only fusion HBx had anchorage-independent growth ability in soft agar whereas wild HBx did not. Fifth, in the presence of the protein-synthesis inhibitor cycloheximide, we determined and compared the half-life of wild HBx and fusion HBx. There was no difference in the half-life of wild HBx and fusion HBx, which was estimated to be about 40 min. Fusion HBx translated from HBV integrant was associated with the development and progression of hepatocellular carcinoma. Fusion HBx could be a universal treatment target for HBV-related HCC.

2. Characteristic mutations in X Gene of hepatitis B virus genotype C increase the risk of hepatocellular carcinoma

Wenwen Li, Ryosuke Muroyama, Norie Kowatari, Naoya Kato

HBV Genotype C, especially subgenotype C2, which is prevalent in Asia can increase the risk of HCC with HBx protein playing a key role in the hepatocarcinogenic process. HBV genotype C sequences were downloaded mainly from 2 online databases: Japan Hepatitis database and BIO HBV database. After selection by related information, sequences were divided into 2 groups: HCC and non-HCC. Subgenotypes were decided by diverse distance of full length genome sequences. Amino acids at X region were compared by Chi-square test and significant changes were analyzed by logistic regression. A total of 653 X gene sequences of genotype C (HCC, 105; non-HCC, 548) were enrolled into analysis. In regression analysis, independent factor for HCC were codon 8, codon 36, codon 38, codon 94, codon 116, codon 127. Specific mutations in HBV X gene would be associated with development of HCC in genotype C. Further studies should be carried out to identify the functional relationship with HCC of these mutations.

3. Hepatitis C virus and interferon stimulated genes

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HCV is a leading cause of chronic liver disease, cirrhosis, and HCC, and there are an estimated 170 million chronically infected people worldwide. One of the important characteristics of HCV is that it develops persistent infection by counteracting the host immune system. The IFN system may play a pivotal role in the host anti-HCV system. Treatment of hepatitis C is based on IFN, although its efficacy remains insufficient. IFN stimulates the expression of several genes with antiviral activities.

One of the most intensively studied interferon (IFN) -stimulated genes (ISGs) with known antiviral activity is double-stranded RNA-activated protein kinase. PKR is activated by binding with double-stranded RNA, a putative replicative intermediate of HCV. Activated PKR phosphorylates the α subunit of eukaryotic initiation factor-2 to inhibit the translation of viral protein. We established stable PKR knockdown (KD) Huh7 cells using RNA interference and investigated the effect of PKR against HCV replication using a subgenomic replicon that expressed luciferase reporter protein and the JFH1 full-length HCV genome. In stable PKR KD cells that harboured a subgenomic replicon, luciferase activity was approximately three times higher than that of control cells, indicating that the subgenomic replicon replicated with a higher efficiency in stable PKR KD cells than that in control cells. Furthermore, stable PKR KD cells secreted significantly more HCV particles than did control cells after transfection with the full-length HCV genome. The replication of the subgenomic replicon was suppressed by the addition of IFN-α in both cells. Although the extent of suppression was significantly lower in stable PKR KD cells than control cells using a low concentration (2.5-5 U/ml) of IFN-α, even 10 U/ml IFN-α suppressed the replication of subgenomic replicon by $>$98% in both cells. PKR plays an important role in suppressing HCV replication in an innate state, but may not be essential in IFN therapy.

Myxovirus resistence-1 (MxA) is also one of the well-known ISGs having antiviral activity.

Therefore, we examined the effect of MxA on HCV replication as well as as PKR. First, stable MxA KD Huh7 cells were established using an RNA interference technique, and the effect of MxA on HCV replication was examined using an HCV subgenomic replicon that expresses luciferase reporter protein or the JFH1 full-length HCV genome. Second, MxA was overexpressed in Huh7 cells harboring JFH1 to examine the effect of MxA on HCV replication. Third, the effect of IFN-α administration on HCV replication was evaluated in MxA KD cells to examine the role of MxA in IFN treatment. HCV replication was determined based on the luciferase activity of the subgenomic replicon, intracellular core protein expression from JFH1, or core protein secretion from JFH1 into the culture supernatant. In stable MxA KD cells, luciferase activity from HCV subgenomic replicon was about 3.8 times higher than that in control cells. In addition, MxA knockdown cells harboring JFH1 secreted up to 100 times more HCV particles compared to control cells. In contrast, MxA overexpression suppressed JFH1 replication. Most importantly, IFN- was less efficient against HCV in MxA KD cells. MxA expression suppressed HCV replication. MxA may play an important role in suppressing HCV replication during IFN administration. This information may be important to improve the efficacy of IFN treatment in patients with refractory hepatitis C.

