Osaka University Research Institute for Microbial Diseases

2011

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Contents

Photo by Hiroshi Nojima.

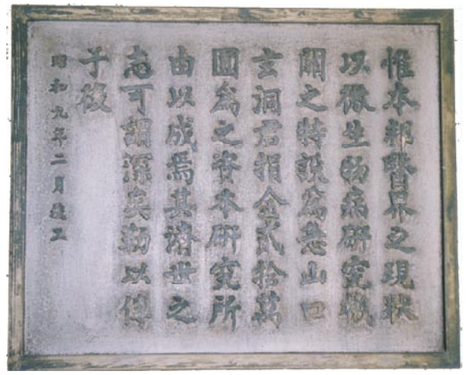
Research Institute for Microbial Diseases

The Research Institute for Microbial Diseases was originally established as a five-department "Research Center for Communicable Diseases" in February, 1934 after a donation from Mr. Gendo Yamaguchi at Dojima in Osaka City. At that time, the Institute was located on Nakanoshima campus of Osaka Medical School, but then became a part of Osaka University in September, 1934. It was moved to its present site on Suita campus in 1967, in accordance with the university's relocation program.

Until 1993, the Institute had grown to include nineteen departments spanning a wide range of diverse subjects, such as infectious disease, immunology, cancer and molecular biology. Additionally, it had three special research facilities, a central laboratory and a library. In 1997, the Research Center for Emerging Infectious Diseases was added to the Institute. In 2005, the Institute was reorganized to constitute three Research divisions; the "Division of Infectious Disease", the "Division of Host Defence" and the "Division of Cellular and Molecular Biology", which represent 15 departments together with three attached centers for specialized research on infectious disease and genome information. Moreover, the Research Collaboration Center on Emerging and Re-emerging Infections was founded in Bangkok in collaboration with the National Institute of Health, Thailand, to defend people against possible emerging and re-emerging infections.

Basic research on infectious disease, immunology, and cell biology is the Institute's principal focus. The results of research in these fields at the Institute have contributed considerably to the diagnosis, prevention, and treatment of infectious diseases, immunological diseases and cancer, as well as to progress in basic biomedical science. The Institute was selected as one of "the 21st century center of excellence programs" on the theme of "combined program on microbiology and immunology" in 2003. Also, it was certified as "joint usage / research center" by MEXT (the Ministry of education, culture, sports, science and technology) in 2009, and started the mission in April, 2010. Moreover, the institute newly established the "Center for Genetic Analysis of Biological Responses" to conserve gene resources and to protect intellectual property.

The Institute accepts and trains Master and Ph.D. candidates in the medical and biological sciences. At present, more than 200 full, associate, and assistant professors, research associates, graduate students and research fellows pursue studies in microbiology, oncology and molecular biology in state of the art facilities at the Institute.



The copper plate recalling that the Research Institute for Microbial Diseases was originally established by a donation from Mr. Gendo Yamaguchi. (At the entrance hall of the main building)

Mr. Gendo Yamaguchi

Born in Onomichi City in 1863 as the eldest son of a physician, he moved to Osaka at the age of 15 and became one of western Japan's most successful businessmen. He retired in 1917, and devoted the rest of his life to religion and the tea ceremony. He donated most of the proceeds of his estate to public enterprise, shrines and temples.

Organization

Director

Faculty Meeting — **Delegate Assembly**

Research Division

- Division of Infectious Diseases
  - Department of Molecular Bacteriology
  - Department of Viral Infections
  - Department of Molecular Virology
  - Department of Pharmacotherapy
  - Department of Pathology
- Division of Host Defense
  - Department of Molecular Immunology
  - Department of Immunoregulation
  - Department of Host Defense
  - Department of Cell Biology
  - Department of Immunochemistry
- Division of Cellular and Molecular Biology
  - Department of Molecular Microbiology
  - Department of Molecular Genetics
  - Department of Oncogene Research
  - Department of Signal Transduction
  - Department of Cellular Regulation

Special Research Facilities

- Research Center for Infectious Disease Control
  - Department of Bacterial Infections
  - Department of Molecular Protozoology
  - Department of Virology
- Genome Information Research Center
  - Department of Experimental Genome Research
  - Department of Genome Informatics
  - Department of Infection Metagenomics
- International Research Center for Infectious Diseases
  - Department of Special Pathogens
  - Department of Infectious Disease Control
  - Pathogenic Microbes Repository Unit
- Animal Resource Center for Infectious Diseases
- DNA-chip Development Center for Infectious Diseases
- Center for Genetic Analysis of Biological Responses

Office of Combined Program on Microbiology and Immunology

- Research Promotion Group
- Education Promotion Group

Research Collaboration Center in Overseas

- Thailand-Japan Research Collaboration Center on Emerging and Re-emerging Infections
  - Section of Bacterial Infections
  - Section of Viral Infections

Endowed Chair

- BIKEN Endowed Department of Dengue Vaccine Development

Common Research Facilities

- Central Instrumentation Laboratory
- Radioisotope Laboratory
- Central Laboratory for Biological Hazardous Microbes
- Library

Administration

- General Affairs Section
- Accounting Section
- Research Cooperation Section

Related Institution

- World Premier International Research Center
- Immunology Frontier Research Center

Former Directors & Professors

Former Directors

Yashiro Kotake, M.D., Professor	1934.9−1940. 6	Michiaki Takahashi, M.D., Professor	1984.4−1986. 3
Arao Imamura, M.D., Professor	1940.8−1943. 7	Toshio Miwatani, M.D., Professor	1986.4−1988. 3
Tenji Taniguchi, M.D., Professor	1943.7−1955. 3	Takeo Kakunaga, D.Pharm., Professor	1988.4−1988. 9
Tsunesaburo Fujino, M.D., Professor	1955.4−1958. 3	Hajime Fujio, M.D., Professor	1988.11−1990.10
Juntaro Kamahora, M.D., Professor	1958.4−1964. 3	Kumao Toyoshima, M.D., Professor	1990.11−1993.10
Tsunehisa Amano, M.D., Professor	1964.4−1968. 3	Akira Hakura, D.Sc., Professor	1993.10−1997.10
Yoshiomi Okuno, M.D., Professor	1968.4−1972. 3	Yoshitake Nishimune, M.D., Professor	1997.10−2001.10
Mitsuo Hori, M.D., Professor	1972.4−1976. 3	Takeji Honda, M.D., Professor	2001.10−2003.10
Junichi Kawamata, M.D., Professor	1976.4−1980. 3	Taroh Kinoshita, D.Med.Sc., Professor	2003.10−2007.10
Shiro Kato, M.D., Professor	1980.4−1984. 3	Hitoshi Kikutani, M.D., Professor	2007.10−

Former Professors

Yashiro Kotake, M.D., Professor	Takahisa Yamanouchi, M.D., Professor
Sadao Yoshida, M.D., Professor	Toshio Miwatani, M.D., Professor
Arao Imamura, M.D., Professor	Michiaki Takahashi, M.D., Professor
Yukichi Satani, M.D., Professor	Hajime Fujio, M.D., Professor
Tenji Taniguchi, M.D., Professor	Tetsuo Taguchi, M.D., Professor
Kota Sera, M.D., Professor	Aizo Matsushiro, D.Sc., Professor
Tatsunori Masayama, M.D., Professor	Atsuo Nakata, D.Sc., Professor
Shohei Otani, M.D., Professor	Hiroto Okayama, M.D., Professor
Teishiro Seki, M.D., Professor	Kumao Toyoshima, M.D., Professor
Masami Suda, M.D., Professor	Teruo Kitani, M.D., Professor
Kaoru Morishita, M.D., Professor	Shin-ichiro Takai, M.D., Professor
Hisashi Yamaguchi, M.D., Professor	Morihiro Matsuda, M.D., Professor
Tsunesaburo Fujino, M.D., Professor	Takashi Kurimura, M.D., Professor
Masakazu Ito, M.D., Professor	Koichi Yamanishi, M.D., Professor
Juntaro Kamahora, M.D., Professor	Akira Hakura, D.Sc., Professor
Shinji Nishimura, M.D., Professor	Tetsu Akiyama, D.Sc., Professor
Mitsuhiko Kato, M.D., Professor	Takeshi Kurata, M.D.,D.Med.Sci., Professor
Masahiko Yoneyama, M.D., Professor	Shigeharu Ueda, M.D.,D.Med.Sci., Professor
Shigeru Shiba, M.D., Professor	Kazunori Shimada, M.D.,D.Med.Sci., Professor
Shozo Inoki, M.D., Professor	Chihiro Sasakawa, M.D., Professor
Mitsuo Hori, M.D., Professor	Akio Sugino, D.Sci., Professor
Yoshiomi Okuno, M.D., Professor	Hiroshi Kiyono, D.D.S.,Ph.D., Professor
Shigeyuki Ishigami, M.D., Professor	Yoshitake Nishimune, M.D., Professor
Tsunehisa Amano, M.D., Professor	Toru Nakano, M.D., D.Med. SC., Professor
Junichi Kawamata, M.D., Professor	Hideo Shinagawa, D.Sc., Professor
Yoshio Okada, M.D., Professor	Shin-ichi Tamura, Ph.D., Professor
Mitsuo Torii, D.Sc., Professor	Michiyuki Matsuda, M.D., D.Med. SC., Professor
Konosuke Fukai, M.D., Professor	Takeshi Honda, M.D., Ph. D.
Tatsuo Mori, M.D., Professor	Naoyuki Taniguchi, M.D., Ph. D.
Tonetaro Ito, M.D., Professor	Tamotsu Yoshimori, M.D., Ph. D.
Takeo Kakunaga, D.Pharm., Professor	Kazuyuki Tanabe, M.D., Ph. D.
Shiro Kato, M.D., Professor	Fumio Imamoto, D.Sc., Professor
Toshio Nakabayashi, M.D., Professor	Atsushi Kumanogo, M.D., Ph. D.

2011.04.01

Director	Professor Hitoshi Kikutani M. D., Ph.D.
Vice Director	Professor Eisuke Mekada Ph.D.
Division of Infectious Diseases	
Department of Molecular Bacteriology	Professor Yasuhiko Horiguchi D. V. M., Ph.D.
Department of Viral Infections	Professor Tatsuo Shioda Ph.D.
Department of Molecular Virology	Professor Yoshiharu Matsuura D. V. M., Ph.D.
Department of Pharmacotherapy	
Department of Pathology	
Division of Host Defense	
Department of Molecular Immunology	Professor Hitoshi Kikutani M. D., Ph.D.
Department of Immunoregulation	Professor Taroh Kinoshita Ph.D.
Department of Host Defense	Professor Shizuo Akira M. D., Ph.D.
Department of Cell Biology	Professor Eisuke Mekada Ph.D.
Department of Immunochemistry	Professor Hisashi Arase M. D., Ph.D.
Division of Cellular and Molecular Biology	
Department of Molecular Microbiology	
Department of Molecular Genetics	Professor Hiroshi Nojima Ph.D.
Department of Oncogene Research	Professor Masato Okada Ph.D.
Department of Signal Transduction	Professor Nobuyuki Takakura M. D., Ph.D.
Department of Cellular Regulation	
Research Center for Infectious Disease Control	Head, Professor Toshihiro Horii Ph.D.
Department of Bacterial Infections	
Department of Molecular Protozoology	Professor Toshihiro Horii Ph.D.
Department of Viorogy	Professor Kazuyoshi Ikuta Ph.D.
Genome Infomation Research Center	Head, Professor Teruo Yasunaga Ph.D.
Department of Experimental Genome Research	Professor Masaru Okabe Ph.D.
Department of Genome Informatics	Professor Teruo Yasunaga Ph.D.
Department of Infection Metagenomics	
International Research Center for Infectious Diseases	Head, Professor Toshihiro Horii Ph.D.
Department of Special Pathogens	
Laboratory of Clinical Research on Infectious Diseases	SA Professor Kazunori Oishi M. D., Ph.D.
Laboratory of Infection Cell Biology	SA Associate Professor Yukako Fujinaga Ph.D.
Laboratory of Viral Pathogenesis and Immunity	SA Associate Professor Takaaki Nakaya M. D., Ph.D.
Department of Infectious Disease Control	
Laboratory of Genomic Research on Pathogenic Bacteria	SA Professor Tetsuya Iida Ph.D.
Laboratory of Malariology	
Laboratory of Combined Research on Microbiology and Immunology	SA Associate Professor Hiroki Nagai, Ph.D.
Animal Resource Center for Infectious Diseases	Head, Professor Masaru Okabe Ph.D.
DNA-chip Development Center for Infectious Diseases	Head, Professor Hiroshi Nojima Ph.D.
Center for Genetic Analysis of Biological Responses	Head, Professor Masaru Okabe Ph.D.
Office of Combined Program on Microbiology and Immunology	Head, Director Hitoshi Kikutani M. D., Ph.D.
Research Promotion Group	Associate Professsor Yoshiko Murakami M. D., Ph.D.
Education Promotion Group	Associate Professsor Hodaka Fujii M. D., Ph.D.
Research Collaboration Center in Overseas	Head, SA Professor Shigeyuki Hamada, D.D.S., Ph.D.
Section of Bacterial Infections	SA Professor Shigeyuki Hamada, D.D.S., Ph.D.
Section of Viral Infections	SA Professor Masanori Kameoka, Ph.D.
BIKEN Endowed Department of Dengue Vaccine Development	
Central Instrumentation Laboratory	Head,Professor Masato Okada Ph.D.
Radioisotope Laboratory	Head,Professor Masato Okada Ph.D.
Central Laboratory for Biological Hazardous Microbes	Head, Professor Tatsuo Shioda Ph.D.
Library	Head, Professor Yoshiharu Matsuura D. V. M., Ph.D.
Administration	Head, Katsumi Uedono

*SA : Specially Appointed

Staff

2011.04.01

Professor	12
Endowed Chair Professor	0
SA Professor	5
Associate Professor	12
Endowed Chair Associate Professor	0
SA Associate Professor	6
SA Lecturer	2
Assistant Professor	26
Endowed Chair Assistant Professor	0
SA Assistant Professor	10
Educational Support Staff	3
Technical Staff	3
Administrative Staff	20
SA Researcher	46
Research Collaborator	4
Part-time General & Technical staff	40
Total	189

SA : Specially Appointed

Graduate Students

2011.04.01

	Doctor Course	Master Course
Graduate School of Medicine	24	2
Graduate School of Science	4	7
Graduate School of Pharmaceutical Science	1	1
Graduate School of Dentistry	0	0
Graduate School of Frontier Biosciences	7	8
Total	36	18

Research Fellows & Research Students

2011.04.01

Special research students	0
Research Students	7
Visiting Research Scholars	1
JSPS Research Fellows	6
Total	14

Department of Molecular Bacteriology

Research Group		
	Professor	Yasuhiko Horiguchi, D. V. M., D. Agr. Sci.
	Assistant Professor	Shigeki Kamitani, D. M. Sc.
	Assistant Professor	Hiroyuki Abe, Ph. D.
	SA Researcher	Aya Fukui, Ph. D.
	SA Researcher	Hirono Toshima, Ph. D.

The objective of this department is to understand the molecular mechanisms by which pathogenic bacterial virulence factors affect host cell functions. Our present research interests include:

(1) Analysis of the structure and function of bacterial protein toxins.
Bacterial protein toxins, which are the most poisonous substances on the earth, are known to act specifically on a particular cell and a particular biomolecule. To understand how bacterial toxins can act so powerfully and specifically, we are analyzing their effects on the host at the systemic, tissue, cellular and molecular levels. The toxins currently under investigation are *Bordetella* dermonecrotic toxin, *Pasteurella* toxin, *Clostridium perfringens* enterotoxin, and *Escherichia coli* cytotoxic necrotizing factor. We are also analyzing the steric structure and molecular localization of the functional domains of these toxins. These approaches together will help to clarify the structure and function of these bacterial toxins.

(2) Analysis of whooping cough pathogenesis.
Bordetella pertussis, a pathogenic bacteria, infects the human respiratory tract and causes whooping cough, which is characterized by paroxysmal coughing. There are two significant questions about the pathogenesis of *B. pertussis* infection. First, why does *B. pertussis* infect humans but no other mammals? Second, how does this bacterium induce the paroxysmal coughing? We are currently examining the pathology of the disease and the function of *B. pertussis* virulence factors by using an animal model of the infection.

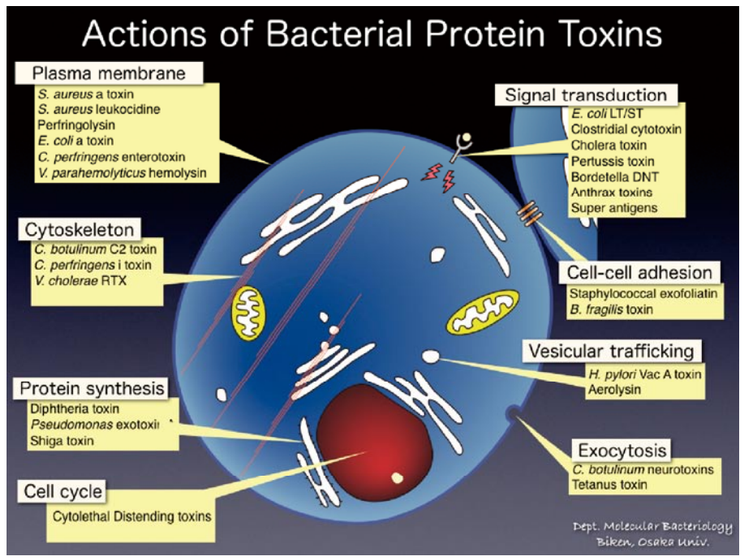


Fig. 1: Bacterial protein toxins with various activities that influence particular cellular functions. Many bacterial protein toxins exert their toxic effects by modifying important functions of the host cells. Significantly, the relevant physiological functions of the cells can also be determined by dissecting the actions of the bacterial toxins.

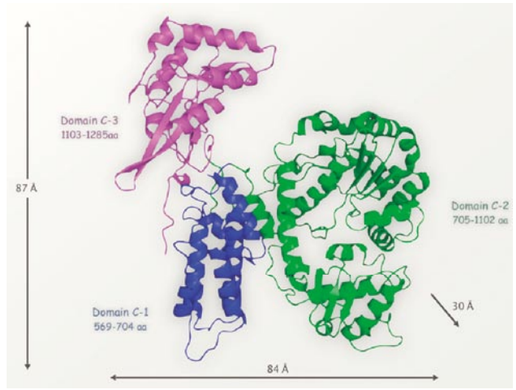


Fig. 2: Overall structure of the intracellular active region of *Pasteurella multocida* toxin, which is composed of three domains and has a Trojan horse-like shape.

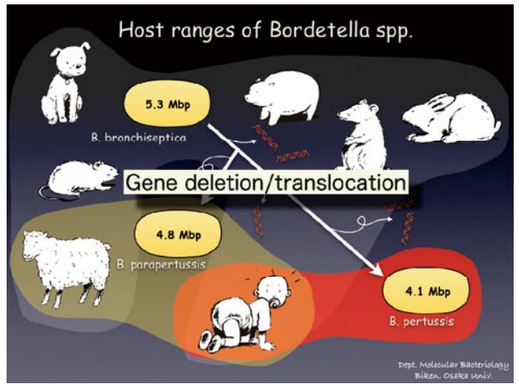


Fig. 3: *Bordetella pertussis*, *B. parapertussis*, and *B. bronchiseptica* are closely-related pathogenic bacteria. *B. bronchiseptica* has the largest genome and the broadest host range, whereas *B. pertussis* has the smallest genome and the narrowest host range. It is believed that *B. pertussis* evolved from a *B. bronchiseptica* lineage through the deletion and/or translocation of a large number of genes.

Recent publications

1. Kimura J, Abe H, Kamitani S, Toshima H, Fukui A, Miyake M, Kamata Y, Sugita-Konishi Y, Yamamoto S, and Horiguchi Y. *Clostridium perfringens* enterotoxin interacts with claudins via electrostatic attraction. J Biol Chem. 2010 Jan 1;285(1):401-8.
2. Miyake M, Sakane S, Kobayashi C, Hanajima-Ozawa M, Fukui A, Kamitani S, and Horiguchi Y. A colorimetric assay for studying effector secretion through the bacterial type III secretion system. FEMS Microbiol Lett. 2008 Jan;278(1):36-42.
3. Ohnishi H, Miyake M, Kamitani S, and Horiguchi Y. FEMS Microbiol Lett. 2008 Feb;279(2):174-9. The morphological changes in cultured cells caused by *Bordetella pertussis* adenylate cyclase toxin. FEMS Microbiol Lett. 2008 Feb;279(2):174-9.
4. Kitadokoro K, Kamitani S, Miyazawa M, Hanajima-Ozawa M, Fukui A, Miyake M, and Horiguchi Y. Crystal structures reveal a thiol protease-like catalytic triad in the C-terminal region of *Pasteurella multocida* toxin. Proc Natl Acad Sci U S A. 2007 Mar 20;104(12):5139-44.
5. Hanajima-Ozawa M, Matsuzawa T, Fukui A, Kamitani S, Ohnishi H, Abe A, Horiguchi Y, and Miyake M. Enteropathogenic *Escherichia coli, Shigella flexneri,* and *Listeria monocytogenes* recruit a junctional protein, zonula occludens-1, to actin tails and pedestals. Infect Immun. 2007 Feb;75(2):565-73.

Department of Viral Infections

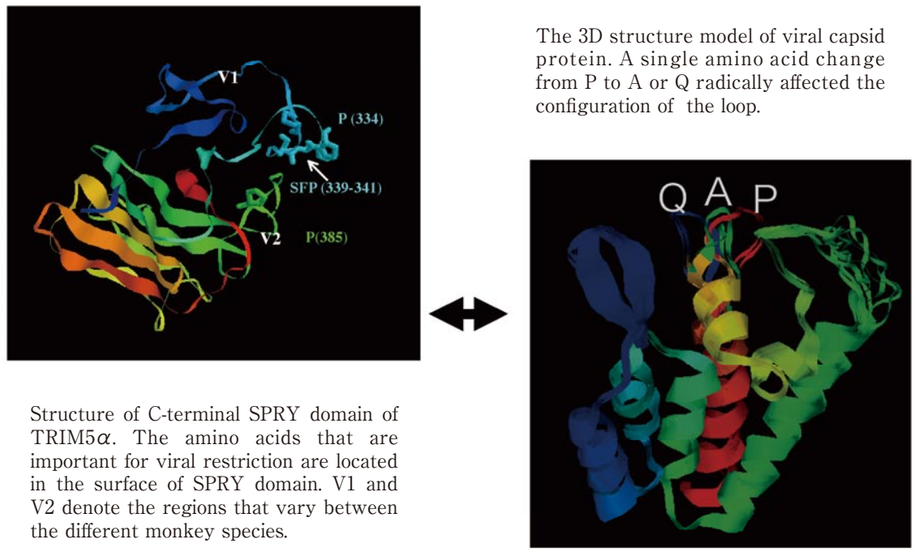
Research Group

Professor	Tatsuo Shioda, D.Med.Sc.
Assistant Professor	Jun-ichi Sakuragi, D.Med.Sc.
Assistant Professor	Emi E. Nakayama, M.D., D.Med.Sc.
Postdoctoral Fellow	Ken Kono, D.Med.Sc.

The main focus in this department is to elucidate the molecular mechanisms of viral diseases including human immunodeficiency virus (HIV) infection. The following projects are currently underway.

