Laboratory Animal Research Center
実験動物研究施設

Professor	Chieko Kai, D.V.M., Ph.D.	教　授	農学博士	甲　斐　知恵子
Associate Professor	Misako Yoneda, D.V.M., Ph.D.	准教授	博士(農学)	米　田　美佐子
Assistant Professor	Tomoko Fujiyuki, Ph.D.	助　教	博士(薬学)	藤　幸　知　子
Assistant Professor	Shotaro Uchida, Ph.D.	助　教	博士(医学)	内　田　翔太郎

Our major research interests are to elucidate molecular mechanisms of pathogenicity and species specificity of negative and single strand RNA viruses (Mononegavirales), and to control viral diseases. For these purposes, we are studying virus replication and identifying viral and host factors important for the expression of pathogenicity using a novel reverse genetics technique. We are also developing new virus vaccines, virus vectors, and oncolytic virus by genetic engineering. In the animal research center, more than 30,000 mice, mainly transgenic or knockout, are kept for research of IMSUT, and the technical staff support their breeding, frozen storage of eggs and microbiological cleaning.

Efficacy of recombinant measles virus expressing highly pathogenic avian influenza virus (HPAIV) antigen against HPAIV infection in monkeys

Fujiyuki T, Horie R, Yoneda M, Kuraishi T[1], Yasui F[2], Kwon HJ, Munekata K[2], Ikeda F, Hoshi M, Kiso Y, Omi M, Sato H, Kida H[3], Hattori S[1], Kohara M[2], Kai C.: [1]Amami Laboratory of Injurious Animals, The Institute of Medical Science, The University of Tokyo. [2]Department of Microbiology and Cell Biology, Tokyo Metropolitan Institute of Medical Science. [3]Research Center for Zoonosis Control, Hokkaido University.

Highly pathogenic avian influenza virus (HPAIV) is a serious threat not only to domestic fowls but also to humans. Vaccines inducing long-lasting immunity against HPAIV are required. In the present study, we generated recombinant measles virus (MV) expressing the hemagglutinin protein of HPAIV without the multibasic site necessary for its pathogenicity in chickens using the backbone of an MV vaccine strain (rMV-Ed-H5HA) or a wild-type MV-derived mutant (rMV-HL-Vko-H5HA). We examined protective efficacy of the candidate vaccines in the monkey infection model by the challenge with a HPAIV (H5N1). Cynomolgus monkeys inoculated with the candidate vaccines produced both anti-H5 HA and anti-MV antibodies. They recovered earlier from influenza symptoms than unvaccinated monkeys after the challenge with the HPAIV strain. Chest radiography and histopathological analyses confirmed less severe pneumonia in the vaccinated monkeys. Vaccination tended to suppress viral shedding and reduced the interleukin-6 levels in the lungs. Furthermore, the vaccination with rMV-Ed-H5HA of monkeys with pre-existing anti-MV immunity induced the production of anti-H5 HA antibodies. These results suggest that both candidate vaccines effectively reduce disease severity in naïve hosts, and that rMV-Ed-H5HA is a particularly good candidate vaccine against HPAIV infection.

The P gene of rodent brain-adapted measles virus plays a critical role in neurovirulence.

Arai T, Terao-Muto Y, Uchida S, Lin C, Honda T, Takenaka A, Ikeda F, Sato H, Yoneda M, Kai C.

In rare cases, MV in children leads to fatal neurological complications such as primary measles encephalitis, post-acute measles encephalitis, subacute

sclerosing panencephalitis and measles inclusion-body encephalitis. To investigate the pathogenesis of MV-induced encephalitis, rodent brain-adapted MV strains CAM/RB and CAMR40 were generated. These strains acquired mutations to adapt to the rodent brain during 40 passages in rat brain. However, it is still unknown which genes confer the neurovirulence of MV. We previously established a rescue system for recombinant MVs possessing the backbone of wild-type strain HL, an avirulent strain in mice. In the present study, to identify the genes in CAMR40 that elicit neurovirulence, we generated chimeric recombinant MVs based on strain HL. As a result, recombinant wild-type MV in which the haemagglutinin (H) gene was substituted with that of CAMR40 caused a non-lethal mild disease in mice, while additional substitution of the HL phosphoprotein (P) gene with that of strain CAMR40 caused lethal severe neurological signs comparable to those of CAMR40. These results clearly indicated that, in addition to the H gene, the P gene is required for the neurovirulence of MV CAMR40.

Nipah and Hendra Virus Nucleoproteins Inhibit Nuclear Accumulation of Signal Transducer and Activator of Transcription 1 (STAT1) and STAT2 by Interfering with Their Complex Formation

Sugai A, Sato H, Takayama I, Yoneda M, Kai C.