4. Characteristic mutations in hepatitis C virus core gene related to the occurrence of hepatocellular carcinoma

Zhongjie Hu, Rhosuke Muroyama, Norie Kowatari, Naoya Kato

Chronic HCV infection often results in HCC. Previous studies have shown that there might be some characteristic mutations in the core region of HCV related to HCC. Thus, we downloaded and analyzed HCV genotype 1b core gene sequences from HCV databases online to identify them. Based on the information of the sequences, 63 from patients with HCC and 188 from non-HCC were enrolled into our analysis. Then, the nucleotides at each position were compared by Chi-square test between the two groups, and 24 polymorphisms were found to be associated with HCC. Further analysis of these 24 polymorphisms by logistic regression indicated that eight were significantly related to the increased HCC risk: A028C, G209A, C219U/A, U264C, A271C/U, C378U/A, G435A/C, and G481A. Moreover, U303C/A was associated with the decreased HCC risk. These mutations could bring about four amino acid substitutions: K10Q, R70Q, M91L, and G161S. In conclusion, eight characteristic mutations in the *HCV-1b* core gene related to the occurrence of HCC were identified. The structural and functional alterations of core protein due to these mutations and the relationship with the occurrence of HCC need to be further studied.

5. Quantitative analysis of hepatitis C virus genotype 1b core codon 70 wild type and mutant and the response to pegylated interferon and ribavirin treatment

Zhongjie Hu, Ryosuke Muroyama, Norie Kowatari, Wenwen Li, Naoya Kato

Substitution of amino acid (aa) 70 in HCV genotype 1b core region was reported to be an important predictor of non-virological response to pegylated interferon plus ribavirin combination therapy (PEG-IFN/RBV). The study was to investigate the wild type (Arg70, HCV-70W) and mutant (Gln/His70, HCV-70M) strains' dynamic response to PEG-IFN/RBV and the effects of wild type or mutant core proteins on interferon stimulated response element (ISRE) and cells' apoptosis. Taqman two step real time RT-PCR based on TaqMan-MGB probes that were designed to distinguish HCV-70W(CGG) and 70M(CAG) was developed. Serial serum samples were collected from 36 patients with HCV 1b treated with PEG-IFN/RBV, and viral loads of both wild type and mutant were quantified. Next, we constructed the plasmids that could express the wild type and mutant core proteins and transfected them into human hepatoma cell line Huh7. Dual luciferase reporter assays and apoptosis PCR array tests were performed. We successfully developed quantitative methods with high sensitivity and specificity for HCV-70W and 70M. Using a dilution series of mixture of wild type and mutant plasmids, the detection limits were identified to be 1 KIU/ml. Cross-reactivity tests confirmed the specificity. The further tests using TA cloning and sequencing confirmed the reliability of the methods. The quantitation of both HCV-70W and HCV-70M from 36 patients' serum samples showed that 80.6% (29/36) of the patients were infected with mixture of wild type and mutant strains before treatment. HCV-70M was dominant in 16.7% (6/36) patients. Analysis of the relationship between the ratio of mutant to wild type and the responses to treatment (Early virological responders, EVRs) showed that there were only 33.3% (2/6) EVRs for patients with dominant mutant while 93.3% (28/30) for patients with dominant wild type ($P=0.003$). However, the tests of serial sera indicated that HCV-70M did

not show resistance to PEG-IFN/RBV therapy independently. When the patients responded to the treatment, titers of HCV-70M decreased as those of HCV-70W. The paired samples t tests showed that the percentage of HCV-70M at week 0 to week 4 and 8 during treatment did not changed significantly. We constructed the expression plasmids successfully and transfected them into Huh7 cells. The results of dual luciferase reporter assays showed that there was no significant difference between the wild type and mutant core protein. Both of them could activate ISRE significantly, whether treated with or without IFN. Furthermore, the results of apoptosis PCR array tests showed that both of them had the same effects on cells' apoptosis. Most patients with HCV 1b were infected with mixture of HCV-70W and HCV-70M. HCV-70M had similar response to PEG-IFN/RBV therapy as HCV-70W, however, the high ratio of HCV-70M to HCV-70W might be related to non-response to antiviral therapy.

6. CD26 in hepatocellular carcinoma

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CD26, also known as dipeptidyl peptidase IV (DPP IV), is a 110-kDa cell surface antigen having an important role in tumor development. To explore the possibility of anti-CD26 treatment against HCC, expression of CD26 was firstly examined in HCC and hepatoblastoma cell lines and also in normal liver cells by FACS and Western blotting of cell lysates. Normal liver cells, HLE, HLF, and SK-Hep1 cells expressed almost no CD26. On the other hand, Hep3B, PLC/PRF/5, and Huh7 cells expressed CD26. CD26 expression pattern was also determined by immunohistochemistry and not membranous but cytoplasmic staining of CD26 was observed in liver cells expressing CD26. Secondly, immunostaining for CD26 was performed using liver tissue microarray containing HCC and noncancerous liver samples of chronic hepatitis and cirrhosis. Although there was no difference in degree of CD26 staining between HCC and non-HCC, zonal CD26 distribution in normal hepatic acinus (intense in zones 2/3 and weak in zone 1) was almost lost in HCC. Evaluating effect of anti-CD26 monoclonal antibody against HCC growth or invasion is ongoing.