(1) Anti-retroviral factors

HIV does not establish a productive infection in any other monkey except for the chimpanzee; this is thought to be due to inhibitors in simian lymphocytes that act at the early stage (reverse transcription) of viral infection. To date, TRIM5*α* and cyclophilin A have been identified as such restriction factors. We had shown that differences in the amino acid sequences in the C-terminal domain of TRIM5*α* of different monkey species affect the species-specific restriction of retrovirus infection (Fig.1, left). We also found that sequence variations in the N-terminal half of the viral capsid protein (Fig. 1, right) determine viral sensitivity to TRIM5*α*-mediated restriction, which indicates that there is an interaction between TRIM5*α* and the virus capsid. In addition, we showed that HIV-2 replication levels in infected individuals are associated with capsid variations, and we suggested that viral sequence analysis can predict AIDS progression. Furthermore, we have succeeded in improving the simian-tropic HIV-1 virus and the methods of monkey genome analysis. These new developments greatly facilitate the generation of an HIV-1 animal model, which would be a highly useful tool in research aiming to understand AIDS pathogenesis and to develop an effective vaccine. We are also seeking to identify the binding surface between the viral capsid protein and TRIM5*α*, as this may be useful for the development of new anti-retroviral drugs.



Structure of C-terminal SPRY domain of TRIM5α. The amino acids that are important for viral restriction are located in the surface of SPRY domain. V1 and V2 denote the regions that vary between the different monkey species.

The 3D structure model of viral capsid protein. A single amino acid change from P to A or Q radically affected the configuration of the loop.

Fig. 1 Structural models of TRIM5*α* (left) and the viral capsid (right).

(2) Host factors that participate in HIV pathogenesis and anti-retroviral drug side-effects.

As an animal model of AIDS has not yet been established, we are utilizing epidemiological procedures to understand the mechanisms of AIDS pathogenesis. There are cases who are not infected despite repeated exposure to HIV. There are also HIV-positive patients who do not develop symptoms of AIDS despite not receiving any anti-retroviral treatment. These cases are suspected to bear a resistance-inducing factor (RIF) against HIV. To characterize these RIFs, we have compared the genome sequences of the cases described above to those of HIV-infected patients and uninfected individuals. We found (a) the deletion mutant CCR5-893 (-), which fails to produce a co-receptor that is needed for HIV entry, (b) a polymorphism in the promoter of the chemokine RANTES, and (c) a polymorphism in the promoter of IL4, which regulates the expression of the co-receptor. We then demonstrated that these mutations affect susceptibility to HIV infection and the rate with which the disease progresses to AIDS.

At present, in collaboration with Thai groups, we are also focusing on the relationship between human genomic variation and anti-retroviral therapy side-effects, with the aim of establishing "tailor-made therapies" that will improve the quality of life of HIV-infected patients.

(3) Molecular mechanisms of HIV particle formation.

The HIV genomic RNA always forms dimers in the mature virion. It was suggested previously that the presence of the dimerized genome in the virion is advantageous for survival, as it provides an extra template that can be used when one RNA molecule is damaged; it may also endow the progeny with genetic variety. We were able to identify the minimal HIV genome region that is sufficient for genome dimerization. Our data suggest that RNA dimerization is part of RNA packaging. We also found that HIV genome dimerization affects the early stage of HIV replication after its entry into cells.

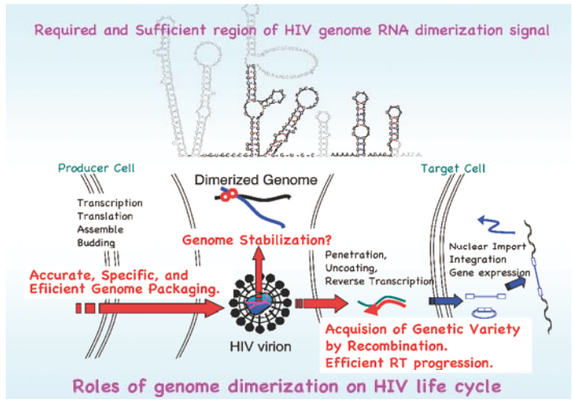


Fig. 2 HIV-1 genome RNA dimeriation.

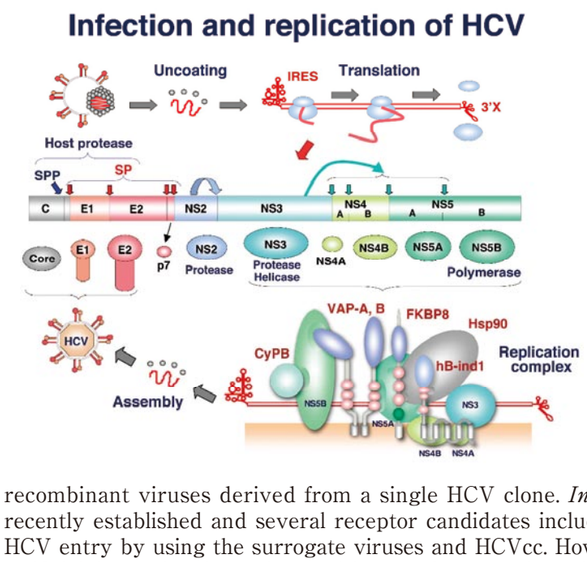
Recent publications

1. The relationship between HIV-1 genome RNA dimerization, virion maturation and infectivity. Ohishi M, Nakano T, Sakuragi S, Shioda T, Sano K, Sakuragi JI. Nucleic Aids Res. 2011Apr 1;39(8):3404-17.
2. Anti-retroviral activity of TRIM5alpha. Nakayama EE, ShiodaT. Rev Med Virol. 2010 Mar;20(2):77-92.
3. TIM1 haplotype may control the disease progression to AIDS in a HIV-1-infected female cohort in Thailand. Wichukchinda N, Nakajima T, Saipradit N, Nakayama EE, Ohtani H, Rojanawiwat A, Pathipvanich P, Ariyoshi K, Sawanpanyalert P, Shioda T, Kimura A. AIDS. 2010 Jul 17;24(11):1625-31.
4. HIV-2 capsids distinguish high and low virus load patients in a West African community cohort. Onyango CO, Leligdowicz A, Yokoyama M, Sato H, Song H, Nakayama EE, Shioda T, de Silva T, Townend J, Jaye A, Whittle H, Rowland-Jones S Cotton M. Vaccine. 2010 May 26; 28 Suppl 2:B60-7.
5. HLA-Cw*04 allele associated with nevirapine-indued rash in HIV-infected Thai patients. Likanonsakul S, Rattanatham T, Feangvad S, Uttayamakul S, Prosithsirikul W, Tunthanathip P, Nakayama EE, Shioda T. AIDS Res Ther. 2009 Oct 21;6:22.

Department of Molecular Virology

Research Group			
	Professor	Yoshiharu Matsuura	DVM, PhD
	Assistant Professor	Eiji Morita	PhD
	SA Assistant Professor	Hiroto Kambara	PhD
	Postdoctoral Fellow	Yuuki Kaname	PhD
	Postdoctoral Fellow	Takasuke Fukuhara	MD, PhD
	Postdoctoral Fellow	Hiroshi Kato	DVM, PhD
	Postdoctoral Fellow	Chikako Ono	PhD

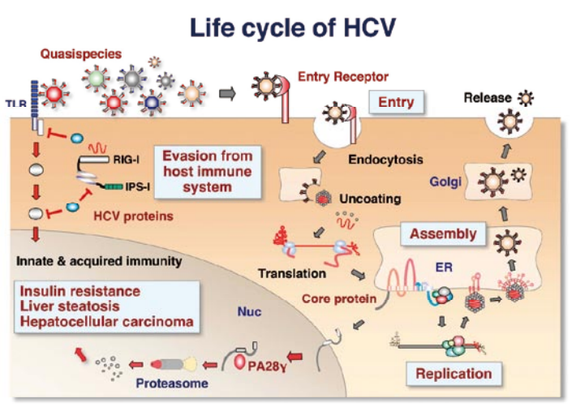
We are working for the understanding of the molecular mechanisms of entry, replication, immune escape, and pathogenesis of hepatitis C virus (HCV) and the development of a novel virus vector for gene delivery.



1. Studies on molecular biology of HCV replication and pathogenesis

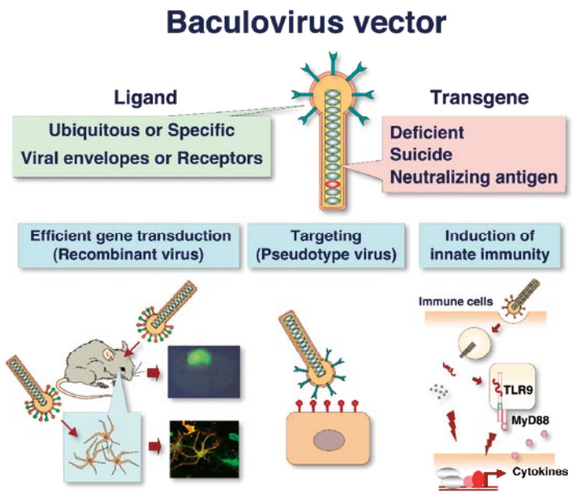
HCV has infected more than 3% of the worldwide population and 80% of these individuals will develop persistent infection. Persistent HCV infection often leads to chronic hepatitis, cirrhosis and hepatocellular carcinoma. The incidence of hepatitis C has significantly decreased since introduction of screening system for anti-HCV antibodies in 1999, however, more than two million people have already been infected with HCV in Japan. The proportion of patients achieving a sustained virological response has increased by the combination therapy of pegylated-IFNα and ribavirin. However, half of the patients with HCV genotype 1 exhibits no response to the combination therapy. HCV exhibits quasispecies heterogeneity and it is difficult to assess infection mechanisms by using surrogate systems such as pseudotype and recombinant viruses derived from a single HCV clone. *In vitro* replication of genotype 2a HCV (HCVcc) has recently established and several receptor candidates including hCD81, SR-BI, and Claudins were identified for HCV entry by using the surrogate viruses and HCVcc. However, a high level of neutralization antibodies to the artificialviruses has been detected in sera from persistently infected patients, suggesting that these antibodies do not play a crucial role in the clearance of HCV. Furthermore, HCV NS3/4A protease was shown to cleave adaptor molecules involved in the TLR- and RIG-I-dependent signaling pathways. HCV particles are internalized into cells through endocytosis. After uncoating, a viral RNA is translated into a large precursor polyprotein composed of 3,000 amino acids. This viral polyprotein is cleaved by signal peptidase (SP), signal peptide peptidase (SPP) and viral encoded proteases, resulting in at least 10 viral proteins. The open reading frame of the polyprotein is flanked at both ends by highly conserved untranslated regions (UTRs), which are required for viral RNA replication. The 5'-UTR harbors an internal ribosome entry site (IRES) that is essential for Cap-independent translation of viral RNA.

Although novel innovative agents in clinical development have been shown to have significant antiviral activity in patients with HCV, drug resistant viruses are easily emerged. Therefore, host proteins indispensable for HCV replication are ideal targets for the development of new therapeutics for chronic hepatitis C with a broad spectrum and a low possibility of emergence of breakthrough viruses against antiviral drugs. HCV belongs to the family of *Flaviviridae* which includes flavivirus such as Japanese encephalitis virus (JEV) that has a robust cell culture system and a small animal model. We are investigating the replication and pathogenesis of JEV as a surrogate model for HCV.



2. Development of baculoviral vectors

Viral vectors are essential tools for the studies on the replication deficient viral infectious diseases, such as HCV. Furthermore development of novel viral vectors is essential for future gene therapy. We are working on the baculovirus *Autographa californica* nucleopolyhedrovirus (AcNPV) as a versatile viral vector for gene delivery not only *in vitro* but also *in vivo*. AcNPV is an insect virus possessing a 134-kb double-stranded circular DNA genome. Due to the strong promoters, baculovirus is commonly used as a tool for the large-scale production of recombinant protein in insect cells. Baculovirus is also capable of entering into a variety of mammalian cells to facilitate the expression of foreign genes under the control of the mammalian promoters without replication of the viral genome. Therefore, baculovirus is a useful viral vector, not only for the abundant expression of foreign genes in insect cells, but also for efficient gene delivery to mammalian cells. AcNPV has a number of unique beneficial properties as a viral vector, including a large capacity for foreign gene incorporation, easy manipulation, and replication competence in insect cells combined with incompetence in mammalian cells. Therefore, the possibility of generating replication-competent revertants expressing baculoviral gene products, which can often lead to harmful immune responses against mammalian cells, is significantly lower than other viral vectors currently in use. Furthermore, intranasal inoculation with AcNPV induces a strong innate immune response, protecting mice from lethal challenges of influenza viruses. We demonstrated that internalization of viral DNA via membrane fusion by the envelope glycoprotein in the endosome is required for the induction of innate immune response by AcNPV through a TLR9/MyD88-dependent pathway. This finding raises the possibility that AcNPV may be harnessed therapeutically to induce host immune response against various infectious diseases, especially caused by the pathogens invading from respiratory tract.



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1. Moriishi K, Shoji I, Mori Y, Suzuki R, Suzuki T, Kataoka C, Matsuura Y. Involvement of PA28gamma in the propagation of hepatitis C virus. Hepatology. 2010 Aug;52(2):411-20.
2. Kaname Y, Tani H, Kataoka C, Shiokawa M, Taguwa S, Abe T, Moriishi K, Kinoshita T, Matsuura Y. Acquisition of complement resistance through incorporation of CD55/decay-accelerating factor into viral particles bearing baculovirus GP64. J Virol. 2010 Apr;84(7):3210-9.
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5. Abe T, Kaname Y, Wen X, Tani H, Moriishi K, Uematsu S, Takeuchi O, Ishii KJ, Kawai T, Akira S, Matsuura Y. Baculovirus induces type I interferon production through toll-like receptor-dependent and -independent pathways in a cell-type-specific manner. J Virol. 2009 Aug;83(15):7629-40.

Department of Molecular Immunology

Research Group		
	Professor	Hitoshi Kikutani, M.D., Ph.D.
	Associate Professor	Teruhito Yasui, Ph.D.
	Postdoctoral fellow	Olivia A. Simma, Ph.D.
	Postdoctoral fellow	Kentaro Morita, Ph.D.
	Postdoctoral fellow	Takeharu Minamitani, Ph.D.
	Postdoctoral fellow	Chiau-Yuang Tsai, Ph.D.

Molecular mechanisms involved in the regulation of immune responses.
T cells are activated by MHC-bound antigenic peptides on antigen-presenting cells. Once activated, the T cells differentiate into functional, helper, or effector T cells. In contrast, antigen-stimulated B cells differentiate into antibody-forming or memory B cells with the help of antigen-specific T cells. Thus, T- and B-cell differentiation requires physiological interactions between T cells and antigen-presenting cells, and between T cells and B cells, respectively. Such cell-cell interactions are mediated by a variety of costimulatory molecules, including CD40, CD40 ligand, B-7 and CD28. In addition, it was revealed recently that several members of the semaphorin family play crucial roles in immune cell interactions. We are currently studying how these molecules function in the regulation of immune responses.

A) Mechanisms by which semaphorin molecules regulate immune responses:
The semaphorin family molecules were first identified as axonal guidance factors that function during neuronal development. However, a series of studies by our laboratory has shown that several semaphorin molecules play crucial roles at various stages of immune responses (Figure 1). For instance, Sema4D/CD100 is involved in the activation of B cells and dendritic cells, while Sema4A participates in both T-cell priming and Th1 differentiation. The interaction between Sema6D and its receptor Plexin-A1 was also shown to participate in cellular immune responses since it activates dendritic cells and promotes bone homeostasis by inducing osteoclastgenesis. Furthermore, we demonstrated recently that Sema7A on activated T cells stimulates macrophages, which then produce inflammatory cytokines; it also triggers inflammatory responses through $\alpha 1\beta 1$ integrin (Figure 2).

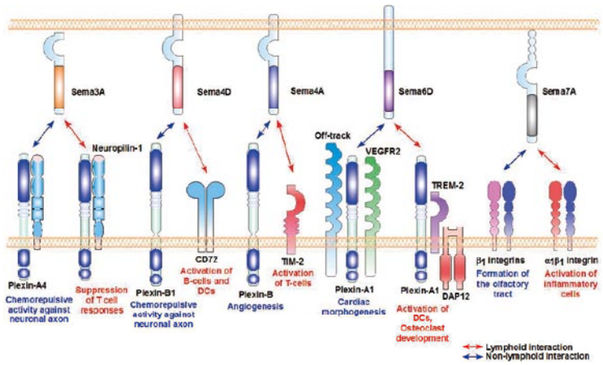

Fig. 1 Representative immune semaphorins
Semaphorins and their receptors have been shown by our research group to participate in immune regulation.

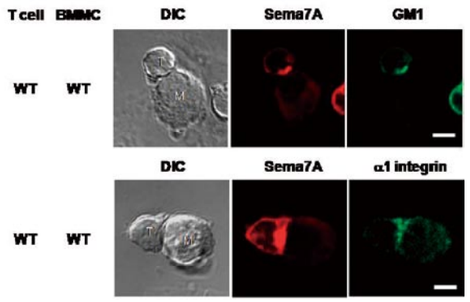

Fig. 2. Accumulation of Sema7A and $\alpha 1$ integrin in the immunological synapse between T cells and macrophages.

B) Elucidation of the molecular mechanism by which B cells survive and differentiate into effector cells:
Effective responses to the invasion of non-self antigen-bearing entities require that B cells differentiate into antibody-secreting cells and memory B cells. B cell survival and differentiation are driven by B cell-antigen receptor (BCR) signaling along with the signals of members of the TNF receptor family, such as CD40 and BAFF-R, on the B cell surface. To date, our group has demonstrated the immunological significance of the molecules that are involved in the signaling pathways downstream of CD40. In particular, we found that TRAF3, which interacts with the cytoplasmic region of both CD40 and BAFF-R, plays a crucial role in B cell survival and differentiation. Furthermore, we identified a PKC family member, PKN1, which is associated with the TRAF family and serves as a negative regulator of Akt in BCR signaling. Our observations together suggest that PKN1 may be responsible for the immunological tolerance that eliminates autoreactive B cells.

Molecular mechanism by which Epstein-Barr virus (EBV) induces immunological disorders.
EBV is a human herpes virus that causes infectious mononucleosis in healthy donors and proliferative disorders in patients who are immunosuppressed because of aging, immunosuppressive therapy, or HIV infection. It appears that EBV infection may be associated with B cell malignancies such as Burkitt's lymphomas and Hodgkin's lymphomas. It may also be linked to autoimmune diseases such as systemic lupus erythematosus (SLE) and multiple sclerosis (MS). EBV infects B cells in a latent fashion and is prevalent worldwide. We are currently studying EBV biology to determine how EBV leads to human carcinogenesis. The outcomes of this study may also reveal attractive therapeutic strategies for EBV-associated immune disorders (Figure 3).

A) The molecular mechanism by which EBV infects the human host:
EBV infection induces B cell growth transformation and immortalization. The mechanism by which EBV invades B cells involves multiple steps, namely virus entry, latency, and lytic infection. We are currently seeking to establish a system that will allow us to trace in vitro the infection dynamics of EBV and the frequency of cell growth transformation. This valuable system involves the production of recombinant EBV particles that carry the gene for GFP, which facilitates the visualization of EBV as it infects human peripheral B cells (Figure 4).

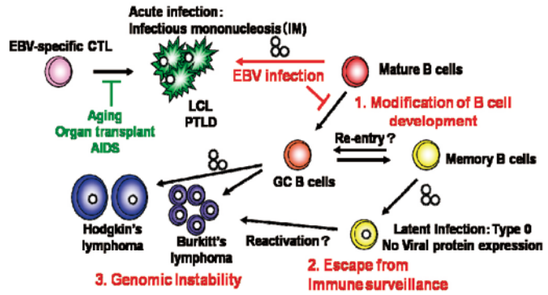

Fig.3. EBV and the host immune system.
The mechanism by which EBV induces human B cell growth transformation is associated closely with the vulnerability of the host immune system.

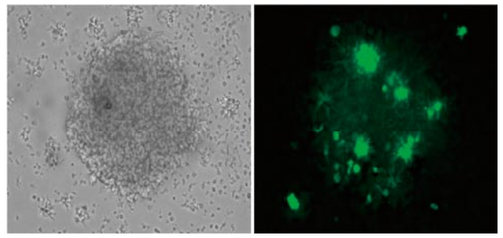

Fig.4. Immortalization of human peripheral blood B lymphocytes by recombinant EBV particles that carry the GFP gene.

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1. Tada S, Okuno T, Yasui T, Nakatsuji Y, Sugimoto T, Kikutani H, Sakoda S. Deleterious effects of lymphocytes at the early stage of neurodegeneration in an animal model of amyotrophic lateral sclerosis. J Neuroinflammation. 2011 Feb 23;8(1):19.
2. Takamatsu H, Takegahara N, Nakagawa Y, Tomura M, Taniguchi M, Friedel RH, Rayburn H, Tessier-Lavigne M, Yoshida Y, Okuno T, Mizui, M, Kang S, Nojima S, Tsujimura T, Nakatsuji Y, Katayama I, Toyofuku T, Kikutani H, Kumanogoh A. Semaphorines guid the entry of dendritic cells into the lymphatics by activating myosin II. Nat. Immunol. 2010 Jul;11(7):594-600.
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5. Suzuki K, Kumanogoh A, Kikutani H. Semaphorins and their receptors in immune cell interactions. Nat Immunol. 2008 Jan;9(1):17-23.

Department of Immunoregulation

Research Group		
	Professor (SUP)	Taroh Kinoshita, D. Med. Sc.
	Associate Professor	Yusuke Maeda, M.D., D. Med. Sc.
	Associate Professor (SUP)	Yoshiko Murakami, M.D., D. Med. Sc.
	Assistant Professor	Yasuhiro Morita, Ph. D.
	SA Assistant Professor	Morihisa Fujita, D. Sc.
	Postdoctoral Fellow (SUP)	Yetao Wang, M.D., D. Med. Sc.
	Postdoctoral Fellow	Noriyuki Kanzawa, D. Med. Sc.

1) Biogenesis, transport and remodeling of GPI-anchored proteins (GPI-APs).
Glycosylphosphatidylinositol (GPI) is a glycolipid that consists of phosphatidylinositol, glucosamine, mannoses and phosphoethanolamines, and acts as a lipid anchor for various plasma-membrane proteins. GPI-APs play important roles in host self-defense, intercellular signal transduction, and other important processes. In addition, some GPI-APs function as receptors for certain viruses and toxins. The GPI-anchor is widely distributed and conserved in various eukaryotes and is essential for the development of higher animals, as well as for the growth of yeasts and protozoan parasites. The modification of proteins due to the attachment of the GPI-anchor functions as a protein localization and sorting signal. Our current project is to identify and clarify the functions of all the genes involved in the biosynthesis of the GPI-anchor in the ER (PIG genes; PhosphatidylInositol glycan) and in the sorting and localization of GPI-APs after their anchorage with GPI (PGAP genes; Post GPI-Attachment to Proteins). We expect that these studies will reveal why many proteins are modified with the GPI-anchor.