Henipaviruses, such as Nipah (NiV) and Hendra (HeV) viruses, are highly pathogenic zoonotic agents within the Paramyxoviridae family. The P gene products of the paramyxoviruses have been well characterized for their interferon (IFN) antagonist activity and their contribution to viral pathogenicity. In this study, we demonstrated that the nucleoprotein (N) of henipaviruses also prevents the host IFN signaling response. Reporter assays demonstrated that the NiV and HeV N proteins (NiV-N and HeV-N, respectively) dose-dependently suppressed both type I and type II IFN responses and that the inhibitory effect was mediated by their core domains. Additionally, NiV-N prevented the nuclear transport of signal transducer and activator of transcription 1 (STAT1) and STAT2. However, NiV-N did not associate with Impα5, Impβ1, or Ran, which are members of the nuclear transport system for STATs. Although P protein is known as a binding partner of N protein and actively retains N protein in the cytoplasm, the IFN antagonist activity of N protein was not abolished by the coexpression of P protein. This suggests that the IFN inhibition by N protein occurs in the cytoplasm. Furthermore, we demonstrated that the complex formation of STATs was hampered in the N protein-expressing cells. As a result, STAT nuclear accumulation was reduced, causing a subsequent down-regulation of interferon-stimulated genes (ISGs) due to low promoter occupancy by STAT complexes. This novel route for preventing host IFN responses by henipavirus N proteins provides new insight into the pathogenesis of these viruses.

Publications

1. Arai T, Terao-Muto Y, Uchida S, Lin C, Honda T, Takenaka A, Ikeda F, Sato H, Yoneda M, Kai C. The P gene of rodent brain-adapted measles virus plays a critical role in neurovirulence. *J Gen Virol.*, 98(7): 1620-1629, 2017
2. de Rie D., The FANTOM Consortium et al. (Kai, C., Sato, H., Yoneda, M. in 66 authors). An integrated expression atlas of miRNAs and their promoters in human and mouse. *Nat Biotechnol.*, 35(9): 872-878, 2017.
3. Sugai, A., Sato, H., Yoneda, M. and Kai, C. Gene end-like sequences within the 3' non-coding region of the Nipah virus genome attenuate viral gene transcription. *Virology.* 508: 36-44, 2017.
4. Sugai A, Sato H, Takayama I, Yoneda M, Kai C. Nipah and Hendra Virus Nucleoproteins Inhibit Nuclear Accumulation of STAT1 and STAT2 by Interfering with Their Complex Formation. *J Virol.*, 91(21). e01136-17, 2017.
5. Noguchi S, The FANTOM Consortium et al. (Kai, C., Sato, H., Yoneda, M. in 178 authors). FANTOM5 CAGE profiles of human and mouse samples. *Sci Data.* 29; 4: 170112, 2017.
6. Fujiyuki T, Horie R, Yoneda M, Kuraishi T, Yasui F, Kwon HJ, Munekata K, Ikeda F, Hoshi M, Kiso Y, Omi M, Sato H, Kida H, Hattori S, Kohara M, Kai C. Efficacy of recombinant measles virus expressing highly pathogenic avian influenza virus (HPAIV) antigen against HPAIV infection in monkeys. *Sci Rep.* 20; 7(1): 12017, 2017.

Amami Laboratory of Injurious Animals
奄美病害動物研究施設

Professor	Chieko Kai, D.V.M., Ph.D.	教　授　農学博士	甲　斐　知恵子
Assistant Professor	Shinichi Yokota, D.V.M., Ph.D.	助　教　博士(人間科学)	横　田　伸　一

The Amami Laboratory of Injurious Animals was established in 1965 at Setouchi-cho in Amami-oshima Island in order to study on endemic diseases involving parasite, arthropods, and venomous snakes in the tropics or subtropics.
The Amami-oshima Island belongs to the Nansei (Southwest) Islands and the fauna is quite different from that in other islands of Japan. Since establishment of the laboratory, trials have been carried out to utilize small mammals found unique in the Amami islands as experimental animals in addition to studies on prevention of Habu bites. As well known, successful eradication of filariasis from this island is one of the monumental works of the laboratory. Our present works are as follows:

1. Research on the Habu control

Shinichi Yokota, Shosaku Hattori, Motonori Ohno[1], Naoko Oda-Ueda[2], Takahito Chijiwa[1], Aichi Yoshida[3], Yoshihiro Hayashi[4], Tomohisa Ogawa[5], and Hiroki Shibata[6],: [1]Department of Applied Life Science, Faculty of Bioscience, Sojo University, [2]Department of Biochemistry, Faculty of Pharmaceutical Science, Sojo University, [3]School of Health Science, Faculty of Medicine, Kagoshima University, [4]National Museum of Nature and Science, Tokyo,[5]Faculty of Agriculture, Tohoku University, [6]Medical Institute of Bioregulation, Kyushu University

Snake bites by the venomous snake Habu, *Protobothrops flavoviridis*, have been reported annually about 60 cases in the population of 100,000 in the Amami Islands. Moreover, there is no indication that the population of the Habu itself has decreased, despite a campaign for capture of snakes by the Kagoshima Prefectural Government. Rat-baited box traps have been introduced to catch the snakes and found to be quite effective. However, maintenance of live rats requires man power and its cost is expensive. Therefore, our effort has been focused on the development of attractant for Habu. The attractant extracted from rats seems ineffective if compared with use of live rats.