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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, structure of viral and host 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 economical 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 dense urban environments. No one can discuss emerging and re-emerging infectious diseases (ERID) 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 Collabora-*

tion on Emerging and Re-emerging Infectious Diseases; MEXT Project Director: Aikichi Iwamoto), IMSUT has established 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 Harbin Veterinary Research Institute (HVRI), the Chinese Academy of Agricultural Science; and IMSUT's project office in Beijing.

Year 2009 was the last year of the 5-year project term. To sum up the research activities of the two joint laboratories in Beijing and one joint program in Harbin, a symposium was to be held in June. But the meeting was postponed until October 21 and 22 because of the pandemic 2009 H1N1 influenza. The symposium was successfully held at the IBPCAS in Beijing under the title *China-Japan Research Collaboration on Emerging and Reemerging Infections: First Five Years and Future.*

Since phase I of the MEXT program (including our project) was to end in March 2010, it was subjected to two evaluations: scientific and political. The latter system was invented by the Democratic Party of Japan, which took power through winning the Lower House election on August 30, 2009. The program survived the two evaluations and is going into phase II from April 2010.

LABORATORIES AND PROJECT OFFICE

a. Laboratory of Structural Virology and Immunology (LSVI)

In the IBP campus, the new building for the National Laboratory of Protein Science (NLPS) was inaugurated in December 2009. Several IBP laboratories moved to the new building, and LSVI accordingly gained additional lab spaces. In LSVI, Z. Matsuda's group members are studying gp41, one of the two subunits of HIV-1 envelope protein. Using the newly engineered pair of reporter proteins called dual split proteins (DSPs), they found that the introduction of the heterologous membrane-spanning domain (MSD) into gp41 exerted allosteric negative effect(s) on the conformational changes of gp120/gp41 during the process of membrane fusion. The intracellular transport of the envelope protein was affected in the other MSD mutants. They also examined the membrane topology of the MSD in both prokaryotic and mammalian systems. The obtained data suggest that gp41 is a type I membrane protein with a single MSD in the steady state. During membrane fusion, however, there was a possibility of alteration in the topology of the MSD. These data suggest that gp41 MSD may play an important role in the function of HIV-1 envelope protein.

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

LMIMM was founded in May 2006, set to work in October 2006 in IMCAS, and moved to an IM's new building in the CAS Olympic Science Park in January 2007. It is an important member of the CAS Key Laboratory of Pathogenic Microbiology and Immunology, which was established in December 2008. Y. Kitamura's group in LMIMM is currently focusing on hepatitis C virus (HCV) and human immunodeficiency virus (HIV), to obtain a better understanding of mechanisms of the viral replication and to develop antiviral drugs. Using the human cell line/infectious HCV RNA system, they are characterizing the adapted virus population and some anti-HCV host genes stimulated by interferon. To investigate the pathogenicity of the viruses in the field, they are analyzing clinical samples from HIV-infected individuals and chronic hepatitis C patients treated in some collaborating hospitals in Beijing. They are also collaborating with Chinese scientists to study some zoonotic viruses such as Porcine Reproductive and Respiratory Syndrome Virus.

c. Collaborative research program with HVRI

H1N1 swine-origin influenza A virus caused a pandemic in 2009. However, H5N1 highly pathogenic avian influenza viruses have continued to cause unprecedented global outbreaks since 2003, and many human cases with a high fatality rate have been reported. For this reason, a joint research program at HVRI (Director, Xiangang Kong) has been conducted on avian influenza viral isolates from all over Asia. HVRI focuses on avian influenza viruses that are circulating in Chinese wild waterfowl and domestic poultry. Specifically, we study type A influenza virus from wild birds, waterfowl, poultry, swine and horses, with an emphasis on viral pathogenicity in various hosts, viral evolution, and viral prevalence in China. In addition, we have conducted molecular surveillance studies of H5N1 viruses isolated throughout Asia.

d. IMSUT Project Office

The office (M. Hayashi, B. Nagano, and K. Yoshiike) 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 (in Japanese) has been presented and updated on the website of the Project (http://www.rcaid.jp/). Faced with the pandemic 2009 H1N1 influenza, the information was particularly valuable to those who live or travel in China.

IMPLEMENTATION OF COLLABORATION

The collaboration was implemented, being based on MOU and Minutes of Meeting between IMSUT and the Chinese institutes. For the joint laboratories the implementation was controlled by the steering committee consisting of Motoharu Seiki, A. Iwamoto, George Fu Gao, and Tao Xu. The collaborative program in Harbin was implemented by the steering committee consisting of M. Seiki, Y. Kawaoka, X. Kong, and H. Chen.

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