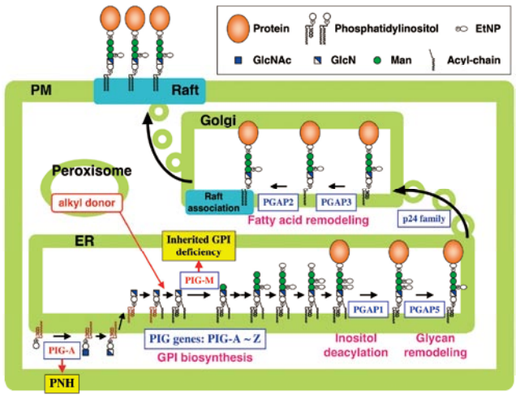

Fig. 1 GPI-anchor biosynthesis and the transport/remodeling of GPI-APs.
PIG genes are involved in the biosynthesis of the GPI-anchor in the ER. Thereafter, GPI-APs are transported to the plasma membrane and enriched in rafts. PGAP genes are involved in these later processes. PGAP1 and PGAP5, which localize in the ER, and PGAP2 and PGAP3, which localize in the Golgi, are involved in the lipid or glycan remodeling of the GPI-anchor. We found that the remodeling affects the sorting of GPI-APs because it alters the physical characteristics of the GPI-anchor.

2) Molecular genetics of acquired (paroxysmal nocturnal hemoglobinuria, PNH) and inherited GPI deficiencies.
PNH is an acquired hematopoietic stem cell disorder in which clonal cells that are defective in GPI biosynthesis are expanded. As a result, abnormal erythrocytes that lack CD59 and DAF/CD55 predominate. CD59 and DAF/CD55 are widely distributed GPI-anchored proteins that inhibit the activation of complement on the host cell surface, and their absence on erythrocytes makes these cells very sensitive to complement and lysis during infections and other events. We are proposing a three-step model of PNH pathogenesis. Step 1 involves the generation of GPI-deficient hematopoietic stem cells due to the somatic mutation of the PIG-A gene. Step 2 involves the immunological selection of GPI-deficient hematopoietic stem cells. In this step, GPI-deficient cells not only survive, but they also proliferate much more frequently than usual to compensate for anemia. This elevated proliferation rate may increase the chance that additional genetic mutations are acquired, which leads to Step 3, where a subclone bearing the growth phenotype is generated (Fig. 2). We identified HMGA2 as the candidate gene for Step 3.
Along with our colleagues in England, we have also identified a novel disease that is characterized by venous thrombosis and seizures, and is caused by a GPI deficiency that has been inherited in an autosomal recessive manner. The patients have a point mutation in the promoter of PIG-M, a mannosyltransferase-encoding gene that plays an essential role in GPI biosynthesis. The point mutation severely reduces PIG-M expression and leads to partial GPI deficiency. While complete GPI deficiency is lethal, partial GPI deficiency could be caused by a partial defect in one of the GPI biosynthesis genes, and the symptoms may vary depending on the defect.

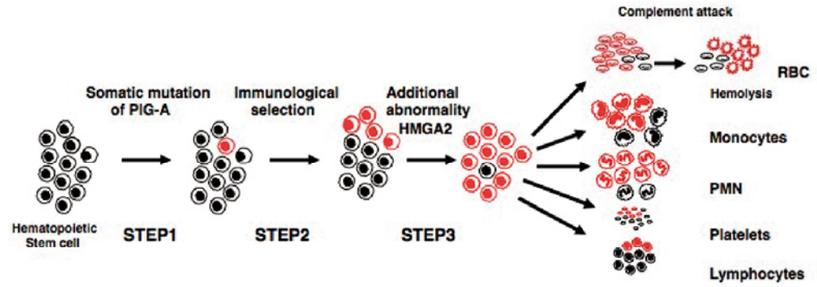

Fig. 2 Pathogenesis of PNH
Step 1 involves the generation of GPI-deficient hematopoietic stem cells due to the somatic mutation of the PIG-A gene. Step 2 involves the immunological selection of GPI-deficient hematopoietic stem cells. In this step, GPI-deficient cells survive and proliferate much more frequently than usual to compensate for anemia. This elevated proliferation may increase the chance that additional genetic mutations occur. Step 3 involves the generation of a subclone bearing the growth phenotype.

3) Glycolipid biosynthesis in pathogens and its use in drug development.
Our research focuses on elucidating the biosynthesis of GPIs in mycobacteria and *Trypanosoma brucei*. *T. brucei* is the causative agent of African sleeping sickness while mycobacteria cause a number of diseases, including tuberculosis. GPIs are located on the cell surface of these pathogens and appear to play key roles in their evasion of host immune attack. In particular, GPI-like molecules found in mycobacteria have anti-inflammatory activities and are thought to be important for the establishment of the infection. We aim first to identify the genes that are involved in these GPI biosynthetic pathways, after which we can create and characterize gene deletion/overexpression mutants. This research will help us to understand the roles these GPI molecules play at the molecular level in cell surface structure maintenance and host immune response modulation. We are also seeking to determine the key enzymes of the biosynthetic pathways and develop a high-throughput screening system that will help us to identify lead compounds in drug libraries.

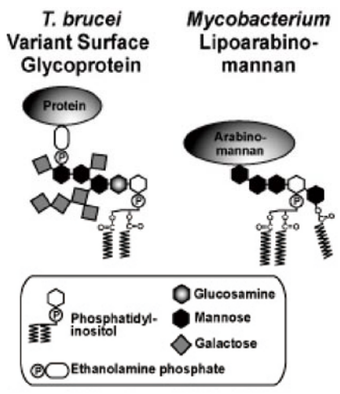

Fig. 3 Structure of the GPIs of pathogens

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2. Kanzawa N, Maeda Y, Ogiso H, Murakami Y, Taguchi R, Kinoshita T. Peroxisome dependency of alkyl-containing GPI-anchor biosynthesis in the endoplasmic reticulum. Proc Natl Acad Sci U S A. 2009 Oct 20;106(42):17711-6.
3. Fujita M, Maeda Y, Ra M, Yamaguchi Y, Taguchi R, Kinoshita T. GPI glycan remodeling by PGAP5 regulates transport of GPI-anchored proteins from the ER to the Golgi. Cell. 2009 Oct 16;139(2):352-65.
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Department of Host Defense

Research Group		
	Professor (SUP)	Shizuo Akira
	Associate Professor	Osamu Takeuchi
	Associate Professor	Taro Kawai
	Associate Professor (SUP)	Satoshi Uematsu
	Assistant Professor	Tatsuya Saito
	SA Assistant Professor	Yutaro Kumagai

Our laboratory studies pathogen recognition by the innate immune system and the mechanisms that regulate innate immune responses. The innate immune system senses invading microbial pathogens, such as bacteria, viruses and parasites, and plays an essential role in inducing inflammatory responses and assisting adaptive immune responses. Pattern-recognition receptors (PRRs) expressed on innate immune cells such as macrophages and dendritic cells recognize pathogen-associated molecular patterns (PAMPs), which are conserved molecular features of microbial pathogens. We are seeking to clarify the complex regulatory mechanisms of the innate immune system.

1) Characterization of the pathogen recognition by Toll-like receptors (TLRs) and their signaling pathways
TLR family members play essential roles in the recognition of pathogens by the innate immune system. Their signaling pathways also play an important role in the gene induction involved in inflammation and immune responses. We have identified many TLR family members and their signaling molecules, and have characterized their functions by generating knockout mice. As a result, we have identified most of the ligands for these TLR family members and their signaling pathways (Figure 1). We also found that the stimulation of TLRs induces not only proinflammatory cytokine genes, but also type I interferon genes. For example, the TRIF-TBK1/IKK-i-IRF-3 pathway plays an important role in the TLR3- and TLR4-mediated induction of IFN-β (Figure 2). Moreover, TLR7 and TLR9 are preferentially expressed in plasmacytoid dendritic cells (pDCs), which produce large amounts of IFN-α upon viral infection. We identified a specific signaling pathway in pDCs that is stimulated by TLR7 and TLR9 ligands and induces IFN-α expression (Figure 2). In summary, TLR signaling is regulated by distinct and complex mechanisms that operate in a ligand- and cell-type specific manner. We are currently expanding our understanding of the in vivo functions of TLRs and their signaling pathways.

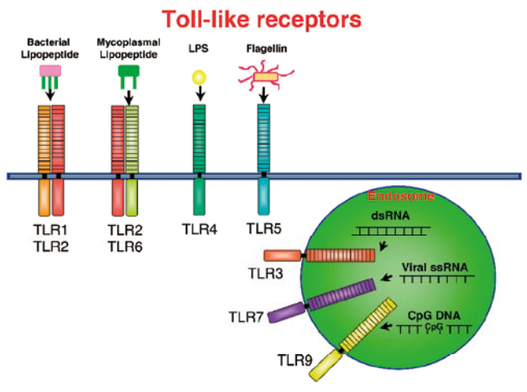

Figure 1: Pathogen recognition by TLRs. TLRs recognize molecular patterns associated with a broad range of pathogens, including bacteria, fungi, protozoa and viruses.

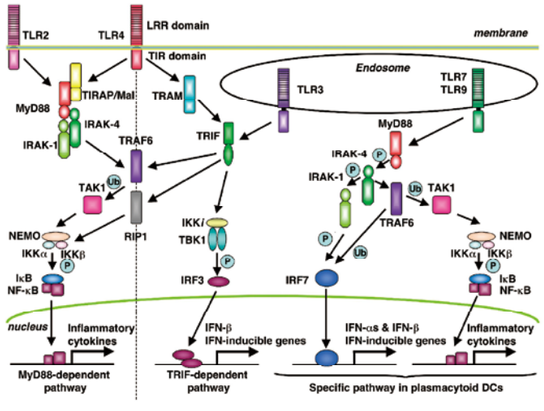

Figure 2: TLR signaling pathways. All TLR family members apart from TLR3 share a common pathway called the MyD88-dependent pathway that induces inflammatory cytokine production. Each TLR family member also has its own specific signaling pathway. Thus, TLR3 and TLR4 operate via a TRIF-dependent pathway while TLR7 and TLR9 act in pDCs via a unique pathway to induce IFN-α expression.

2) Therapeutic applications of TLR agonists and antagonists
Appropriate agonist-induced stimulation of TLRs could stimulate an innate immune response that boosts host resistance to cancer, allergy, and infectious diseases. This approach could also be used to promote the development of an adaptive immune response to a co-administered vaccine. TLR antagonists may also have therapeutic potential, as they could prevent or ameliorate the inappropriate or exaggerated TLR stimulation that leads to deleterious outcomes such as autoimmune diseases, sepsis or atherosclerosis.

3) Investigation of pathogen recognition mechanisms by cytoplasmic receptors.
Infection with pathogens such as viruses induces type I IFNs in both a TLR-dependent and a TLR-independent manner. RIG-I and MAD5, which are RNA helicases that recognize viral RNAs, recognize different viruses and are important for host antiviral responses (Figure 3). We also identified a new adaptor molecule, IPS-1, which plays an essential role in RIG-I- and MDA5-mediated antiviral responses (Figure 3). We are currently exploring these TLR-independent mechanisms further by generating knockout mice.

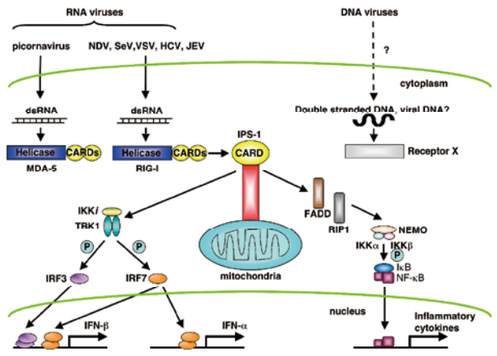

Figure 3: Signaling pathways employed by anti-viral RNA helicases. Viruses produce dsRNA during their replication in the host cell cytoplasm. RIG-I and Mda5 recognize this viral RNA and initiate antiviral signaling. In this signaling pathway, IPS-1 interacts with RIG-I and Mda5 via the CARD-like domain, and this leads to the TBK1- and IKKi-dependent phosphorylation and activation of IRF3 and IRF7. IPS-1 also activates NF-kB via FADD/RIP1-dependent pathways. In addition, synthetic dsDNA activates type I IFN promoters, although the receptor responsible for the dsDNA recognition has not yet been identified.

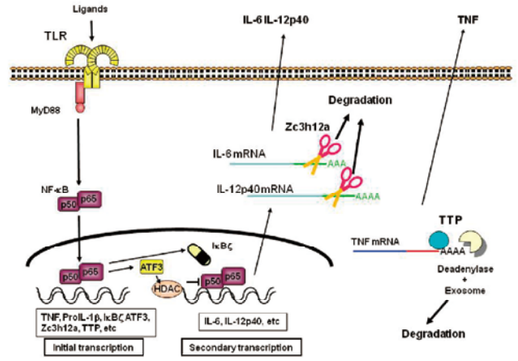

Figure 4: Mechanisms that regulate the inflammatory responses generated by TLR-inducible genes. TLR-inducible IkBz induces the transcription of genes such as Il6 via NFkBp50. In contrast, Zc3h12a, another TLR-inducible gene, functions as an Rnase that degrades the mRNAs for Il6 and Il12, among others, and negatively regulates inflammatory responses. TTP is also known to be involved in the degradation of TNF mRNA.

4) Investigation of the mechanisms that regulate inflammatory responses.
The inflammatory responses that are elicited by the activation of innate immunity are properly regulated by various mechanisms. Our recent studies revealed that TLR signal-inducible molecules further positively and negatively regulate inflammatory responses in response to infection. For example, the TLR-inducible nuclear factor IkBz functions as a transcriptional modulator that is responsible for inflammatory cytokine production (Figure 4). In contrast, TLR-inducible Zc3h12a, an RNase, destabilizes a set of mRNAs such as Il6 and negatively regulates inflammation. Mice that lack Zc3h12a spontaneously develop severe autoimmune inflammatory diseases. Currently, we are examining the posttranscriptional regulation of inflammatory responses.

Recent publications

1. Saitoh T, Satoh T, Yamamoto N, Uematsu S, Takeuchi O, Kawai T, Akira S. Antiviral protein Viperin promotes Toll-like receptor 7- and Toll-like receptor 9-mediated type I interferon production in plasmacytoid dendritic cells. Immunity. 2011 Mar 25;34(3):352-63.
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3. Satoh T, Takeuchi O, Vandenbon A, Yasuda K, Tanaka Y, Kumagai Y, Miyake T, Matsushita K, Okazaki T, Saitoh T, Honma K, Matsuyama T, Yui K, Tsujimura T, Standley DM, Nakanishi K, Nakai K, Akira S. The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. Nat Immunol. 2010 Oct;11(10):936-44.
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5. Takeuchi O, Akira S. Pattern Recognition Receptors and Inflammation. Cell. 2010 Mar 19;140(6):805-20. Review.

Department of Cell Biology

Research Group

Professor	Eisuke Mekada, Ph.D.
Associate Professor	Ryo Iwamoto, Ph.D.
Assistant Professor	Hiroto Mizushima, Ph.D.
SA Researcher	Takashi Nakamura, Ph.D.
SA Researcher	Tomoya Hikita, Ph.D.
SA Researcher	Mizuho Sato, Ph.D.

We are studying cell growth and differentiation mechanisms that involve growth factors and adhesion molecules presented at cell-cell contact sites. In particular, we are focusing on the mode of action of HB-EGF, a membrane-anchored EGF family of growth factors, and the molecules to which they bind, namely the tetraspanin family. These proteins function in morphogenesis and tissue maintenance and repair by regulating cell proliferation, migration and adhesion. They are also involved in the growth, invasion and metastasis of cancer cells.

1) Mode of action of HB-EGF
HB-EGF is a member of the EGF family of growth factors and binds to and activates EGFR and ErbB4. It is synthesized as proHB-EGF, a membrane-anchored precursor protein, and is cleaved on the cell surface to yield the soluble growth factor (sHB-EGF). The conversion of proHB-EGF into the soluble form is critical for the activity of this growth factor, and therefore this process is tightly regulated. HB-EGF is secreted by various tissues and cells and functions in several physiological processes. For example, it maintains heart muscle function, suppresses the cell proliferation involved in heart valve and lung alveolar development, promotes the cell migration that participates in wound healing and eyelid closure, supports blastocyst attachment to the uterus during implantation, and promotes the cell proliferation involved in skin hyperplasia. ProHB-EGF is not only a precursor of the soluble form, it is also a biologically active molecule that regulates the growth of neighboring cells in a juxtacrine fashion. How is the conversion of the membrane-anchored form into the soluble form regulated? How does HB-EGF function in the manifold physiological processes that are dependent on this molecule? What roles do sHB-EGF and proHB-EGF play in such physiological processes? Do they participate in pathological processes? These questions are currently being analyzed at the molecular level.

2) Development of anti-cancer drugs targeting HB-EGF
HB-EGF is involved in the growth, invasion and metastasis of various cancers. We are developing new anti-cancer drugs that target HB-EGF, and pre-clinical and clinical studies assessing an anti-HB-EGF monoclonal antibody and a non-toxic mutant protein of diphtheria toxin CRM197 are currently in progress.

3) CD9 and tetraspanin function
CD9, a member of the tetraspanin superfamily, is a membrane protein with four transmembrane domains. It associates with proHB-EGF and upregulates proHB-EGF function. CD9 is also involved in cell signaling, growth, motility, and adhesion, and in tumor cell metastasis and sperm-egg fusion. In addition, the Caenorhabditis elegans tetraspanin TSP-15 is essential for the epidermal integrity of the worm. We are analyzing the physiological activity of CD9 and other tetraspanins by using genetically engineered mice or C. elegans worms that lack CD9 or other tetraspanins.

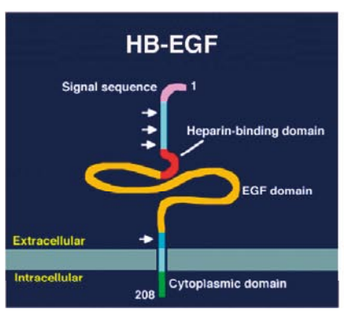

Fig. 1. Structure of proHB-EGF.

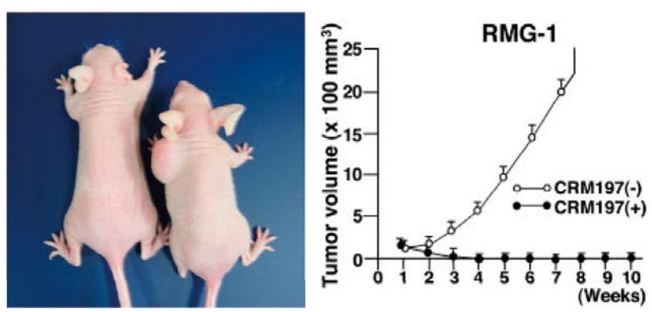

Fig. 2. Tumorigenesis in nude mice explanted with ovarian cancer cells (left) and inhibition of this tumorigenesis by CRM197, a non-toxic mutant of diphtheria toxin (right).

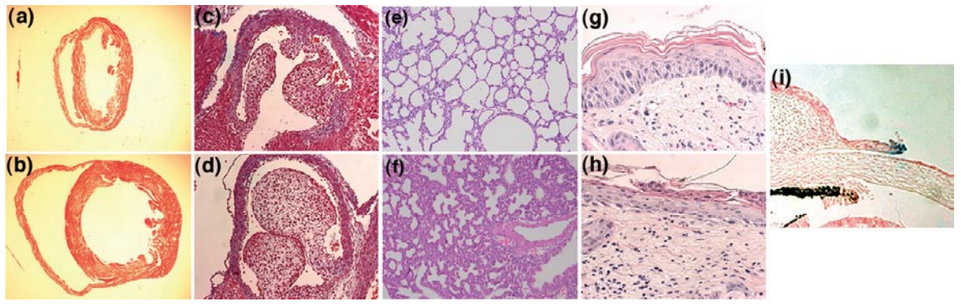

Fig. 3. HB-EGF KO mice exhibit several tissue abnormalities. Unlike WT mice (a, c, e, g), KO mice (b, d, f, h) show abnormal phenotypes in the heart (a, b), heart valves (c, d), and lung alveoli (e, f), as well as retinoid-induced skin hyperplasia (g, h). HB-EGF KO embryos also show defects in eyelid closure because HB-EGF is normally expressed at the tip of the leading edge of migrating epithelium (i).

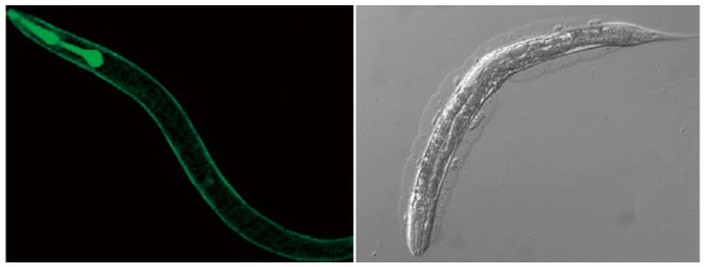

Fig. 4. Expression of TSP-15 in C. elegans (left) and reduction of tsp-15 function in C. elegans by RNA interference (right), which induces abnormalities of the hypodermis, including dissociation of the cuticle and degeneration of the hypodermis.

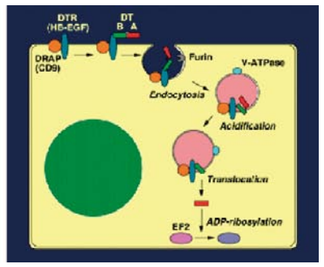

Fig. 5. Entry mechanism of diphtheria toxin.

Recent publications

1. Membrane type 1-matrix metalloproteinase cleaves off the NH2-terminal portion of heparin-binding epidermal growth factor and converts it into a heparin-independent growth factor. Koshikawa N, Mizushima H, Minegishi T, Iwamoto R, Mekada E, Seiki M. Cancer Res. 2010 Jul 15;70(14):6093-103.
2. HB-EGF function in cardiac valve development requires interaction with heparan sulfate proteoglycans. Iwamoto R, Mine N, Kawaguchi T, Minami S, Saeki K, Mekada E. Development. 2010 Jul;137(13):2205-14.
3. Mizushima H, Wang X, Miyamoto S, Mekada E. Integrin signal masks growth-promotion activity of HB-EGF in monolayer cell cultures. J Cell Sci. 2009 Dec 1;122(Pt 23):4277-86.
4. Miyado K, Yoshida K, Yamagata K, Sakakibara K, Okabe M, Wang X, Miyamoto K, Akutsu H, Kondo T, Takahashi Y, Ban T, Ito C, Toshimori K, Nakamura A, Ito M, Miyado M, Mekada E, Umezawa A. The fusing ability of sperm is bestowed by CD9-containing vesicles released from eggs in mice. Proc Natl Acad Sci U S A. 2008 Sep 2;105(35):12921-6.
5. Takeda Y, He P, Tachibana I, Zhou B, Miyado K, Kaneko H, Suzuki M, Minami S, Iwasaki T, Goya S, Kijima T, Kumagai T, Yoshida M, Osaki T, Komori T, Mekada E, Kawase I. Double deficiency of tetraspanins CD9 and CD81 alters cell motility and protease production of macrophages and causes COPD-like phenotype in mice. J Biol Chem. 2008 Sep 19;283(38):26089-97.

Department of Immunochemistry

Research Group

Research Group	
Professor	Hisashi Arase, M.D., Ph.D., SUP
Assistant Professor	Tadahiro Suenaga, M.D., Ph.D., SUP
Assistant Professor	Junji Uehori, Ph.D., SUP
SA Researcher	Fumiji Saito, Ph.D.
SA Researcher	Kouyuki Hirayasu, Ph.D.
SA Researcher	Masako Kouyama, Ph.D.
Postdoctoral Fellow	Jing Wan, Ph.D.