It was known that the Habu survived the injection of the Habu venom since early times, because some proteins in the serum of the Habu blood combine to the elements of the Habu venom. The research of these binding proteins has been initiated with an objective of clinical trials. Phospholipase A_2 and its isozymes isolated from Habu venom have myonecrotic activity and hemorrhagic activity, and metal protease has hemorrhagic activity. The binding proteins isolated from serum of Habu inhibit myonecrotic activity of phospholipase A_2 and its isozymes. We found that protein-HSF and peptide-AHP isolated from the Habu serum effectively control the hemorrhage caused by venom of the Habu, *Ovophis okinavensis, Agkistrodon blomhoffi brevicaudus, Calloselasma rhodostoma, Bitis arietans, Bothrops asper*, and, *Trimeresurus stejnegeri.*

Further, a statistics analysis and the simulation were done with the snakes captured by the Government, and the analysis of population dynamics of Habu was attempted. As a result of investigating the individual measurement data of the captured Habu over 9 years, we were able to obtain the generous age composition of the Habu. From analyzing of the age pyramid of the Habu and the result of questionnaire surveys for the inhabitant in the Amami-oshima Island, the total population of the Habu which lives in this island was estimated at about 80,000. By the analysis of the measured data

of last nine years, the snake sizes were miniaturized, and the population of young snakes decreased. According to these investigations, the population of the Habu is expected to decrease in the near future.

These studies are supported by grants from the Ministry of Land, Infrastructure and Transport and the Kagoshima Prefectural Government.

2. Reproduction of squirrel monkeys and owl monekys.

Shinichi Yokota, Shosaku Hattori, Kumiko Ikeda, and Chieko Kai

The squirrel monkey (*Saimiri boliviensis*) and the owl monkey (*Aotus lemurinus griseimenbra*) were widely distributed in the tropical rainforest in Central and South America. The advantage of using both species for medical researches resides in its small size and gentle behavior. In this laboratory, squirrel monkeys have a breeding season between winter and early spring. They are polygamy. Their puberty is 3-4 years old in females and 4-5 years old in males. Their gestation period is about 150 days. In contrast, the owl monkey is annual breeding animals. They are monogamy. Their puberty is 3 years old for both sex. Their gestation period is about 130 days. Two newborns were given in reproductive groups of squirrel monkeys in 2017, and were nursed by laboratory staffs because of neglect of their mothers. On the other hand, 2 newborns were given in a female owl monkey in 2017.

3. Comparison of morphological specificities of the retinal photoreceptor cells in the New World Monkeys, Aotus lemurinus and Saimiri boliviensis

Takeshi Kusakabe[8], Shinichi Yokota, Shosaku Hattori, Midori Yoshizawa[9], Chieko Kai, and Yasuo Kiso[8]: [8]Joint Faculty of Veterinary Science, Yamaguchi University, [9]Graduate School of Agricultural Science, Utsunomiya University

The New World monkey Aotus spp. (night monkeys) are the only one species possessing the nocturnal lifestyles among the simian primates, and are expected for use of valuable experimental animal with the close species of Saimiri spp. (squirrel monkeys). We examined the histological appearance and electroretinogram (ERG) reacivity of the retina of the owl monkeys by comparison with squirrel monkeys, taxonomically close-species and expressing diurnal behavior. In conclusion, it was demonstrated that although the owl monkeys has typical traits to nocturnal lifestyles, such as large pupil and eyeball sizes and rod-cell dominant retina, the ability for scotopic vision was not developed enough.

4. Estimation of the therapeutic effect of transplantation of DFAT cells sheet on corneal epithelial defects in squirrel monkey (*Saimiri boliviensis*)

Shinichi Yokota, Shosaku Hattori, Akane Tanaka[10], Hiroshi Matsuda[10], and Chieko Kai: [10]Division of animal life science, Institute of agriculture, Tokyo University of Agriculture and Technology

Dedifferentiated fat (DFAT) cells are seemed to be a good candidate source of adult stem cells in regenerative medicine, because these cells exhibit multilineage potential as adipose-derived stem/stromal cells (ASCs). We isolated squirrel monkey DFAT cells from a small amount of adipose tissue by the ceiling culture method, and estimated the therapeutic effect of transplantation of DFAT cells sheet on corneal epithelial defects. We are currently preparing to submit the results of this study to the scientific journal.

Publications

Matsuu, A,. Yokota, S,. Ito, K,. and Masatani, T. Seroprevalence of Toxoplasma gondii in free-ranging and feral domestic cats on Amami Oshima Island, Japan. J Vet Med Sci. 79(11) 1853-1856, 2017.

Kumiyoshi, N,. Yoshida, Y,. Itoh, Y,. Yokota, S,. Kuraishi, K,. Hattori, S,. Kondo, T,. Yoshizawa, M,. Kai, C,. Kiso, Y,. Kusakabe, K. Morphological specificities of the retinal photoreceptor cells in the nocturnally adapted owl monkeys. J Vet Med Sci. in press.