Our department analyzes how pathogens such as viruses have acquired the ability to evade the immune system and how host immune systems have acquired resistance to various pathogens. In particular, we are elucidating a fundamental mechanism of host defense against various pathogens by analyzing various immune regulatory receptors. Of particular interest are 'paired receptors' that consist of activating and inhibitory receptors expressed on various immune cells (Figure 1). We have suggested that these 'paired receptors' have evolved with pathogens by identifying the host ligands and viral ligands they recognize. In addition, we have found that these receptors are also involved in viral entry into cells. These studies will help to elucidate fundamental mechanisms of immune evasion by pathogen and the host factors that influence resistance to various infections. This research will help to build the foundation required for the development of new vaccines and therapies for infectious diseases.

(1)Analysis of recognition by 'paired receptors'

Immune cells express various receptors that consist of activating and inhibitory receptors that are highly homologous to each other. The inhibitory receptors recognize self-antigens such as MHC molecules. In contrast, the activating receptors generally do not recognize self-antigens and ligands for most of them have remained unclear. We have found that one of these 'paired receptors' recognizes cytomegalovirus protein and showed that 'paired receptors' play an important role in determining host resistance to pathogens (Figure 2). We are continuing to analyze the functions of these receptors to elucidate the interactions between pathogens and the host immune system.

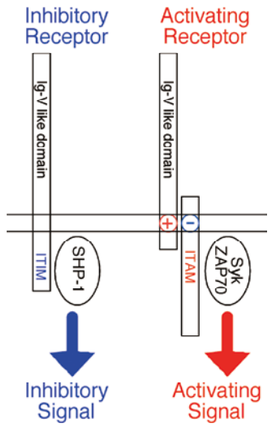

Figure 1. Paired receptors
Paired receptors consist of inhibitory and activating receptors that are highly homologous to each other. The inhibitory receptors transduce inhibitory signal via ITIM in their cytoplasmic domain whereas the activating receptors transduce activating signals by association with ITAM bearing adaptor molecules.

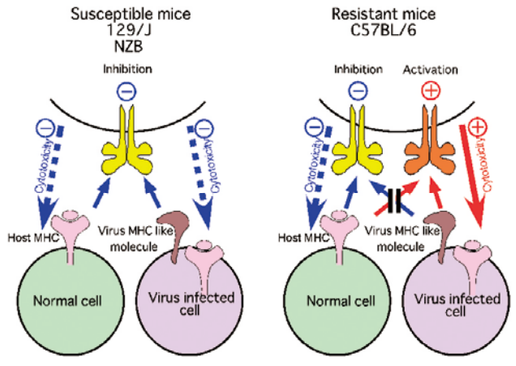

Figure 2. Recognition of cytomegalovirus-infected cells by inhibitory and activating paired receptors
Viruses have acquired MHC-like molecules that serve as ligands for inhibitory receptors expressed on the NK cells of susceptible mice strains. As a result, virus-infected cells are not killed by NK cells despite their low MHC expression (left). In contrast, NK cells from resistant mouse strains do not express inhibitory receptors that recognize virus MHC-like molecules. Instead, they express activating receptors that do recognize virus MHC-like molecules and therefore these cells can efficiently eliminate virus-infected cells (right) (Arase et al. Science 2002).

(2)Entry mechanism of virus into cells

As described above, several viruses, which show persistent infection, downregulate immune response by expressing ligands for inhibitory receptors. Interestingly, we have found that some viruses exploit inhibitory receptors to enter the cells. Interaction between immune receptors and viral proteins was found to play an important role in entry mechanism of herpes simplex virus (HSV) (Figure 3). Because there is a possibility that other viruses also use similar receptors to enter cells, we further investigate molecular mechanisms involved in viral entry into cells.

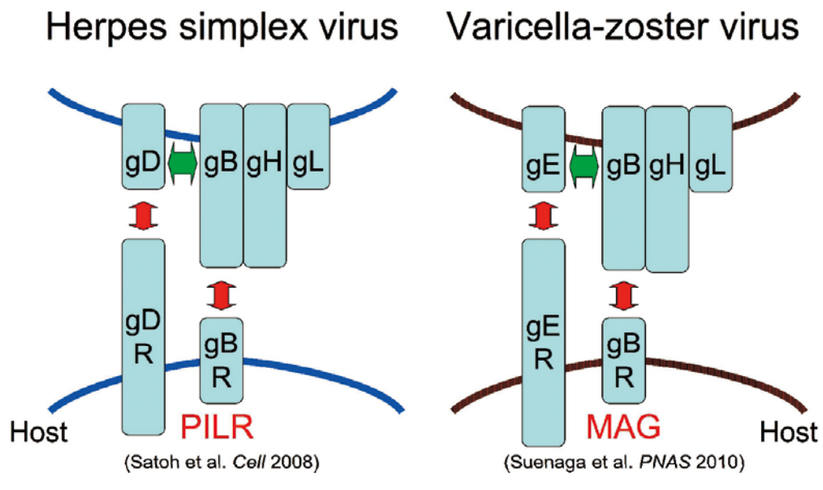

Figure 3. Entry mechanism of virus into cells
Some viruses express ligands for inhibitory receptors and downregulate immune response. We found that PILR *α*, one of inhibitory paired receptors, recognizes herpes simplex virus (HSV) infected cells. Molecular cloning of the ligands for PILR*α* revealed that PILR*α* recognizes HSV glycoprotein B that plays an essential role in HSV infection. Furthermore, interaction between PILR*α* and glycoprotein B was found to be involved in viral entry into cells. On the other hand, we also found that glycoprotein B of varicella-zoster virus associates with myelin associated glycoprotein (MAG, Siglec-4), one of paired receptors, and the association mediates VZV entry into cells. In this way, paired receptors play important roles not only in immune regulation but also in viral entry into cells.

Recent publications

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2. Wang J, Fan Q, Satoh T, Arii J, Lanier LL, Spear PG, Kawaguchi Y, Arase H.Binding of herpes simplex virus glycoprotein B (gB) to PILR *α* depends on specific sialylated O-linked glycans on gB. J Virol. 2009 Dec;83(24):13042-5.
3. Orr MT, Sun JC, Hesslein DG, Arase H, Phillips JH, Takai T, Lanier LL. Ly49H signaling through DAP10 is essential for optimal natural killer cell responses to mouse cytomegalovirus infection. J Exp Med. 2009 Apr 13;206(4):807-17.
4. Satoh T, Arii J, Suenaga T, Wang J, Kogure A, Uehori J, Arase N, Shiratori I, Tanaka S, Kawaguchi Y, Spear PG, Lanier LL, Arase H. PILR *α* is a herpes simplex virus-1 entry co-receptor that associates with glycoprotein B. Cell. 2008 Mar 21;132(6):935-44
5. Wang J, Shiratori I, Satoh T, Lanier LL, Arase H. An essential role of sialylated O-linked sugar chains in the recognition of mouse CD99 by paired immunoglobulin-like type 2 receptor (PILR). J Immunol. 2008 Feb 1;180(3):1686-93.

Department of Molecular Genetics

Research Group

Professor	Hiroshi Nojima, Ph.D.
Assistant Professor	Norikazu Yabuta, Ph.D.
Assistant Professor (SUP)	Daisuke Okuzaki, Ph.D.
Postdoctoral Fellow	Yoko Naito, Ph.D.

We are studying the eukaryotic cell cycle to understand the mechanism that is responsible for the chromosome instability in cancer cells. Chromosome instability is observed in cancer cells, but not in normal cells. Indeed, many human cancer cells exhibit mitotic defects (such as centrosome aberrations, abnormal spindle formation, and chromosome missegregation), and the resulting chromosome instability has been shown to be a major cause of malignant tumor progression. We are focusing on functional analyses of the Ser/Thr kinases Lats (large tumor suppressor) and GAK (cyclin G-associated kinase). These kinases localize at the centrosome, regulate mitotic progression in response to DNA damage, and cause chromosome instability when their functions are disrupted. Both Lats (Lats1 and Lats2) and GAK form complexes with Mdm2. In turn, Mdm2 controls the stability of p53, which is a transcriptional regulator of the Lats2, cyclin G1 and Mdm2 genes. Thus, the Lats and GAK complexes have intimate correlation in their function (Fig. 1).
In addition, to inhibit spontaneous metastasis and the growth of malignant tumors by inhibiting connexin 26, our laboratory has developed safe oleamide-derivative drugs that are associated with few side effects.

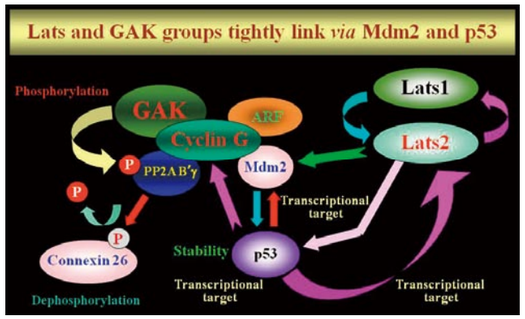

Fig. 1: The functions of the Lats and GAK complexes correlate closely.

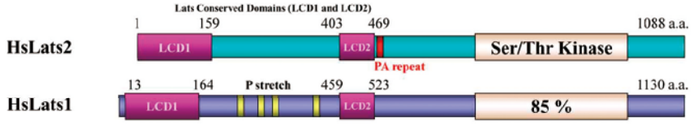

Fig. 2: The structures of the Lats1 and Lats2 proteins are similar.

Our research subjects are as follows:
(1) Lats Group: Lats1 and Lats2, which belong to the Lats kinase family, are highly conserved across species and localize at the centrosome during the cell cycle (Fig. 2). Two miRNAs, miRNA-372 and -373, function as potential novel oncogenes in testicular germ cell tumors by inhibiting *LATS2* expression, which suggests that Lats2 is an important tumor suppressor (Voorhoeve *et al.*, Cell, 2006). Lats2 binds Mdm2, thereby inhibiting its E3 ligase activity and activating p53; in turn, p53 rapidly and selectively upregulates Lats2 expression in G2/M cells. This positive feedback loop constitutes a novel checkpoint pathway that plays a critical role in the maintenance of proper chromosome numbers (Aylon *et al.*, Gene Dev., 2006). We have discovered the following: (A) *Lats2* knockout mice are embryonic lethal, which indicates the essential role of Lats2 in the development and differentiation of mammalian germ cells. (B) *Lats2*$^{-/-}$ mouse embryonic fibroblasts (MEF) display an enhanced growth rate, centrosome fragmentation (Fig. 3), misalignment of the chromosome at M phase, abnormal chromosome segregation, and aberrant cytokinesis. These results indicate the essential role Lats2 plays in proper M phase progression. (C) Aurora-A phosphorylates Lats2 on three distinct serines during mitosis; Lats2 localizes at the centrosome, the mitotic spindle, or the spindle midzone during the cell cycle depending on which site is phosphorylated. (D) Down-regulation of Lats2 by siRNA causes the mislocalization of the chromosomal passenger complex (CPC) during the metaphase/anaphase transition, with the consequence that cytokinesis fails. These observations suggest that the Aurora-A-Lats2-CPC axis is a novel pathway that regulates proper cytokinesis. (E) *Lats1*/*Lats2* knockout mice show arrested development at a very early stage of embryogenesis.

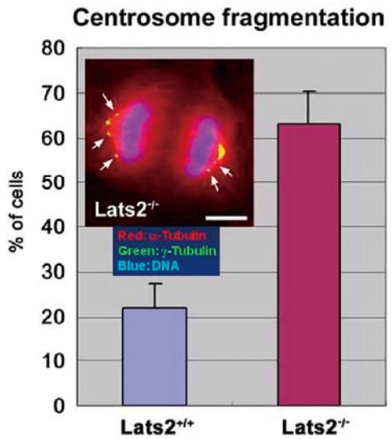

Fig. 3: Loss of Lats2 leads to centrosome fragmentation.

(2) GAK Group:
GAK is an association partner of clathrin heavy chain (CHC) and is essential for clathrin-mediated membrane trafficking. Unlike neuron-specific auxilin, which plays a similar role in neural cells, GAK has a kinase domain (Fig. 4) whose function has remained unclear. We have discovered the following: (A) GAK forms the KBG (KBG1 and KBG2) complex with PP2A B' γ^1 and cyclin G (cyclin G1 and cyclin G2), which regulate the dephosphorylation activity of PP2A. (B) GAK localizes not only in the cytoplasm but also at the nucleus, where it has two additional functions, namely the maintenance of proper centrosome maturation and mitotic chromosome congression. (C) GAK knockdown by siRNA causes cell cycle arrest at the metaphase, which indicates that GAK is required for proper mitotic progression. This impaired mitotic progression was found to be due to the activation of the spindle assembly checkpoint (SAC), which senses protruding, misaligned, or abnormally condensed chromosomes in knockdown cells. (Fig. 5) (D) CHC is involved in this regulatory process since GAK functions cooperatively with clathrin during mitotic progression as well as during endocytosis. (Fig. 6)

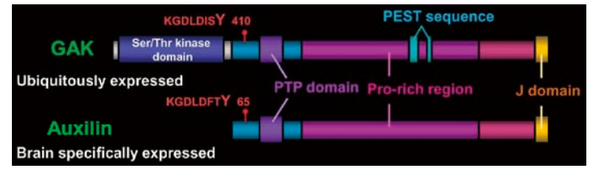

Fig. 4: GAK is similar to auxilin except for bearing a kinase domain.

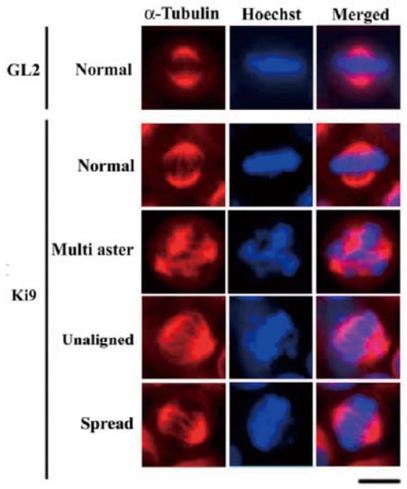

Fig. 5: GAK knockdown by siRNA (Ki9) generates abnormal chromosomes.

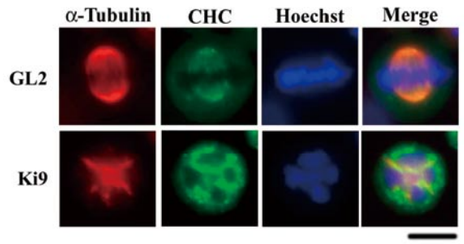

Fig. 6: GAK knockdown by siRNA (Ki9) causes the abnormal localization of CHC.

Recent publications

1. Okada N, Yabuta N, Suzuki H, Aylon Y, Oren M, Nojima H. A novel Chk1/2-Lats2-14-3-3 signaling pathway regulates P-body formation in response to UV damage. J. Cell Sci. 2011 Jan 1;124(1):57-67.
2. Shigehisa A, Okuzaki D, Kasama T, Tohda H, Hirata A, Nojima H. Mug28, a Meiosis-specific Protein of Schizosaccharomyces pombe, Regulates Spore Wall Formation. Mol Biol Cell. 2010 Jun 15;21(12):1955-67.
3. Aylon Y, Ofir-Rosenfeld, Y., Yabuta N, Lap, E. Nojima H, Lu, X. and Oren M. The Lats2 tumor suppressor augments p53-mediated apoptosis by promoting the nuclear proapoptotic function of ASPP1. Genes Dev., 2010 Nov 1;24(21):2420-9.
4. Shimizu H, Nagamori I, Yabuta N, Nojima H. GAK, a regulator of clathrin-mediated membrane traffic, also controls centrosome integrity and chromosome congression. J Cell Sci. 2009 Sep 1;122(17):3145-52.
5. Ohtaka A, Okuzaki D, Nojima H. Mug27 is a meiosis-specific protein kinase that functions in fission yeast meiosis II and sporulation. J Cell Sci. 2008 May 1;121(9):1547-58.

Department of Oncogene Research

Research Group

Professor	Masato Okada, D.Sc.
Associate Professor	Shigeyuki Nada, D.Sc.
Assistant Professor	Chitose Oneyama, D.Sc.
SA Researcher	Kentaro Kajiwara, D.Sc.

Cancers arise, evolve and develop progressively due to the accumulations of mutations and/or modifications in the genomic DNA. Loss-of-function mutations in "tumor suppressor genes" induce cell immortalization, while gain-of-function mutations in "proto-oncogenes" induce cell transformation. Cell immortalization prevents the induction of apoptosis and/or senescence, which is a defense mechanism against cancer development. Cell transformation involves the gain of autonomous cell growth, the loss of cell communication, morphological changes, and the elevated production of matrix proteases and growth factors that participate in invasion, metastasis and angiogenesis. These cellular events thus lead to the malignant conversion of cancer cells. The primary focus of this department is to understand the molecular basis of the cell transformation that is induced by the gain-of-function mutations in proto-oncogenes. As a representative proto-oncogene, we have focused on the c-Src proto-oncogene, which encodes a non-receptor tyrosine kinase. To date, we have analyzed its physiological roles in development and the mechanisms by which its specific regulators, such as Csk and Cbp, regulate it. To obtain a full picture of the cell signaling pathways that lead to c-Src–mediated cell transformation and to search for new therapeutic targets that will block c-Src–mediated cancer progression, the following projects are currently in progress:

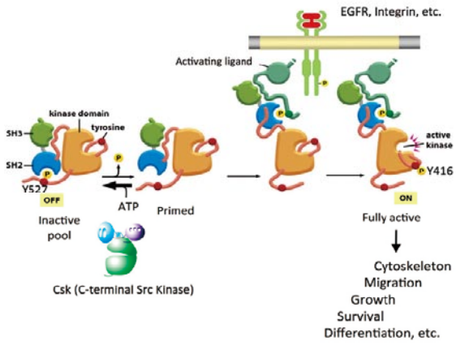

Fig. 1. Functions and regulation of c-Src.

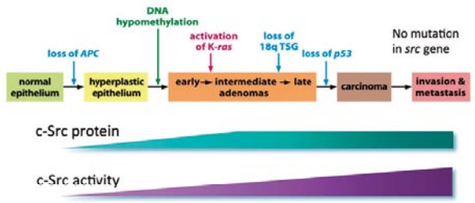

Fig. 2. c-Src and human cancer.

I. Molecular mechanisms that suppress c-Src–mediated cell transformation.

In normal cells, c-Src is present in an inactive form that is phosphorylated by its negative regulatory kinase Csk. Extracellular stimuli transiently activate it (Fig. 1), after which it in turn activates downstream pathways such as the MAPK pathway, thereby inducing the gene expression that is required for cell growth and the phenotypic changes that are involved in cell transformation (Fig. 2). While the c-Src gene is rarely mutated in human cancers, its protein is frequently hyperactivated and overexpressed. This aberrant activation of c-Src is suggested to contribute to cancer malignancy (Fig. 2).

Recently, we found a new system by which the aberrant activation of c-Src could be suppressed. We previously showed that the inactivation of c-Src is facilitated when Csk is recruited to lipid rafts by the specific adaptor Cbp. Further analysis then revealed that Cbp can recruit activated c-Src to lipid rafts directly, and that this is sufficient for suppressing cell transformation. Furthermore, we found that the expression of Cbp is substantially downregulated in various human cancers, which suggests that the Cbp gene serves as a tumor suppressor gene (ref. 3, Fig. 3). Currently, we are analyzing the molecular mechanisms behind the downregulation of the Cbp gene in cancer cells.

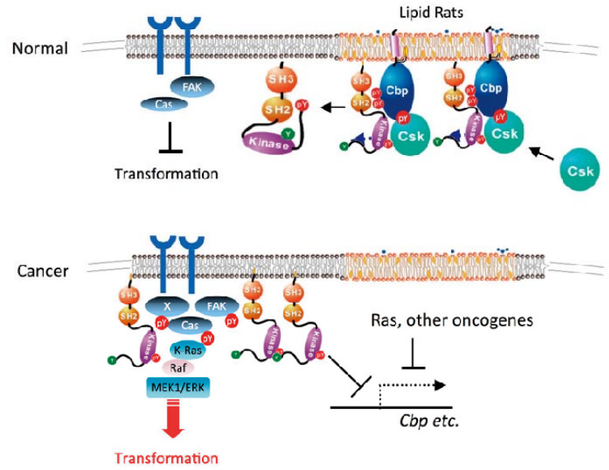

Fig. 3. Tumor-suppressing role of Cbp.

II. Cell signaling pathway of c-Src–mediated cell transformation.

To elucidate the main pathway by which c-Src induces malignant transformation, we searched for new targets of c-Src. Recently, one such potential c-Src target was found to be the novel adaptor protein p18, which is exclusively localized at the lipid rafts of late endosomes. p18 can recruit a branch of MAPK pathway to late endosomes by directly binding the p14/MP1 complex, which is known to be a specific scaffold of MEK1. Analyses of p18 KO mice (embryonic lethal), p18 KO cells, and epidermis-specific p18 KO mice revealed that p18 plays a pivotal role in endosome dynamics by regulating membrane fusion between endosomal vesicles. More recently, we also found that the p18-dependent MAPK pathway is essential for the cell transformation induced by Src, K-Ras and Pak1 (ref. 2, Fig. 4). We are currently analyzing the details of this pathway. We have also started a project that seeks to identify anti-cancer agents that target the p18-dependent pathway.

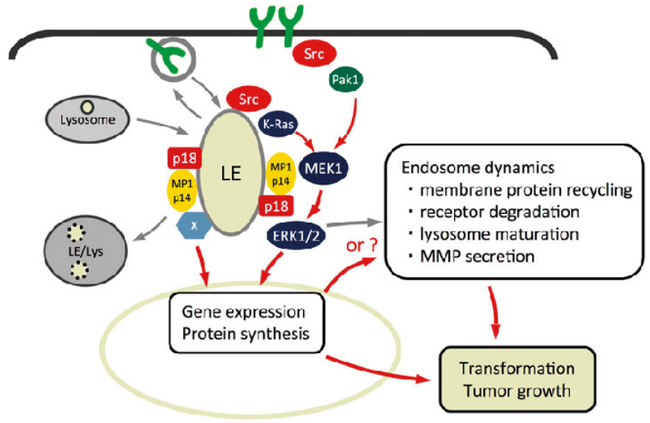

Fig. 4. Roles played by the p18-MAPK pathway in endosome dynamics and cancer growth.

Recent publications

1. Oneyama C, Iino T, Saito K, Suzuki K, Ogawa A, Okada M. Transforming potential of Src family kinases is limited by the cholesterol-enriched membrane microdomain. Mol Cell Biol. 2009 Dec;29(24):6462-72.
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3. Oneyama C, Hikita T, Enya K, Dobenecker MW, Saito K, Nada S, Tarakhovsky A, Okada M. The lipid raft-anchored adaptor protein Cbp controls the oncogenic potential of c-Src. Mol Cell. 2008 May 23;30(4):426-36.
4. Oneyama C, Hikita T, Nada S, Okada M. Functional dissection of transformation by c-Src and v-Src. Genes Cells. 2008 Jan;13(1):1-12.
5. Yagi R, Waguri S, Sumikawa Y, Nada S, Oneyama C, Itami S, Schmedt C, Uchiyama Y, Okada M. C-terminal Src kinase controls development and maintenance of mouse squamous epithelia. EMBO J. 2007 Mar 7;26(5):1234-44.

Department of Signal Transduction

Research Group

Professor	Nobuyuki Takakura, M.D., Ph.D.
Research Associate	Hiroyasu Kidoya, Ph.D.
Postdoctoral Fellow	Yumi Kinugasa, Ph.D.
Postdoctoral Fellow	Hisamichi Naito, M.D., Ph.D.
Postdoctoral Fellow	Tomomi Mohri, Ph.D.
Postdoctoral Fellow	Yinglu Han, Ph.D.

It is well known that the development of normal tissues and organs requires the generation of tissue-specific cells from stem cells. The maintenance of this stem cell system also requires the generation of an appropriate microenvironment. Blood vessels are the most essential structures in tissues and organs, as without blood vessel formation, almost all tissues cannot develop (there are some exceptions). In our research group, we are analyzing the molecular mechanisms by which blood vessels form in physiological and pathological conditions, including in cancers and inflammation. We are also elucidating the mechanisms that cause stem cells to associate closely with blood vessels. Ultimately, we wish to employ our results to establish strategies that will inhibit the malignant progression of various diseases. Our specific research projects are as follows:

I. Analysis of the molecular mechanism of blood vessel formation
1) Molecular analysis of sprouting angiogenesis from preexisting vessels, with a particular focus on the Tie2 receptor.
2) Identification and characterization of adult endothelial stem cells (the CD31-positive side population cells).
3) Molecular characterization of arterio-venous patterning, with a particular focus on the apelin/APJ system.
4) Development of a system that delivers drugs into blood vessels.

II. Molecular analysis of self-renewal in normal and cancer stem cells
1) Mechanism of stem cell reprogramming.
2) Analysis of cell cycle regulation in stem cells, with a special focus on Galectin-3 and the GINS complex.
3) Bioimaging of the niches that are inhabited by living cancer and normal stem cells.
4) Establishment of a strategy that can inhibit the formation of the vascular niche inhabited by cancer stem cells.

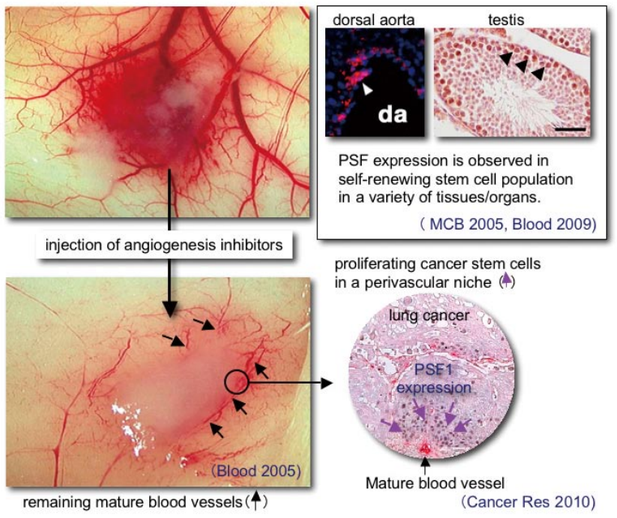


Figure 1. Vascular niche of cancer stem cells.
After treatment of a tumor with angiogenesis inhibitors, the mature blood vessels at the tumor edge persist (left panels). Cancer stem cells marked by PSF1 (a member of the GINS DNA replication factor family that is expressed by the self-renewing normal stem cell population in a variety of tissues and organ) are present and proliferate in the vascular niche represented by these mature blood vessels (right panels). Molecular analysis of vascular niche formation may be highly useful for the development of a new therapy for cancer stem cells.

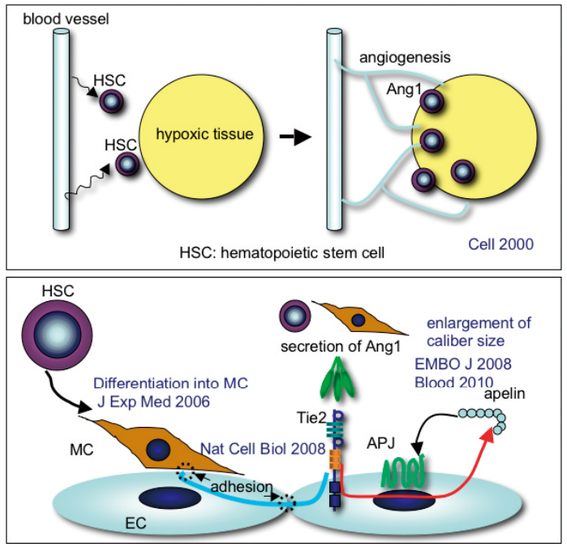


Figure 2. Maturation of blood vessels at the tumor edge.
Hematopoietic stem cells (HSCs) migrate into hypoxic tissues, produce angiopoietin-1 (Ang1), and stimulate Tie2 on endothelial cells (ECs), which results in EC migration and proliferation (upper panel). Many HSCs accumulate at the edge of the tumor during the early stage of tumorigenesis and a portion of HSCs may differentiate into mural cells (MCs) and stabilize blood vessels. Ang1 from HSCs and MCs promotes the production of apelin by ECs, thereby stimulating the apelin receptor APJ on ECs. The activation of APJ induces the proliferation and assembly of ECs, resulting in blood vessel enlargement.

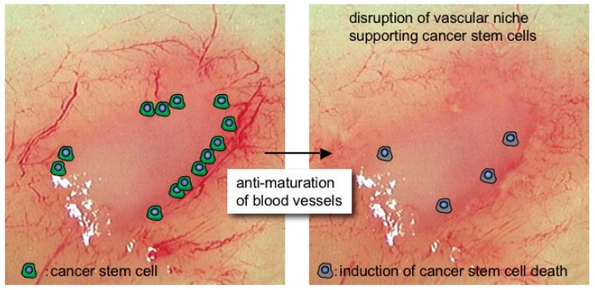


Figure 3. Disruption of the vascular niche in the tumor limb.
Our goal is to completely inhibit tumor growth by destroying mature blood vessels in the tumor environment. For this purpose, we need a drug delivery system that will deliver drugs that specifically block the blood vessel maturation functions of ECs in the tumor.

Recent publications

1. Kidoya H, Naito H, Takakura N. Apelin induces enlarged and nonleaky blood vessels for functional recovery from ischemia. Blood. 2010 Apr 15;115(15): 3166-74.
2. Nagahama Y, Ueno M, Miyamoto S, Morii E, Minami T, Mochizuki N, Saya H, Takakura N. PSF1, a DNA replication factor expressed widely in stem and progenitor cells, drives tumorigenic and metastatic properties. Cancer Res. 2010 Feb 1;70(3):1215-24
3. Ueno M, Itoh M, Sugihara K, Asano M, Takakura N. Both alleles of PSF1 are required for maintenance of pool size of immature hematopoietic cells and acute bone marrow regeneration. Blood. 2009 Jan 15;113(3):555-62.
4. Kidoya H, Ueno M, Yamada Y, Mochizuki N, Nakata M, Yano T, Fujii R, Takakura N. Spatial and temporal role of the apelin/APJ system in the caliber size regulation of blood vessels during angiogenesis. EMBO J. 2008 Feb 6;27(3):522-34.
5. Yamada Y, Takakura N. Physiological pathway of differentiation of hematopoietic stem cell population into mural cells. J Exp Med. 2006 Apr 17;203(4):1055-65.

Department of Molecular Protozoology

Research Group		
	Professor	Toshihiro Horii, Ph. D.
	Invited Professor	Kazuyuki Tanabe, Ph. D.
	Assistant Professor	Nobuko Arisue, Ph. D.
	Assistant Professor	Takahiro Tougan, Ph. D.
	Postdoctorial Fellow	Masanori Yagi, Ph. D
	Postdoctorial Fellow	Hajime Honma, Ph. D.

Malaria is a serious threat to global human health. More than 40% of the world's population lives in malaria-endemic areas and two million people succumb to the disease annually (Fig. 1). Controlling malaria has become more challenging since the emergence of drug-resistant malaria parasites. This has intensified the need for novel drug target strategies and an effective malaria vaccine. Our department is focused on the development of both anti-malarial vaccines and drugs. We are also seeking to understand the mechanisms that the malaria parasite uses to survive in the host.

(1) Development of a recombinant vaccine based on the malaria protein SERA.

We are developing a malaria vaccine that is based on SE36, which is a recombinant protein that spans an amino acid sequence in the serine repeat antigen (SERA) of malaria parasites (Fig. 2). We and co-researchers in malaria-endemic areas have demonstrated that naturally acquired immunity against malaria correlates exclusively with the development of anti-SERA IgG3 antibodies. We have also shown that, after vaccination with SE36, many types of animals, including chimpanzees, develop antibodies that inhibit the growth of malaria parasites (Fig. 3). Together with the Kanonji Institute of the Research Foundation of Osaka University, we have constructed a system by which the SE36 malaria vaccine can be mass-produced (Fig. 4). In 2005, we conducted a phase I clinical trial in Japan with SE36 to assess its safety and immunogenicity. All vaccine-administered volunteers were sero-converted and showed no serious adverse events. We are currently in the process of conducting additional phase Ib clinical trials in an endemic region in Uganda. This project is under taken in collaboration with the Research Foundation for Microbial Diseases of Osaka University.

We are also studying the function of the SERA molecule in the parasite and characterizing the host immune response against SERA. In addition, in collaboration with colleagues in Uganda, Thailand, Indonesia and the Solomon Islands, we have started a new research project that aims to develop a *Plasmodium vivax* vaccine.

Fig. 1: Patients waiting at the Out-patient Department of Apac Hospital in Northern Uganda: major victims of malaria are children under the age of 5 years.

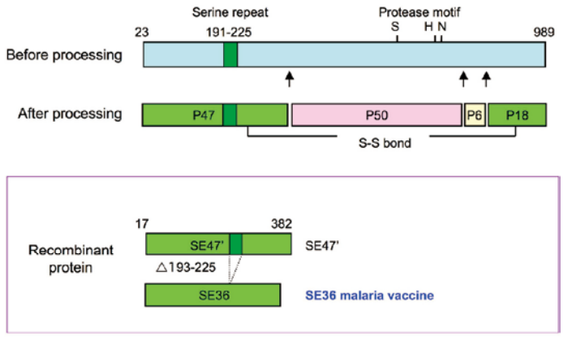


Fig. 2: Processed fragments of *P. falciparum* SERA and the structure of the recombinant SE36.

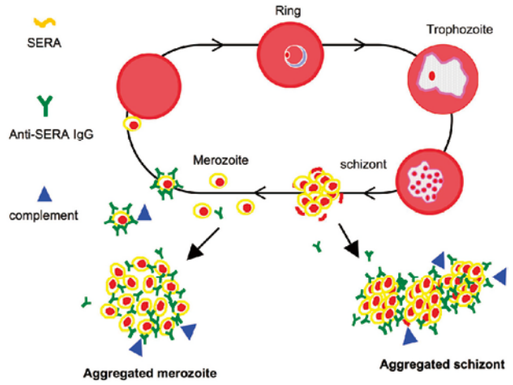


Fig. 3: Model that explains how anti-SERA IgG inhibits erythrocytic P. falciparum growth.

Fig. 4: The SE36 malaria vaccine for clinical trials is produced under Good Manufacturing Practices (GMP) at the Kanonji Institute of the Research Foundation for Microbial Diseases of Osaka University.

(2) Identification of SERA genes from several Plasmodium species.

The Plasmodium SERA gene family consists of several gene members. To trace the evolution of the SERA genes, we identified the SERA genes of several Plasmodium species and constructed the SERA gene family tree (Fig. 5). Transcription and polymorphic analyses are being used to search for functional or vaccine target molecules in the Plasmodium SERA genes.

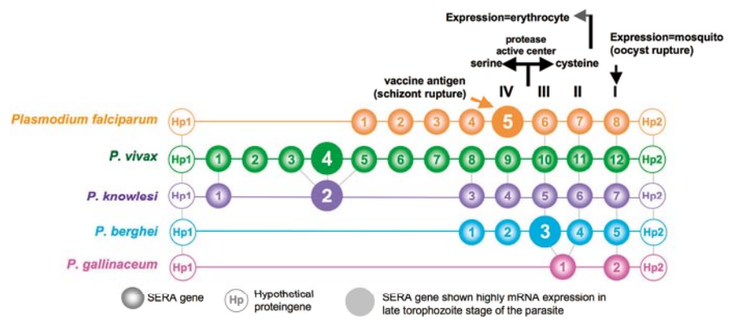


Fig. 5: An overview of the Plasmodium SERA gene family.

Recent publications

1. Clues to evolution of the SERA multigene family in 18 *Plasmodium* species. Arisue N, Kawai S, Hirai M, Palacpac NM, Jia M, Kaneko A, Tanabe K, Horii T. PLoS One. 2011 Mar 15;6(3):e17775.
2. Evidences of protection against blood-stage infection of *Plasmodium falciparum* by the novel protein vaccine SE36. Horii T, Shirai H, Jie L, Ishii KJ, Palacpac NQ, Tougan T, Hato M, Ohta N, Bobogare A, Arakaki N, Matsumoto Y, Namazue J, Ishikawa T, Ueda S, Takahashi M. Parasitol Int. 2010 Sep;59(3):380-6.
3. *Plasmodium falciparum* accompanied the human expansion out of Africa. Tanabe K, Mita T, Jombart T, Eriksson A, Horibe S, Palacpac N, Ranford-Cartwright L, Sawai H, Sakihama N, Ohmae H, Nakamura M, Ferreira MU, Escalante AA, Prugnolle F, Björkman A, Färnert A, Kaneko A, Horii T, Manica A, Kishino H, Balloux F. Curr Biol. 2010 Jul 27;20(14):1283-9.
4. Big bang in the evolution of extant malaria parasites. Hayakawa T, Culleton R, Otani H, Horii T, Tanabe K. Mol Biol Evol. 2008 Oct;25(10):2233-9.
5. Phylogeny and evolution of the SERA multigene family in the genus *Plasmodium*. Arisue N, Hirai M, Arai M, Matsuoka H, Horii T. J Mol Evol. 2007 Jul;65(1):82-91.

Department of Virology

Research Group		
	Professor	Kazuyoshi Ikuta, Ph.D.
	Assistant Professor	Takeshi Kurosu, D.V.M., Ph.D.
	Assistant Professor	Yohei Watanabe, D.V.M., Ph.D.
	SA Assistant Professor	Tadahiro Sasaki, Ph.D.
	SA Assistant Professor	Mayo Yasugi-Ueda, D.V.M., Ph.D.
	SA Assistant Professor	Azusa Asai, Ph.D.
	Postdoctoral Fellow	Yuji Inoue, Ph.D.

The research in this department focus to several viruses and prion that target to immune, respiratory, or central nervous systems, and aims to understand the mechanisms for their replication and pathogenesis of viral infections, as well as the applied researches to develop to control them, to remove them from the blood products, and to establish the novel rapid diagnosis.

(1) Infections to the immune system

We are characterizing the mechanism for the pathogenesis how dengue virus (DENV) derived from Souse Asian countries induces dengue fever and dengue hemorrhagic fever. DENV infection induces strong humoral immunity. The produced antibodies (Abs) regulate the virus infection positively (antibody-dependent enhancement; ADE) and negatively (neutralization). We prepare and analyze human monoclonal Abs derived from DENV-infected patients to understand the pathogenicity that could lead to development of the therapeutics.

(2) Infections to the respiratory system

We recently succeed to prepare the human neutralizing monoclonal Abs against influenza virus that induces typical acute infectious disease at the respiratory region. Since the epitope region recognized with the monoclonal Abs is highly conserved and forms conformational structure, we are working for the possible development of a new type vaccine with this conformation as collaboration with several companies.

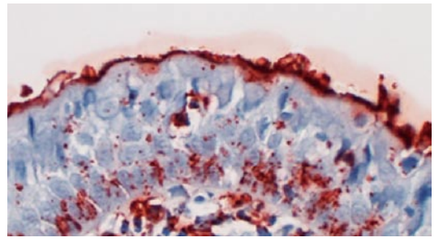
We are also executing a collaboration work with Alexandria University, about highly pathogenic avian influenza H5N1 viruses circulating in Egypt. Recently, we found that some of new H5 sublineages in Egypt have acquired an enhanced binding capacity to human-type receptor beyond expectation. We are focusing to search the pandemic potential of H5N1 viruses in Egypt.

(3) Blood-borne infections

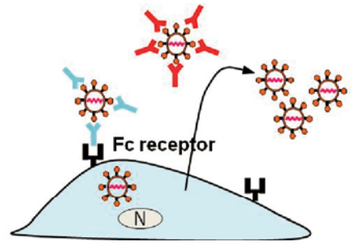
We are working to establish how to remove infectious agents such as parvovirus B19, SARS-corona virus, hepatitis E virus, and prion that were contaminating in blood products as collaboration with a company.

(4) Rapid diagnosis kits

There are many techniques for the diagnosis of virus infections: immunofluorescence, ELISA, Western blot, and PCR assays. We are currently working for the development of rapid diagnosis kits against several infectious diseases, as collaboration with several companies.



Some of new H5 sublineages in Egypt have acquired an increased attachment to and infectivity in human lower respiratory tract (H5N1 virus attached to human tracheal epithelia is stained red in the panel). We are currently investigating the mechanism(s) underlying the possible emergence of pandemic H5N1 viruses in Egypt and its risk in the field, with Alexandria University.



Dengue virus infection induces strong humoral immunity and the production of Abs. These Abs regulate infection positively by antibody-dependent enhancement (ADE) and negatively by neutralization, respectively. We analyze the monoclonal Abs from DENV-infected patients to investigate the pathogenicity and develop therapeutics.

Recent publications

1. Watanabe Y, Ibrahim MS, Ellakany HF, Kawashita N, Mizuike R, Hiramatsu H, Sriwilaijaroen N, Takagi T, Suzuki Y, Ikuta K. Acquisition of human-type receptor binding specificity by new H5N1 influenza virus sublineages during their emergence in birds in Egypt. PLoS Pathog. 2011 in press.
2. Ibrahim MS, Watanabe Y, Ellakany HF, Yamagishi A, Sapsutthipas S, Toyoda T, Abd El-Hamied HS, Ikuta K. Host-specific genetic variation of highly pathogenic avian influenza viruses (H5N1). Virus Genes. 2011 Feb 17.
3. Mizuike R, Sasaki T, Baba K, Iwamoto H, Shibai Y, Kosaka M, Kubota-Koketsu R, Yang CS, Du A, Sakudo A, Tsujikawa M, Yunoki M, Ikuta K. Development of two types of rapid diagnostic test kits to detect the hemagglutinin or nucleoprotein of the swine-origin pandemic influenza A virus H1N1. Clin Vaccine Immunol. 2011 Mar;18(3):494-9. Epub 2011 Jan 12.
4. Kurosu T, Khamlert C, Phanthanawiboon S, Ikuta K, Anantapreecha S. Highly efficient rescue of dengue virus using a co-culture system with mosquito/mammalian cells. Biochem Biophys Res Commun. 2010 Apr 2;394(2):398-404. Epub 2010 Mar 7.
5. Yamashita A, Kawashita N, Kubota-Koketsu R, Inoue Y, Watanabe Y, Ibrahim MS, Ideno S, Yunoki M, Okuno Y, Takagi T, Yasunaga T, Ikuta K. Highly conserved sequences for human neutralization epitope on hemagglutinin of influenza A viruses H3N2, H1N1 and H5N1: Implication for human monoclonal antibody recognition. Biochem Biophys Res Commun. 2010 Mar 19;393(4):614-8. Epub 2010 Feb 10.

Department of Experimental Genome Research

Research Group		
	Professor	Masaru OKABE, Ph.D.
	Associate Professor	Takeshi MIWA, Ph.D.
	Associate Professor	Masahito IKAWA, Ph.D.
	Assistant Professor	Hidetoshi HASUWA, Ph.D.
	Assistant Professor	Naokazu INOUE, Ph.D.
	Assistant Professor	Ayako ISOTANI, Ph.D.
	SA Assistant Professor	Yuhkoh SATOUH, Ph.D.

In the past, naturally-mutated animals have been used to elucidate the mechanisms of various diseases. In the "post-genome project era", however, genetically manipulated animals play a key role in such investigations through providing animal models for human diseases. Our laboratory assists other research facilities in generating such genetically manipulated animals, as shown on our web page.
http://kumikae01.gen-info.osaka-u.ac.jp/EGR/index.cfm
This objective is carried out in collaboration with the Animal Resource Center for Infectious Diseases

Research Projects
We have succeeded to produce a first genetically altered "green mouse" that glows in the dark in the world. These mice are highly useful for many types of research projects, such as those involving stem cell transplantation and regeneration study. Utilizing one of these animals, we demonstrated that it was possible to determine the sex of the embryos at their preimplantation stage of the mouse. These mice were used to study fertilization (Fig. 1) and sex determination mechanism in germ cells.
We are also interested in the mechanism of fertilization from the context of self-nonself recognition. Utilizing the homologous recombination technology, we have shown protein IZUMO1 is essential for sperm to fuse with eggs as a first sperm factor and, we recently found sperm protein SPESP1 is necessary to produce the fully "fusion competent" sperm (Fig. 2 and 3) (4).

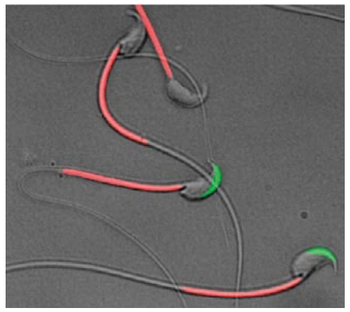


Fig. 1. A transgenic mouse line whose sperm express green fluorescent protein (GFP) in their acrosome and red fluorescent protein (RFP) in their mitochondria. This sperm make it possible to observe live image.

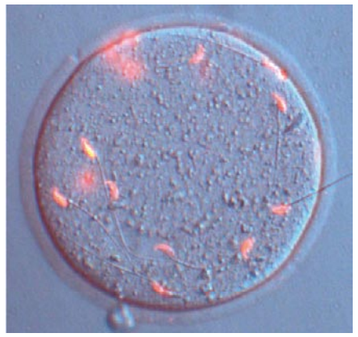


Fig. 2. Accumulation of many Izumo1 KO sperm in the perivitelline space of the eggs, because Izumo1 KO sperm is not able to fuse with egg.

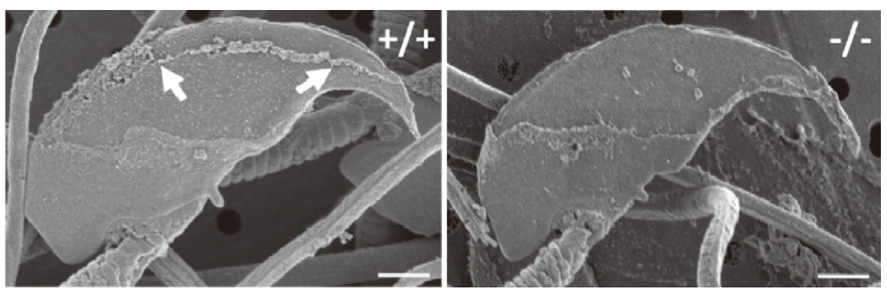


Fig. 3. In almost all the acrosome reacted Spesp1 deficient sperm, the membrane of the entire equatorial segment area was detached from sperm (right) (4).

In addition to the study the mechanism of sperm-egg interaction, we are studying the placenta from the point of view that the organ has important roles in feto-maternal immune tolerance. Since we think the gene-functions has to be observed in live animals, we tried to find a method for the gene manipulate in placenta and developed a method using Lentiviral vectors. With this method it was demonstrated that the placenta could be gene manipulated without affecting the embryos (Fig. 4) (2). Recently, to understand the biological function of non coding RNA, we are also examining the role(s) of miRNA in live animals using miRNA knock-out technique.

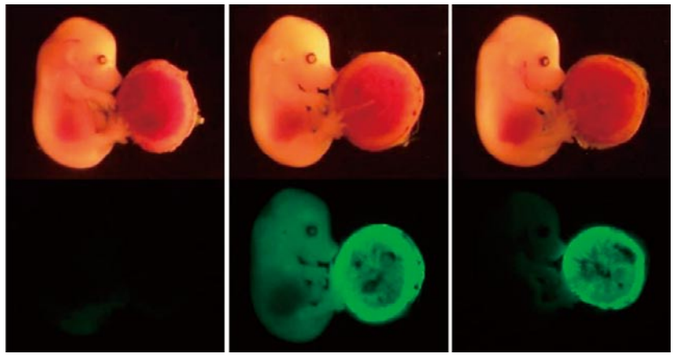


Fig. 4. Placenta-spesific gene manipulation. GFP Transgene expression at E14.5 after gene manipulation. untransduced embryos (left), embryos with normal transgenic procedure (middle) and embryos with newly developed method for placenta specific gene manipulation (2).

Miwa's group is investigating the molecular biological mechanisms involved in human diseases, especially cardiovascular diseases, by using genetically manipulated animals. To understand the cellular and molecular aspects of vascular smooth muscle (SM) cell growth in atherosclerotic plaques, we have characterized the transcriptional mechanisms of one SM-specific gene, human SM alpha-actin (SmaA) gene. Since SmaA is also expressed in many tissues during acute inflammation, we are analyzing its gene expression system and its role (Fig. 5) (5). We have also developed a diastolic heart failure model using Dahl salt-sensitive rats and analyzed the molecular pathogenic mechanisms. We are currently investigating how the endothelin and renin-angiotensin systems contribute to diastolic heart failure through the development of excessive hypertrophy and fibrosis.

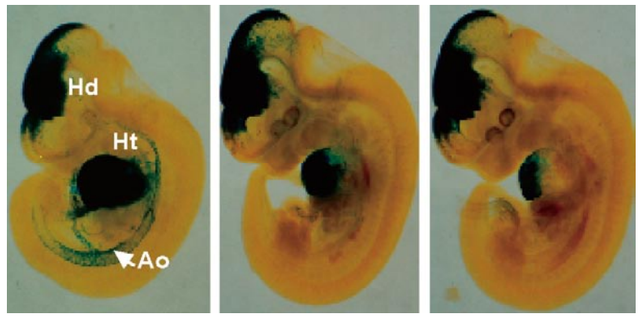


Fig. 5. The human SM alpha-actin promoter (left) expressed in embryonic aorta, but those including -1M (center) and 4M (right) point mutants in the enhancer region did not specifically express (5).

Recent publications

1. Isotani A, Hatayama H, Kaseda K, Ikawa M, Okabe M. Formation of a thymus from rat ES cells in xenogeneic nude mouse↔rat ES chimeras. Genes Cells. 2011 Apr;16(4):397-405.
2. Kumasawa K, Ikawa M, Kidoya H, Hasuwa H, Saito-Fujita T, Morioka Y, Takakura N, Kimura T, Okabe M. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. Proc Natl Acad Sci U S A. 2011 Jan 25;108(4):1451-5.
3. Ikawa M, Tokuhiro K, Yamaguchi R, Benham AM, Tamura T, Wada I, Satouh Y, Inoue N, Okabe M. Calsperin is a testis-specific chaperone required for sperm fertility. J Biol Chem. 2011 Feb 18;286(7):5639-46.
4. Fujihara Y, Murakami M, Inoue N, Satouh Y, Kaseda K, Ikawa M, Okabe M. Sperm equatorial segment protein 1, SPESP1, is required for fully fertile sperm in mouse. J Cell Sci. 2010 May 1;123(Pt 9):1531-6.
5. Kamimura D, Ohtani T, Miwa T, et al.Ca2+ Entry mode of Na+/Ca2+ exchanger as a new therapeutic target for heart failure with preserved ejection fraction. European Heart J. 2011 Apr 13;(in press)

Department of Genome Informatics

Research Group		
	Professor	Teruo Yasunaga, Ph.D.
	Professor (SUP)	Tatsuya Takagi, Ph.D.
	Assistant Professor	Naohisa Goto, Ph.D.
	Assistant Professor	Shota Nakamura, Ph.D.
	SA Researcher	U. Chandimal de Silva, M.Sc.

We study the genome information of various organisms by using high performance computers in an effort to identify new biological phenomena and to understand how organisms evolve. In addition, we develop software tools for bioinformatics and molecular biology. We also operate a computer system for genome sequence data analyses that is made available to researchers in our university, and hold training courses for genome analysis at least once every year.

(1) Large-scale analysis of genomes

The complete genome sequences of more than 1,000 organisms are available today. We analyze this enormous body of genome data by using bioinformatics and molecular evolution techniques. We also develop software and algorithms for large-scale genome analysis. We developed CONSERV, a conserved sequence finder, and by analyzing the complete genome sequences of 266 organisms, identified invariant sequences that may have been present in the last common ancestor of all extant life forms (Goto et al, 2007; Fig. 1). Also, we strive to understand the evolutionary pathways of the influenza virus by the comprehensive analysis of its genome (Fig. 2).

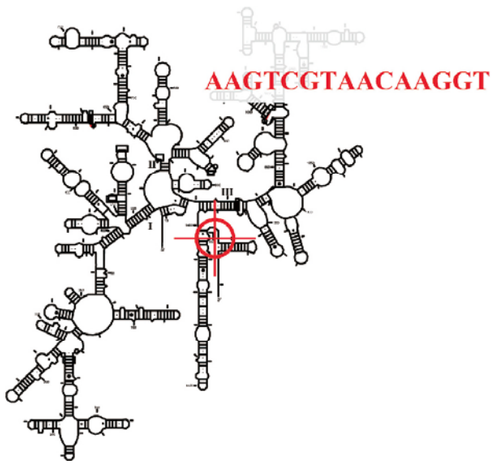


Figure 1. Large-scale genome analysis of 266 organisms: A sequence conserved in almost all known genomes.

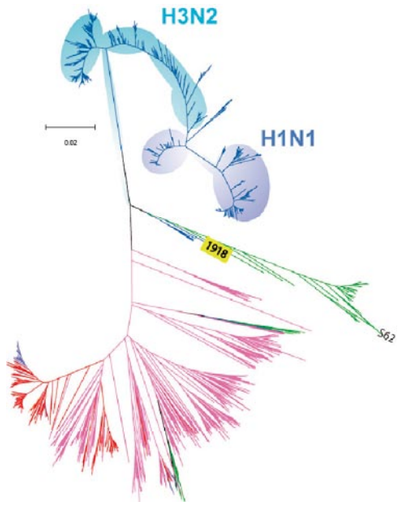


Figure 2. Comprehensive analysis of the influenza virus genome.

(2) Next generation sequencer data analysis

The recently developed "next generation sequencing" technology has made it possible to sequence the whole genome of any microorganism in one sequencer run, producing a massive amount of nucleotide sequence data in each run. We develop software to handle this data and have set up an analysis system, which is used for collaborative sequencing projects of microorganisms with other laboratories (Fig. 3).

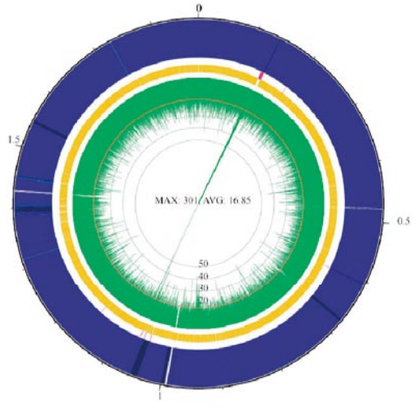


Figure 3. Next generation sequencing enables sequencing of a whole genome in one run.

Figure 4. Genome Information Research Center Computer System.

(3) Operation of computer system for genome information analysis at Osaka University.

We provide computer resources for researchers in our university. We also provide mirrored access to major nucleotide, protein, and genome databases through our servers (Fig.4) which are fully synchronized with the mother servers and kept up to date at all times.

Recent publications

1. Yamashita A, Kawashita N, Kubota-Koketsu R, Inoue Y, Watanabe Y, Ibrahim MS, Ideno S, Yunoki M, Okuno Y, Takagi T, Yasunaga T, Ikuta K. Highly conserved sequences for human neutralization epitope on hemagglutinin of influenza A viruses H3N2, H1N1 and H5N1: Implication for human monoclonal antibody recognition. Biochem Biophys Res Commun. 2010 Mar 19;393(4):614-8.
2. Nakamura S, Yang CS, Sakon N, Ueda M, Tougan T, Yamashita A, Goto N, Takahashi K, Yasunaga T, Ikuta K, Mizutani T, Okamoto Y, Tagami M, Morita R, Maeda N, Kawai J, Hayashizaki Y, Nagai Y, Horii T, Iida T, Nakaya T. Direct metagenomic detection of viral pathogens in nasal and fecal specimens using an unbiased high-throughput sequencing approach. PLoS One. 2009;4(1):e4219.
3. Yamashita A, Goto N, Nishiguchi S, Shimada K, Yamanishi H, Yasunaga T. Computational search for over-represented 8-mers within the 5'-regulatory regions of 634 mouse testis-specific genes. Gene. 2008 Dec 31;427(1-2):93-8.
4. Yoshida M, Yamashita A, Idoji Y, Nishiguchi S, Shimada K, Yasunaga T, Yamanishi H. In silico study of a novel gene evolved from an ancestral SVIP gene and highly expressed in the adult mouse testes. Int J Mol Med. 2008 Aug;22(2):143-8.
5. Goto N, Kurokawa K, Yasunaga T. Analysis of invariant sequences in 266 complete genomes. Gene. 2007 Oct 15;401(1-2):172-80.

Department of Infection Metagenomics

Research Group		
	Professor (SUP)	Toshihiro Horii, Ph.D.
	Professor (SUP)	Teruo Yasunaga, Ph.D.
	SA Professor (SUP)	Tetsuya Iida, Ph.D.
	SA Associate Professor (SUP)	Takaaki Nakaya, Ph.D.
	Assistant Professor (SUP)	Naohisa Goto, Ph.D.
	Assistant Professor (SUP)	Shota Nakamura, Ph.D.

1. RAPID (Robotics Assisted Pathogen IDentification)

Under the aegis of the Program of Research Centers for Emerging and Re-emerging Infectious Diseases of MEXT, Japan, we are constructing, in collaboration with the Omics Science Center, RIKEN, a framework called "RAPID" that will facilitate the emergency diagnosis of infectious diseases. We are also cooperating with the research centers of eight countries in Asia and Africa in an effort to identify the causative agents in naturally occurring outbreaks.

2. Metagenomic Diagnosis of Infectious Diseases

Metagenomic analysis allows us to diagnose many of the major human infectious diseases (including respiratory tract infections, enteric infections, and blood-borne infections) by using a single common protocol. In addition, to pre-empt zoonotic disease outbreaks, we are seeking to identify new pathogenic microorganisms in animal-derived samples that may have zoonotic potential.

3. Metagenomic Analysis of the Intestinal Microbiome

The intestinal microbiome plays an important role in protecting the host from pathogen invasion. We are currently analyzing the intestinal microbiome of patients with diarrheal diseases to elucidate how the human host, the intestinal microbiome, and pathogenic microorganisms interact. This will help us to understand the changes that take place in the intestinal microbiome during the course of infection.

4. Development of Novel Methods for Pathogen Detection

To develop more efficient and comprehensive methods of identifying pathogens, we are studying the efficacy of different methods that amplify the genome of pathogenic microorganisms and subtract the host genome.

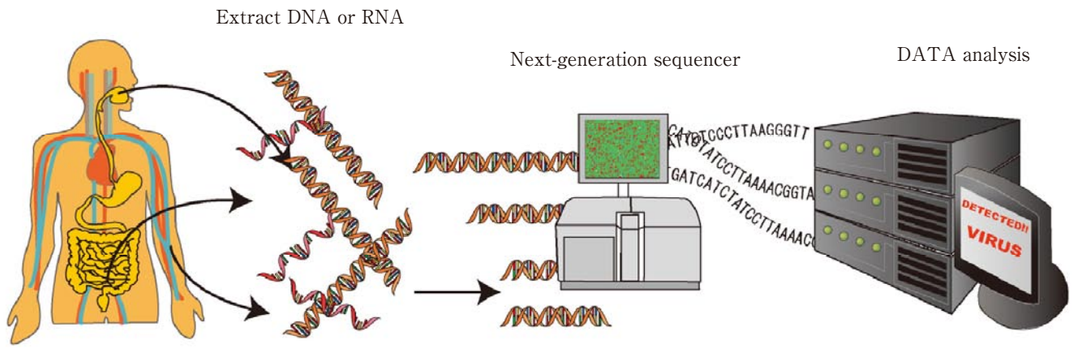


Fig. 1. Metagenomic diagnosis of infectious diseases using a next-generation sequencer.

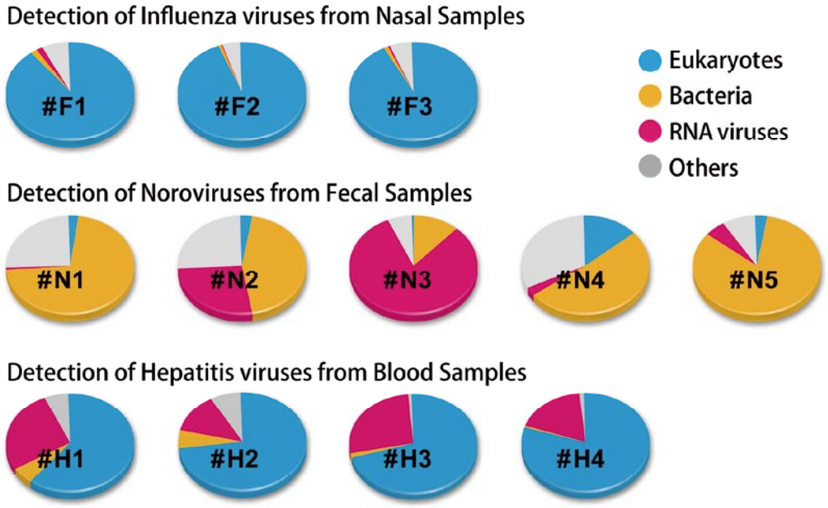


Fig. 2. Distribution of detected organisms in viral infection cases

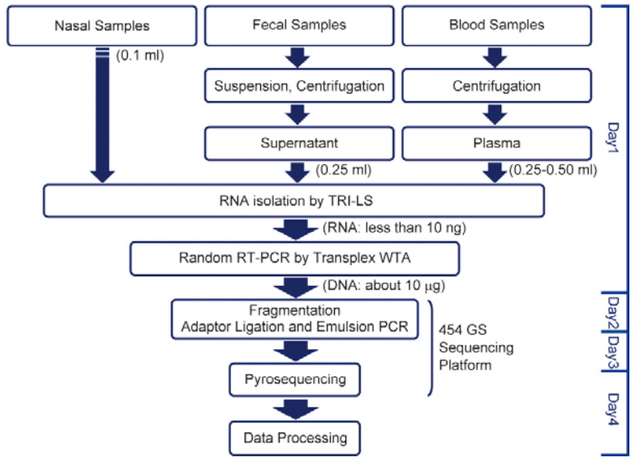


Fig. 3. Standard operating protocol for RAPID

Fig. 4. Next-generation sequencer, 454 GS Junior System

Recent publications

1. Nakamura S, Yang CS, Sakon N, Ueda M, Tougan T, Yamashita A, Goto N, Takahashi K, Yasunaga T, Ikuta K, Mizutani T, Okamoto Y, Tagami M, Morita R, Maeda N, Kawai J, Hayashizaki Y, Nagai Y, Horii T, Iida T, Nakaya T. Direct metagenomic detection of viral pathogens in nasal and fecal specimens using an unbiased high-throughput sequencing approach. PLoS One. 2009;4(1):e4219.
2. Nakamura S, Maeda N, Miron IM, Yoh M, Izutsu K, Kataoka C, Honda T, Yasunaga T, Nakaya T, Kawai J, Hayashizaki Y, Horii T, Iida T. Metagenomic diagnosis of bacterial infections. Emerg Infect Dis. 2008 Nov;14(11):1784-6.

Laboratory for Clinical Research on Infectious Diseases

Research Group

SA Professor	Kazunori Oishi M.D., Ph.D.
Assistant Professor	Yukihiro Akeda Ph.D.
Postdoctoral Fellow	Tatsuya Nakayama Ph.D.
Postdoctoral Fellow	Zhenyu Piao Ph.D.
Research Staff	Yumi Hattori
	Yuka Koizumi
	Michiyo Hayakawa Ph.D.

The research activities in our department aim to 1) investigate the epidemiology, pathogenesis and prevention by vaccines of pneumonia and invasive bacterial infections, 2) elucidate the mechanisms by which dengue viruses induce disease, and 3) analyze the protein secretion systems of pathogenic bacteria. In addition, our group is registered as a member of the World Health Organization (WHO)/Global Outbreak Alert & Response Network (GOARN) and, when necessary, will join the outbreak response team in the global effort to control emerging and re-emerging infectious diseases.

1) Epidemiology, pathogenesis, and vaccine-mediated prevention of pneumonia and invasive bacterial infections

1. Study of pneumonia in Thailand
We have conducted a study project entitled "Surveillance of emerging respiratory infections and analysis of mechanism of secondary bacterial pneumonia in Thailand". This project allowed us to investigate how virus-host-bacteria interactions promote secondary bacterial infections in pediatric patients with pneumonia. In 2009, we examined the clinical features of 24 adult cases of pandemic H1N1 influenza-associated severe community-acquired pneumonia at Buddachinaraj Hospital, Phitsanulok.

2. Clinical applications of the 23-valent pneumococcal polysaccharide vaccine (PPV) and the development of new pneumococcal vaccines
a) Clinical application of 23-valent PPV
We found that the combined PPV and influenza vaccine (IV) vaccination program reduced the incidence of acute exacerbation in patients with chronic obstructive pulmonary diseases (Vaccine, 2008). An open-label, randomized study was conducted involving 786 Japanese subjects older than 65 years of age who were receiving a routine IV. Concomitant PPV vaccination significantly reduced the number of admissions and medical costs for all-cause pneumonia for subjects older than 75 years. We also started a project in 2008 that examines the effects of PPV in combination with IV on long-term–care residents. Our goal is the nationwide and routine vaccination of the elderly in Japan.
b) Development of a nasal mucosal pneumococcal vaccine
Pneumococcal surface protein A (PspA) is known to elicit protective antibodies in animals. We have demonstrated the effects of PspA plus TLR agonist on bacterial clearance in a mouse model of pneumococcal pneumonia (Vaccine, 2009). In addition, we have shown that the PspA nasal vaccine is effective in a mouse model of secondary pneumonia after influenza virus infection.

3. Research on *Streptococcus suis* infections, which are prevalent in Thailand
Streptococcus suis is an important zoonotic pathogen that causes invasive infections such as meningitis in humans who are in close contact with infected pigs or contaminated pork-derived products. The number of such human cases is rapidly increasing in Thailand because of a tradition of consuming raw pork or blood in the north. We showed that the clinical manifestations of serotype 2 infections are related to the genotypic profiles of the isolates; we also reported the clonal dissemination in humans of serotype 14, which has been a rarely occurring serotype up until now (Figure 1). We are currently developing an epidemiological study of S. suis infections in Phayao Province as a project of RCC-DMSc.

2) Mechanisms by which dengue virus infections lead to thrombocytopenia
Dengue illness has become a major public health concern, particularly in tropical countries. We have conducted a number of clinical studies in the Philippines and recently found in an ex vivo setting that patients with thrombocytopenia during acute phase secondary dengue virus infections showed increased phagocytosis of platelets (AJTMH, 2009). Since we found that IVIG treatment did not significantly hasten the recovery from thrombocytopenia in such patients (AJTMH, 2007), this suggests that the Fcγ receptor is not involved in platelet phagocytosis by macrophages. We are currently elucidating the novel mechanism by which the platelets are phagocytosed in this disease.

3) Protein secretion systems of pathogenic bacteria
The development of bacterial infections requires many virulence factors. Since most are proteins that are secreted by the pathogenic bacteria, it is essential to study the mechanisms by which proteins are secreted by pathogenic bacteria during the course of infection. We are using the food-borne pathogen *Vibrio parahaemolyticus* and a causative agent of pneumonia, *Streptococcus pneumoniae*, to study such protein secretion systems and the secreted virulence factors.

4) Response to emerging and re-emerging infectious diseases
The objective of GOARN is to combat the international spread of infectious disease outbreaks by ensuring that appropriate technical assistance reaches the affected areas rapidly and by promoting long-term epidemic preparedness. Our group is registered as a member of GOARN and our team will join the WHO-organized response team when there is an outbreak of infectious diseases in developing countries.

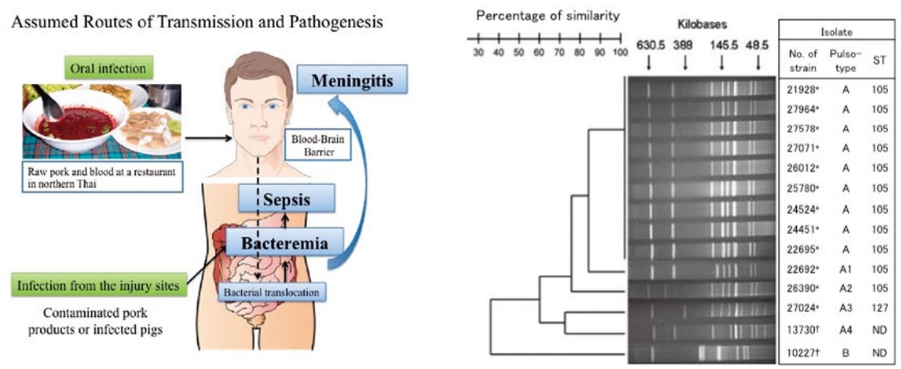


Figure 1. Assumed routes of transmission and pathogenesis of S. suis infection, and the clonal dissemination of serotype 14 infections in Thailand (J Med Microbiol, 2009).

Recent publications

1. Kerdsin A, Dejsirilert S, Puangpatra P, Sripakdee S, Chumla K, Boonkerd N, Polwichai P, Tanimura S, Takeuchi D, Nakayama T, Nakamura S, Akeda Y, Gottschalk M, Sawanpanyalert P, Oishi K. Genotypic profile of *Streptococcus suis* serotype 2 and clinical features of infection in humans, Thailand. Emerg Infect Dis. 2011 May 17(5):835-42.
2. Ezoe H, Akeda Y, Piao Z, Aoshi T, Koyama S, Tanimoto T, Ken J. Ishii KJ, Oishi K. Intranasal vaccination with pneumococcal surface protein A plus poly(I:C) protects against secondary pneumococcal pneumonia in mice. Vaccine. 2011 Feb 17;29(9):1754-61.
3. Kawakami K, Ohkusa Y, Kuroki R, Tanaka T, Koyama K, Harada Y, Iwanaga K, Yamaryo T, Oishi K. Effectiveness of pneumococcal polysaccharide vaccine against pneumonia and cost analysis for the elderly who receive seasonal influenza vaccine in Japan. Vaccine. 2010 Oct 8;28(43):7063-9.
4. Kerdsin A, Oishi K, Sripakdee S, Boonkerd N, Polwichai P, Nakamura S, Uchida R, Sawanpanyalert P, Dejsirilert S. Clonal dissemination of *Streptococcus suis* serotype 14 in Thailand. J Med Microbiol. 2009 Nov;58(Pt 11):1508-13.
5. Honda S, Saito M, Dimaano EM, Morales PA, Alonzo MTG, Suarez LC, Koike N, Inoue S, Kumatori A, Matias RR, Natividad FF, Oishi K. Increased platelet phagocytosis from patients with secondary dengue virus Infection by human macrophages. Am J Trop Med Hyg. 2009 May;80(5):841-5.

Laboratory for Infection Cell Biology

Research Group
SA Associate Professor Yukako Fujinaga, Ph.D.
SA Assistant Professor Yo Sugawara, Ph.D.
SA Assistant Professor Takuhiro Matsumura, Ph.D.

Many bacterial toxins are able to severely damage the host, even at very low concentrations. Most are enzymes that act catalytically and with high specificity on functional host cell molecules, thereby markedly modulating host homeostasis. The toxins are also often highly efficient in accessing their target molecule in the host. The ingenious transport systems involved often exploit the fundamental membrane trafficking machinery and the functions of intracellular organelles. Therefore, studies seeking to elucidate toxin trafficking could provide us with valuable information about basic cellular function, as well as aiding our understanding of the pathology induced by these toxins and helping us to develop effective therapeutic strategies against them. We are currently engaged in studying the transport mechanisms of the botulinum neurotoxin complex, which must pass down the digestive tract and cross the epithelial barrier lining the intestine to cause food-borne botulism.

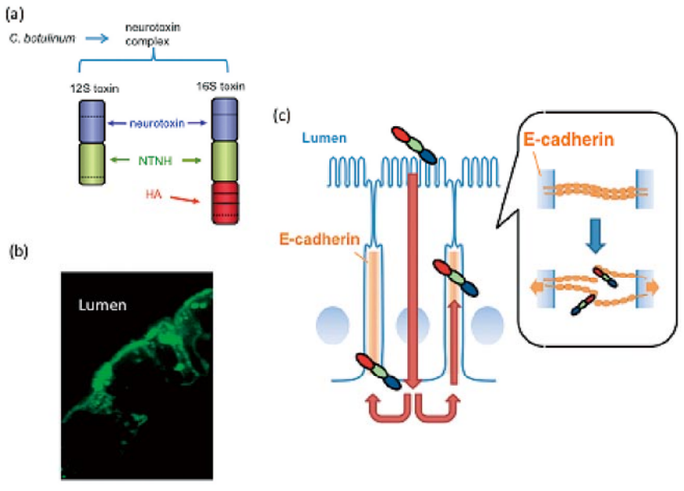


Figure (a) Schematic depiction of the botulinum neurotoxin complex (16S and 12S toxins). Orally ingested neurotoxin complexes cross the intestinal epithelial barrier to cause food-borne botulism. (b) 16S toxin (green) penetrates the intestinal epithelium. (c) Interaction of botulinum neurotoxin complexes with the intestinal epithelial barrier. The HA of the botulinum neurotoxin complex binds E-cadherin and disrupts E-cadherin–mediated cell-to-cell adhesion, thereby disrupting the epithelial paracellular barrier.

Recent publications

1. Sugawara Y, Fujinaga Y. The botulinum toxin complex meets E-cadherin on the way to its destination. Cell Adh Migr. 2011; 5(1): 34-36. [Review]
2. Sugawara Y, Matsumura T, Takegahara Y, Jin Y, Tsukasaki Y, Takeichi M, Fujinaga Y. Botulinum HA disrupts the intercellular epithelial barrier by directly binding E-cadherin. J Cell Biol. 2010; 189 (4), 691-700
3. Jin Y[1], Takegahara Y[1], Sugawara Y, Matsumura T, Fujinaga Y. Disruption of the epithelial barrier by botulinum hemagglutinin (HA) proteins - Differences in cell tropism and the mechanism of action between HA proteins of types A or B, and HA proteins of type C. Microbiology. 2009; 155(Pt 1): 35-45.[1] These authors are contributed equally.
4. Matsumura T, Jin Y, Kabumoto Y, Takegahara Y, Oguma K, Lencer WI, Fujinaga Y. The HA proteins of botulinum toxin disrupt intestinal epithelial intercellular junctions to increase toxin absorption. Cell Microbiol. 2008; 10(2): 355-364.
5. Matsumura T[1], Fujinaga Y[1], Jin Y, Kabumoto Y, Oguma K. Human milk SIgA binds to botulinum type B 16S toxin and limits toxin adherence on T84 cells. Biochem Biophys Res Commun. 2007; 352(4), 867-872.

Laboratory for Viral Pathogenesis and Immunity

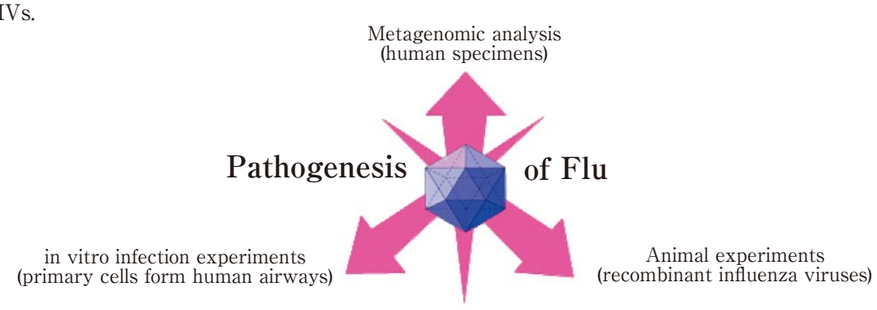
Research Group
SA Associate Professor Takaaki Nakaya, Ph.D.
SA Assistant Professor Tomo Daidoji, D.V.M., Ph.D.

◆ Molecular mechanism of H5N1 avian influenza virus pathogenesis

In recent years, the highly pathogenic avian influenza virus (AIV) H5N1 emerged from southeast Asia and raised serious worldwide concern about the risk of an influenza pandemic. However, how H5N1 induces disease remains poorly understood. We are using in vitro and animal experiments to study the role the viral glycoprotein hemagglutinin (HA) plays in viral growth and cell toxicity. Recent achievements are listed below:

By genetically changing H5 AIV by recombinant DNA techniques, H5N1-HA has been shown to be one of the major viral factors that determine lethality in mice. We confirmed that the pathogenicity of HA depends on its cleavage sequence, which is consistent with previous observations. However, our further experiments suggest that other region(s) and amino acids of H5N1-HA may also participate in the pathogenicity of H5N1.

That H5N1-HA can induce significant cellular toxicity was demonstrated by in vitro experiments. We showed that H5N1-HA–specific cell toxicity (apoptosis) was observed in porcine as well as human primary airway epithelial cells. Similar results were also observed in primary cells from a water fowl known to be a natural AIV host. In contrast, HA proteins from previously isolated AIVs, including the H5 subtype, did not induce this severe cell toxicity. Thus, the genotype of HA may be critical for the pathogenicity and/or cellular toxicity of H5N1 AIVs.



◆ Metagenomic analysis of viral pathogens in humans: Development of Pathogen Identification System by using a high-throughput “Next-Generation” DNA sequencer (RAPID system; Department of Infection Metagenomics)

We are establishing a protocol to rapidly obtain the whole genome information of viral pathogens. This is expected to significantly accelerate the speed with which pathogens can be identified. Using this protocol, we have successfully demonstrated the presence of pathogenic microbes in clinical human samples without resorting to conventional selective procedures for specific pathogens.

Recent publications

1. Daidoji T, Kaihatsu K, Nakaya T. Curr Chem Biol [Review] 2010 4: 208-18. The role of apoptosis in influenza virus pathogenesis ad the mechanisms involved in anti-influenza therapies.
2. Okumura Y, Takahashi E, Yano M, Ohuchi M, Daidoji T, Nakaya T, Bottcher E, Garten W, Klenk HD, Kido H. J Virol. 2010 84(10): 5089-96. Novel type II transmembrane serine proteases, MSPL and TMPRSS13, Proteolytically activate membrane fusion activity of the hemagglutinin of highly pathogenic avian influenza viruses and induce their multicycle replication.
3. Ueda M, Daidoji T, Du A, Yang C-S, Ibrahim M-S, Ikuta K, Nakaya T. J Virol. 2010 84(6):3068-78. Highly pathogenic H5N1 avian influenza virus induces extracellular Ca2+ influx, leading to apoptosis in avian cells.
4. Nakamura S, Yang C-S, Sakon N, Ueda M, Tougan T, Yamashita A, Goto N, Takahashi K, Yasunaga T, Ikuta K, Mizutani T, Okamoto Y, Tagami M, Morita R, Maeda N, Kawai J, Hayashizaki Y, Nagai Y, Horii T, Iida T, Nakaya T. PLoS ONE. 2009 4(1):e4219. Direct metagenomic detection of viral pathogens in nasal and fecal specimens using an unbiased high-throughput sequencing approach.
5. Daidoji T, Koma T, Du A, Yang C-S, Ueda M, Ikuta K, Nakaya T. J Virol. 2008 82(22): 11294-307. H5N1 avian influenza virus induces apoptotic cell death in mammalian airway epithelial cells.

Laboratory of Genomic Research on Pathogenic Bacteria

Research Group

SA Professor Tetsuya Iida, Ph.D.
Postdoctoral Fellow Shigeaki Matsuda, Ph.D.
Postdoctoral Fellow Hirotaka Hiyoshi, Ph.D.

This research group is studying pathogenic bacteria from the genomic point of view.

Our main research targets are as follows:

1. Characterization of the mechanism(s) used by bacterial pathogens to infect host organisms by identifying infection-related changes in pathogen genome expression: To understand the molecular mechanisms by which bacterial pathogens infect host organisms, we are using DNA microarrays and other molecular methods to investigate the changes in the genome expression pattern of various bacterial pathogens that occur during their interaction with their target host.

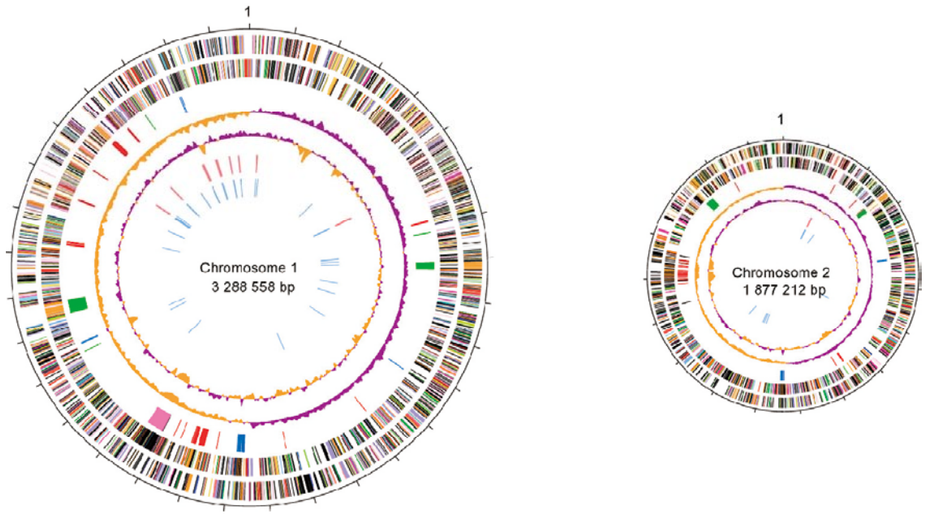


Figure 1. Whole genome sequence of *Vibrio parahaemolyticus*

2. Analysis of the mechanism(s) that lead to the emergence of new infectious diseases: The unique features of various newly emerged bacterial pathogens are being studied by analyzing their genomes and comparing them with those of other bacterial strains.

3. Investigation of the life cycles of bacterial pathogens in their natural environment: Based on what is currently understood about various bacterial pathogens, we are seeking to characterize their life cycles in their natural habitats.

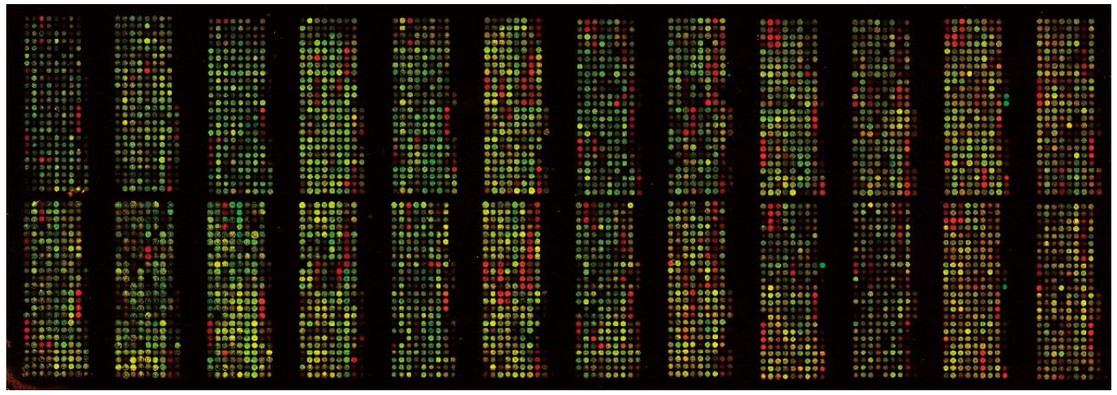


Figure 2. Characterization and comparison of the genomes of pathogenic bacteria by using DNA microarrays

4. Development of new methods for the rapid identification of bacterial pathogens based on genomic information: To rapidly diagnose bacterial infections, a novel system for identifying bacterial pathogens by large-scale DNA sequencing is being developed.

Recent publications

1. Kodama T, Gotoh K, Hiyoshi H, Morita M, Izutsu K, Akeda Y, Park KS, Cantarelli VV, Dryselius R, Iida T, Honda T. Two regulators of *Vibrio parahaemolyticus* play important roles in enterotoxicity by controlling the expression of genes in the Vp-PAI region. PLoS One. 2010 Jan 13;5(1):e8678.
2. Okada N, Iida T, Park KS, Goto N, Yasunaga T, Hiyoshi H, Matsuda S, Kodama T, Honda T. Identification and characterization of a novel type III secretion system in *trh*-positive *Vibrio parahaemolyticus* strain TH3996 reveal genetic lineage and diversity of pathogenic machinery beyond the species level. Infect Immun. 2009 Feb;77(2):904-13.
3. Dryselius R, Izutsu K, Honda T, Iida T. Differential replication dynamics for large and small *Vibrio* chromosomes affect gene dosage, expression and location. BMC Genomics. 2008 Nov 26;9:559.
4. Nakamura S, Maeda N, Miron IM, Yoh M, Izutsu K, Kataoka C, Honda T, Yasunaga T, Nakaya T, Kawai J, Hayashizaki Y, Horii T, Iida T. Metagenomic diagnosis of bacterial infections. Emerg Infect Dis. 2008 Nov;14(11):1784-6.
5. Kodama T, Rokuda M, Park KS, Cantarelli VV, Matsuda S, Iida T, Honda T. Identification and characterization of VopT, a novel ADP-ribosyltransferase effector protein secreted via the *Vibrio parahaemolyticus* type III secretion system 2. Cell Microbiol. 2007 Nov;9(11):2598-609.

Laboratory of Combined Research on Microbiology and Immunology

Research Group SA Associate Professor Hiroki Nagai, Ph.D

Protein secretion is a process of fundamental importance for bacterial pathogenesis. Whether they deliver toxins or directly inject effector proteins into the cytoplasm of host cells, bacterial protein secretion systems play a central role in modulating eukaryotic cell functions. *Legionella pneumophila* are Gram-negative bacteria that are found ubiquitously in soil and freshwater environments. Once inhaled by humans, *Legionella* infections can result in a severe form of pneumonia known as Legionnaires' disease. *Legionella* use a type IV secretion system to deliver effector proteins, and this mediates the establishment of a replicative niche in host cells. The goal of our research is to understand at the molecular level how *Legionella* subvert host cellular functions to accomplish their successful intracellular replication. To this end, the following research projects are currently in progress.

(1) Analysis of the structure and function of the type IV secretion apparatus.

There is essentially nothing known about the substrate transfer across eukaryotic and bacterial membranes that occurs via type IV secretion systems. Furthermore, the macromolecular structure of the type IV secretion apparatus is largely unknown. To address these questions, we are working towards the structural and functional analysis of the type IV secretion apparatus from *Legionella*.

(2) Analysis of effector proteins that translocate from *Legionella* to host cells.

We previously demonstrated that RalF is translocated by the type IV secretion system of *Legionella* into host cells and is required for the recruitment of host ARF proteins to *Legionella*-containing vacuoles. We also recently demonstrated that the effector LubX acts as an E3 ligase and targets another effector for proteasomal degradation within host cells. LubX is the first effector protein that has been shown to target and regulate another effector within host cells.

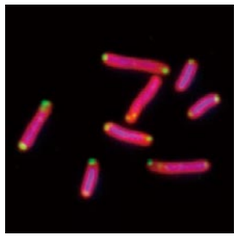


Fig. 1 Type IV apparatus localizes to bacterial poles (Green).

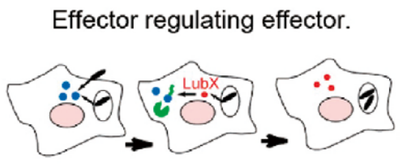


Fig. 2 Discovery of metaeffector.

Recent publications

1. Kubori T, Shinzawa N, Kanuka H, Nagai H. *Legionella* metaeffector exploits host proteasome to temporally regulate cognate effector. PLoS Pathog. 2010;**6**(12),e1001216.
2. Nakano N, Kubori T, Kinoshita M, Imada K, Nagai H. Crystal structure of *Legionella* DotD: insights into the relationship between type IVB and type II/III secretion systems. PLoS Pathog. 2010;**6**(10),e1001129.
3. Kubori, T., Hyakutake, A. and Nagai, H. Legionella translocates an E3 ubiquitin ligase that has multiple U-boxes with distinct functions. Mol. Microbiol. 2008;**67**(6),1307-1319.
4. Nagai, H., Cambronne, E.D., Kagan, J.C., Amor, J.C., Kahn, R.A. and Roy, C.R.. A C-terminal translocation signal required for Dot/Icm-dependent delivery of the *Legionella* RalF protein to host cells. Proc. Natl. Acad. Sci. USA. 2005;**102**,826-831.
5. Amor,J.C., Swails, J., Roy, C.R., Nagai, H., Ingmundson, A., Cheng, X., and Kahn, R.A. The structure of RalF, an ARF guanine nucleotide exchange factor from *Legionella pneumophila,* reveals the presence of a cap over the active site. J. Biol. Chem, 2005;**280**, 1392-1400.

Office of Combined Program on Microbiology and Immunology

Research Group
Research promotion group Associate Professor Yoshiko Murakami, M.D., Ph.D.
Education promotion group Associate Professor Hodaka Fujii, M.D., Ph.D.

Office activities

Our institute and the Immunology Frontier Research Center are world premier institutes in the fields of microbiology and immunology, respectively. These institutes are located next to each other. To take maximum advantage of this situation, our office works to encourage cross-disciplinary microbiology and immunology research as follows:

Research promotion

To directly promote cross-disciplinary microbiology and immunology research, we are implementing the following plans.

1. Organization of the Awaji international forum on infection and immunology, which is held in September annually.
2. Organization of the research progress report, which is produced every month by our institute.
3. Organization of the symposium and the research presentation, which are held annually.
4. Organization of interactive projects that involve the Institut Pasteur in France, Chonnam University in Korea, and the Research Collaboration Center on Emerging and Re-emerging Infections in Thailand.

These activities aim to facilitate cooperative research on microbiology and immunology by promoting research collaboration, information exchange, personal exchange between laboratories, and preparing the research environment.

Education promotion

To facilitate seamless cross-disciplinary research on microbiology and immunology, we also direct a multidisciplinary graduate program on microbiology and immunology. This task includes designing the curriculum and its contents. We also organize an Open House of the institute and guide new students.

Laboratory of Immunoregulation

Research Group Associate Professor Yoshiko Murakami, MD. Ph.D

I have an additional appointment in the Department of Immunoregulation, where I serve as the leader of the PNH group. This group is performing the following studies (see details on the Department page):

1. Investigation of the pathogenesis of paroxysmal nocturnal hemoglobinuria (PNH), an acquired glycosylphosphatidylinositol (GPI) deficiency.
2. Investigation of the pathogenesis of inherited GPI deficiency.
3. Investigation of the functional significance of GPI-anchored proteins by using the Pgap3 KO mouse in which GPI-anchored proteins fail to localize within the raft due to defective GPI anchor remodeling.

Recent publications

1. Sena CB, Fukuda T, Miyanagi K, Matsumoto S, Kobayashi K, Murakami Y, Maeda Y, Kinoshita T, Morita YS. Controlled expression of branch-forming mannosyltransferase is critical for mycobacterial lipoarabinomannan biosynthesis. J Biol Chem. 2010 Mar 9. doi: 10.1074/jbc.M109.077297
2. Kanzawa N, Maeda Y, Ogiso H, Murakami Y, Taguchi R, Kinoshita T. Peroxisome dependency of alkyl-containing GPI-anchor biosynthesis in the endoplasmic reticulum. Proc Natl Acad Sci U S A. 2009 Oct 20;106(42):17711-6.
3. Almeida AM*, Murakami Y*, Baker A, Maeda Y, Roberts IA, Kinoshita T, Layton DM, Karadimitris A. Targeted therapy for inherited GPI deficiency. N Engl J Med. 2007 Apr 19;356(16):1641-7(* equally contributed).
4. Almeida AM*, Murakami Y*, Layton M, Hillmen P, Sellick G, Maeda Y, Richards S, Patterson S, Kotsianidis I, Mollica L, Crawford D, Baker A, Ferguson M, Roberts I, Houlston R, Kinoshita T, Karadimitris A. Hypomorphic promoter mutation in the mannosyltransferase-encoding PIG-M gene causes inherited glycosylphosphatidylinositol deficiency. Nat. Med., 12:846-851. 2006(* equally contributed).
5. Inoue N, Izui-Sarumaru T, Murakami Y, Endo Y, Nishimura J, Kurokawa K, Kuwayama M, Shime H, Machii T, Kanakura Y, Meyers G, Wittwer C, Chen Z, Babcock W, Frei-Lahr D, Parker C, Kinoshita T. Molecular basis of clonal expansion of hematopoiesis in two patients with paroxysmal nocturnal hemoglobinuria (PNH). Blood 2006 108:4232-4236.

Fujii Group

Research Group
Associate Professor Hodaka Fujii, M.D., Ph.D.
Assistant Professor Toshitsugu Fujita, Ph.D.

We are developing novel technologies to address important questions in biology. In addition, we are analyzing mechanisms that regulate the immune system by using state-of-the-art transgenic/knock-out/knock-in technologies.

I. Development of novel technologies to elucidate fundamental principles of the immune system
(1) We developed the inducible translocation trap (ITT) system to identify the signal-induced nuclear translocation of signaling proteins (Fig. 1). ITT is the first non-protein–specific technology that can identify nuclear-translocating proteins; it also enables the analysis of the "translocatome", namely the entire set of proteins that translocate to the nucleus in response to a defined extracellular stimulus. We will use ITT to (i) identify and characterize signal-induced nuclear-translocating proteins, (ii) perform high-throughput screening of small compounds that affect the nuclear translocation of particular signaling proteins, and (iii) screen an RNAi library to identify proteins that regulate the nuclear translocation of signaling proteins.
(2) We are developing the insertional chromatin immunoprecipitation (iChIP) system to isolate specific genomic regions that retain their in vivo conformation. This system will enable us to perform unbiased molecular biological and biochemical analyses of the chromatin structure of specific genomic regions and to identify the molecules (proteins, DNA, RNA, and others) that interact with these regions. iChIP will also help us to elucidate molecular transcriptional regulation, cell differentiation, and lineage commitment mechanisms, especially those involved in lymphocyte development.

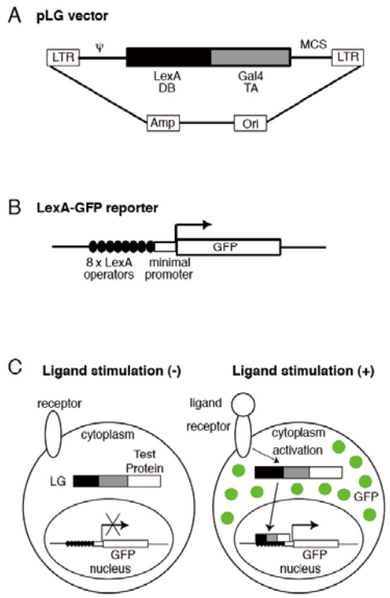


Figure 1. The inducible translocation trap system.

II. Analysis of immune regulation mechanisms and development of therapies for autoimmune diseases
(1) We identified a novel phosphorylated nuclear protein, Cyclon, whose expression is induced in T cells when they are activated. We found that Cyclon regulates the activation-induced cell death of T cells by modulating the expression levels of Fas (Fig. 2). We are currently using transgenic and gene-deficient mice to analyze the in vivo functions of Cyclon and the molecular mechanisms by which it regulates Fas expression. The knowledge generated by these studies will be used to develop effective therapies of autoimmune diseases.
(2) We identified GARP, an activated T-regulatory cell (T-reg)-specific cell surface molecule, and showed that it plays an important role in the immune suppression that is mediated by T-regs. We are currently elucidating the in vivo function of GARP by using transgenic and gene-deficient mice.

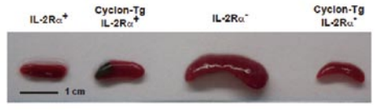


Figure 2. Normalization of the splenomegaly in interleukin-2 receptor α-chain–deficient mice by the transgenic expression of Cyclon.

Recent publications

1. Hoshino A, Fujii H. Insertional chromatin immunoprecipitation: a method for isolating specific genomic regions. J Biosci Bioeng. 2009 Nov;108(5):446-9.
2. Saint Fleur S, Hoshino A, Kondo K, Egawa T, Fujii H. Regulation of Fas-mediated immune homeostasis by an activation-induced protein, Cyclon. Blood. 2009 Aug 13;114(7):1355-65.
3. Wang R, Kozhaya L, Khaitan A, Fujii H, Unutmaz D. Expression of GARP selectively identifies activated human Foxp3+ regulatory T cells. Proc Natl Acad Sci U S A. 2009 Aug 11;106(32):13439-44.
4. Singh AP, Buscaglia CA, Wang Q, Levay A, Nussenzweig DR, Walker J, Winzeler EA, Fujii H, Fonoura BMA, Nussenzweig, V. Plasmodium circumsporozoite protein promotes the development of the liver stages of the parasite. Cell. 2007 Nov 2;131(3):492-504.
5. Hoshino A, Hirst JA, Fujii H. Regulation of cell proliferation by interleukin-3-induced nuclear translocation of pyruvate kinase. J Biol Chem. 2007 Jun 15;282(24):17706-11.

Thailand - Japan Research Collaboration Center on Emerging and Re-emerging Infections

Director SA Professor Shigeyuki Hamada, D.D.S., Ph.D.

It was believed until recently that infectious diseases could be conquered through the development of chemotherapies and vaccines. However, the recent worldwide emergence of new infectious diseases and reemergence of infectious diseases that were once considered to be controlled has seriously challenged this notion. Under these circumstances, intensive research that closely monitors and rapidly analyzes emerging and re-emerging infections is urgently required. Since a variety of infectious diseases can spread rapidly across national borders, it is obvious that these diseases cannot be controlled by the independent efforts of individual nations.

To this end, Osaka University founded the Research Collaboration Center on Emerging and Re-emerging Infections (RCC-ERI) in the Thai National Institute of Health (NIH), Department of Medical Sciences, Ministry of Public Health of Thailand in 2005.

The facility consists of P2 and P3 biohazard containment laboratories and various other equipment and facilities on 600-m² of floor space. Previously, most of the research projects conducted abroad were short-term, with the researchers only staying for a few months to complete their experiments. Due to the installation of the RCC-ERI, researchers are now able to stay for longer periods of time. The RCC-ERI aims to carry out research projects on both emerging and re-emerging infections in close collaboration with the researchers at the NIH, while at the same time developing the talents of young scientists from Japan and Southeast Asian countries in the field of infection.

To conduct basic and applied research and to develop human resources, the RCC-ERI consists of two sections that are devoted to bacterial and viral infection research. In addition, we aim to establish an effective system that would (i) provide information that would help to prevent the emergence of emerging and re-emerging infections, and (ii) promptly activate a variety of countermeasures for such a disease emerged, including developing therapeutics or vaccines. Finally, we wish to begin collaborations with laboratories from the nations that neighbor Thailand so that we can be at the frontline with the capacity to quickly respond to any globally spreading infectious disease.

P2-level laboratory

P3-level laboratory

Section of Bacterial Infections

Research Group		
	SA Professor	Shigeyuki Hamada, D.D.S., Ph.D.
	SA Associate Professor	Yumi Kumagai, Ph.D.
	SA Researcher	Kazuhisa Okada, Ph.D.
	SA Researcher	Takashi Nozawa, Ph.D.
	Research Fellow	Amonrattana Roobthaisong, M.Sc.
	Research Fellow	Chetsada Boonthimat, M.Sc.

The Section of Bacterial Infections pays special attention to emerging and reemerging bacterial diseases that are prevalent or are broken out in Asian countries. We study the molecular epidemiology of enteric or systemic bacterial infections. Moreover, in collaboration with the National Institute of Health, Department of Medical Sciences, Ministry of Public Health of Thailand, we develop detection and identification techniques for the diagnosis of bacterial diseases.

It has been reported that pneumonia, tuberculosis, and acute diarrheal diseases are associated with high morbidity and mortality rates in Thailand. Consequently, in the Program of Promotion of Research Network for Emerging and Reemerging Infectious Diseases during the 2010–2014 fiscal years, we prioritize research on enteric infectious diseases in Thailand that are caused by *Vibirio cholerae* and diarrheagenic *E. coli*.

In addition, *Streptococcus suis*, which is generally pathogenic and frequently isolated from diseased pigs, has been found to cause several systemic (zoonotic) infectious diseases in humans, namely meningitis, infective endocarditis, and toxic shock-like syndrome. This has mainly been observed in Asian countries, including Northern Thailand. We survey this emerging zoonotic infection closely, and elucidate the molecular pathogenesis of *S. suis* infections.

Cholera surveillance in the border of Thailand and Myanmar

Recent publications

1. Okada K, Chantaroj S, Roobthaisong A, Hamada S, Sawanpanyalert P. A Cholera Outbreak of the Vibrio cholerae O1 El Tor Variant Carrying Classical CtxB in Northeastern Thailand in 2007. Am J Trop Med Hyg, 2010. May; 82(5):875-8.
2. Puiprom O, Chantaroj S, Gangnonngiw W, Okada K, Honda T, Taniguchi T, Sawanpanyalert P. Identification of colonization factors of enterotoxigenic Escherichia coli with PCR-based technique. Epidemiol Infect 2010.Apr;138(4):519-24.
3. Okada K, Chantaroj S, Taniguchi T, Suzuki Y, Roobthaisong A, Puiprom O, Honda T, Sawanpanyalert P. A rapid, simple, and sensitive loop-mediated isothermal amplification method to detect toxigenic Vibrio cholerae in rectal swab samples. Diagn Microbiol Infect Dis 2010. Feb;66(2):135-9.
4. Maruyama F, Kobata M, Kurokawa K, Nishida K, Sakurai A, Nakano K, Nomura R, Kawabata S, Ooshima T, Nakai K, Hattori M, Hamada S, Nakagawa I. Comparative genomic analyses of Streptococcus mutans provide insights into chromosomal shuffling and species-specific content. BMC Genomics 2009. Aug 5;10:358.
5. Kasai S, Okada K, Hoshino A, Iida T, Honda T. Lateral transfer of the lux gene cluster. J Biochem 2007. Feb;141(2):231-7.

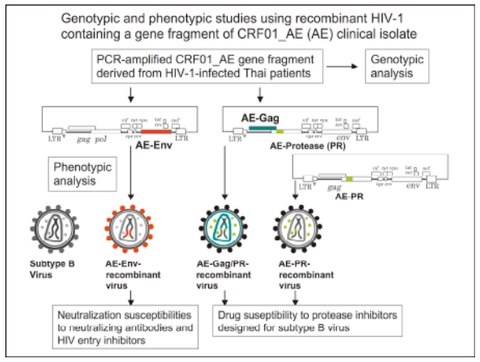
Section of Viral Infections

Research Group		
	SA Professor	Naokazu Takeda, Ph.D.
	SA Professor	Masanori Kameoka, Ph.D.
	SA Researcher	Natsuko Kishishita, Ph.D.
	Postdoctoral Fellow	Uamporn Siripanyaphinyo, Ph.D.
	Postdoctoral Fellow	Sompong Sapsutthipas, Ph.D.
	Postdoctoral Fellow	Nitchakarn Noranate, Ph.D.
	Research Fellow	Piraporn Utachee, M.Sc.
	Research Fellow	Chris Verathamjamras, M.Sc.
	Research Fellow	Samatchaya Boonchawalit, M.Sc.
	Research Fellow	Uranan Tumkosit, M.Sc.

Intestinal infectious diseases (enteroviral infections): It has recently become difficult to isolate viruses from patients with hand, foot and mouth diseases, and it has been suggested that enteroviruses other than Enterovirus 71 and Coxsackie virus 16 may be involved. To grasp the prevalence of these viruses in Thailand, molecular epideomiological studies are currently underway.

Blood-borne infectious diseases (HIV diseases/AIDS): We are performing basic studies that examine the virological and immunological characteristics of the HIV-1 CRF01_AE strains that are prevalent in Southeast Asia, including Thailand. In addition, the mechanism by which HIV acquires viral drug resistance to anti-retroviral drugs is being studied.

Mosquito-borne infectious diseases (dengue fever): We are constructing infectious molecularly cloned viruses to identify the viral genes that are involved in viral pathogenesis.



Recent publications

1. Kanai Y, Chittaganpitch M, Nakamura I, Li GM, Bai GR, Li YG, Ikuta K, Sawanpanyalert P. Distinct propagation efficiencies of H5N1 influenza virus Thai isolates in newly established murine respiratory region-derived cell clones. Virus Res. 2010 Nov;153(2):218-25.
2. Soonthornsata B, Tian YS, Utachee P, Sapsutthipas S, Isarangkura-na-Ayuthaya P, Auwanit W, Takagi T, Ikuta K, Sawanpanyalert P, Kawashita N, Kameoka M. Design and evaluation of antiretroviral peptides corresponding to the C-terminal heptad repeat region (C-HR) of human immunodeficiency virus type 1 envelope glycoprotein gp41. Virology. 2010 Sep 15;405(1):157-64.
3. Kanai Y, Boonsathorn N, Chittaganpitch M, Bai G, Li Y, Kase T, Takahashi K, Okuno Y, Jampangern W, Ikuta K, Sawanpanyalert P. The impact of antigenic drift of influenza A virus on human herd immunity: Sero-epidemiological study of H1N1 in healthy Thai population in 2009. Vaccine. 2010 Jul 26;28(33):5437-44.
4. Li YG, Chittaganpitch M, Waicharoen S, Kanai Y, Bai GR, Kameoka M, Takeda N, Ikuta K, Sawanpanyalert P. Characterization of H5N1 influenza viruses isolated from humans in vitro. Virol J. 2010 Jun 1;7:112.
5. Utachee P, Nakamura S, Isarangkura-Na-Ayuthaya P, Tokunaga K, Sawanpanyalert P, Ikuta K, Auwanit W, Kameoka M. Two N-linked glycosylation sites in the V2 and C2 regions of human immunodeficiency virus type 1 CRF01_AE envelope glycoprotein gp120 regulate viral neutralization susceptibility to the human monoclonal antibody specific for the CD4 binding domain. J Virol. 2010 May;84(9):4311-20.

Frontier Biomedical Science Underlying Organelle Network Biology

Research program

Our main goal is to create an interdisciplinary research center, which will coordinate work in cell biology, microbiology/immunology, and glycobiology in order to converge on a greater knowledge of the organellar network.
The subjects under study will range widely:

- modes of communication between organelles
- interactions of pathogens with the organelle network
- the roles of glycosylation in determining organelle function
- the effects of abnormal glycosylation on disease

By combining these fundamental studies with clinical research, we will drive the creation and development of the new field of organelle network medicine. To this end, we will conduct research focused on achieving an integrated understanding of human disease, and on developing technological solutions to clinical problems.
On the road to these goals, we will ask clinically relevant questions, such as:

- What is the mechanism leading from invasion by a pathogen to establishment of a full-fledged infection?
- How might we interfere in the interactions between pathogens and the organelle network?
- How does disruption of organelle network result in neurodegenerative diseases? To what extent does ER quality control play a role?
- Can we exploit changes in protein and sugar chain modifications to develop novel diagnostic tools?

Diseases are not merely the consequences of single causes or single gene mutations. We recognize that diseases are multifactorial conditions, in which many genes and environmental factors intertwine and interact in a complex way. Based on this recognition, our Center will encourage biological and clinical research that is committed to understanding disease at the level of both the molecular and organelle networks.
Ultimately, our greater understanding of the organelle network, and of clinically important issues in organelle biology, will allow us to develop novel therapeutic strategies that accelerate the medicine of the 21st century.

Education program

Young scientists will someday become the biologists and clinical researchers of the future. Therefore, we are committed to establishing a world-class training environment in which junior scientists are fully supported and actively trained to take global leadership roles in 21st century science.
Our training program has many aspects, all focused on training the future leaders of the new field of organelle network medicine:

- **Researcher development program** - Through a series of courses taught by domestic and international academics and industrialists, young scientists will improve their management capabilities, technical writing, English proficiency and grant application skills.
- **Interdisciplinary graduate curriculum** - Investigators will have the opportunity to study across multiple university schools, including the Schools of Medicine, Pharmaceutical Sciences, Dental Sciences, Science and Frontier Biosciences, and the Research Institute for Microbial Disease.
- **Support for interdisciplinary projects** - We will support research that breaks through barriers between departments and fields, and encourage young researchers to be creative and flexible in their work.
- **Meetings for international young investigators** - Our young researchers will themselves organize meetings, held in a "training camp" format, to facilitate bottom-up international exchanges.
- **Construction of an international network** - We will have a special staff devoted to the task of expanding cooperation with overseas centers
- **Securing career paths** - We will provide Ph.D.-level researchers with space and funding, in order to help move them along the road to independence. After completion of graduate training, we will cooperate with other research centers to help our alumni achieve tenured positions.
- **Research assistants (RAs)** - RA positions will be available in order to provide financial assistance to graduate students. Our students will also be actively encouraged and well supported to attend international meetings.
- **Overseas practical training program** - In order to advance clinical training, we will create practical courses using overseas centers.
- **Graduate student exchange** - Providing opportunities to study abroad.

We hope to create an environment where traditional barriers between departments and fields essentially don't exist - where young scholars can quickly and efficiently obtain advice from researchers in different fields.
Major changes like this don't happen by themselves - which is why our faculty will include a specially appointed associate professor whose primary responsibility is the cultivation of our collaborative networks with other institutions.

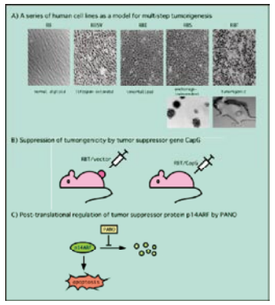
Members

Name Affiliated department, Position title, Specialized field, Academic degree	Division of roles
Yoshihiro Yoneda Graduate School of Frontier Biosciences (Department of Frontier Biosciences), Professor, Cell Biology, M.D., Ph.D.	Coordination of a center establishment and elucidation of the organelle network
Tatsushi Toda Graduate School of Medicine (Division of Medicine), Visiting Professor, Mol.Genetics & Neurology, M.D., Ph.D.	Sugar chain modifications and neurodegeneration diseases
Yoshihide Tsujimoto Graduate School of Medicine (Division of Medicine), Professor, Medical Genetics, Ph.D.	Mechanism of cell death and organelle
Kiyoshi Takeda Graduate School of Medicine (Division of Medicine), Professor, Immunology, M.D., Ph.D.	Analysis of activity regulation mechanism of the natural immunity system
Kazunori Tomono Graduate School of Medicine (Division of Medicine), Professor, Clinical Microbiology, M.D., Ph.D.	Analysis of antibacterial activity and development of new antimicrobial agents
Masaya Tohyama United Graduate School of Child Development, (Department of Child Development) Professor, Anatomy & Neurosci, M.D., Ph.D.	Neurological function abnormalities and organella
Toshikazu Nakamura Office for University-Industry Collaboration, SA Professor, Biochemistry, Molecular Biology, Ph.D.	Treatment strategies against neurodegenerative and renal diseases with HGF
Tetsuo Takehara Graduate School of Medicine (Division of Medicine), Professor, Gastroenterology & Hepatology, M.D., Ph.D.	Hepatitis onset mechanism and treatment strategies
Eiji Miyoshi Graduate School of Medicine (Division of Health Sciences), Professor, Clinical and Laboratory Medicine, M.D., Ph.D.	Development of biomarkers using sugar chain technologies
Yoshinao Wada Graduate School of Medicine (Division of Medicine), Visiting Professor, Mass Spectrometry, M.D., Ph.D.	Development of sugar chain analysis methods
Yukari Fujimoto Graduate School of Science (Department of Chemistry), Associate Professor, Organic Chemistry, Ph.D.	Recognition system of bacteria with its glycoconjugate
Naoyuki Taniguchi RIKENCSystems Glycobiology Research Group), Group Director, Biochemistry, M.D., Ph.D.	Functional analyses of sugar chains/proteins
Tamotsu Yoshimori Graduate School of Frontier Biosciences (Department of Frontier Biosciences), Professor, Cell Biology, Ph.D.	Analyses of roles of membrane traffic in infection/immunity
Hitoshi Kikutani Research Institute for Microbial Diseases, Professor, Immunology, M.D., Ph.D.	Study on the dynamics of the acquired immunity
Eisuke Mekada Research Institute for Microbial Diseases, Professor, Cell Biology, Ph.D.	Analyses of factors related to toxicity manifestation of the diphtheria toxin
Yoshiharu Matsuura Research Institute for Microbial Diseases, Professor, Virology, Ph.D.	Infection mechanism of Hepatitis C virus and studies on the control methods
Tatsuo Shioda Research Institute for Microbial Diseases, Professor, Virology, Ph.D.	Studies on host factors related to HIV infection
Yasuhiko Horiguchi Research Institute for Microbial Diseases, Professor, Bacteriology, Ph.D.	Analyses of functions and structures of bacterial virulence factors
Kazuyoshi Ikuta Research Institute for Microbial Diseases, Professor, Virology, Ph.D.	Studying emerging viral infections and their pathogenesis
Toshihiro Horii Research Institute for Microbial Diseases, Professor, Parasitology, Ph.D.	Development of malaria vaccines and analyses of the host-parasite interactions
Shizuo Akira WPI Immunology Frontier Research Center, Professor, Immunology, M.D., Ph.D.	Studies on innate immunity
Taroh Kinoshita WPI Immunology Frontier Research Center, Professor, Immunology, Ph.D.	Analysis of significance of GPI anchor in the host-pathogen interactions
Hisashi Arase WPI Immunology Frontier Research Center, Professor, Immunology, M.D., Ph.D.	Studies on mechanism of immunoregulation by pathogens
Atsushi Kumanogoh Graduate School of Medicine (Division of Medicine), Professor, Immunology, M.D., Ph.D.	Studies of immunoregulation/regulatory molecules of cell migration
Tadashi Suzuki RIKEN (Systems Glycobiology Research Group), Team Leader, Biochemistry, D. Sc.	Quality controls of free sugar chains and glycoproteins
Yoshiki Yamaguchi RIKEN (Syetems Glycobiology Research Group), Team Leader, Structural Biology, D. Pharm.	Structural analyses of glycoconjugates by NMR

Cancer Cell Research Group

Research Group　Associate Professor　Masuo Yutsudo, Ph.D.
Assistant Professor　Shinji Higashiyama, Ph.D.

(1) Analyses of the CapG tumor suppressor gene
We isolated a series of cell lines from a human diploid fibroblast that had been transformed in various ways; these lines included immortalized, anchorage-independent, and tumorigenic cell lines (Figure). Analysis of their gene expression profiles revealed that the tumorigenic cell line had lost CapG protein expression. Analysis of a variety of cancer cell lines revealed that several had also lost CapG expression. When these tumorigenic human cancer cell lines were transfected with CapG cDNA, they all became non-tumorigenic. We also identified a protein that interacts with CapG: this protein is an oncogene product that forms a complex with another tumor suppressor protein. Thus, CapG may suppress tumorigenicity by modulating the activity of a particular oncoprotein/tumor suppressor protein complex.



(2) Cellular dedifferentiation involved in tumorigenesis
It is well known that cancer cells often express genes that are usually only expressed by less differentiated cells. We found that during the malignant progression of our model cell lines, fibroblast-specific gene expression was shut off and the expression of several new genes was switched on. We are currently studying how the alteration of differentiation status relates to tumorigenesis.

(3) Post-translational regulation of tumor suppressor protein p14ARF by PANO
We isolated a novel apoptosis-inducing gene called PANO, which encodes a nucleolar protein. Our studies then revealed that PANO up-regulates the expression of tumor suppressor protein p14ARF by inhibiting its degradation, and that this may induce apoptosis. We are currently investigating whether this gene participates in human tumorigenesis.

Recent publications

Watari, A., Takaki, K., Higashiyama, S., Li, Y., Satomi, Y., Takao, T., Tanemura, A., Yamaguchi, Y., Katayama, I., Shimakage, M., Miyashiro, I., Takami, K., Kodama, K., and Yutsudo, M. (2006). Suppression of tumorigenicity, but not anchorage-independece, of human cancer cells by new candidate tumor suppressor gene CapG. Oncogene 25, 7373-7380.

Germ Cell Group

Research Group　Associate Professor　Masami Nozaki, Ph. D.

(1) DNA methylation during spermatogenesis.
Many of the testicular germ cell-specific genes are retroposons, most of which contain a CpG-rich region within their ORFs. We discovered that the methylation of the CpG dinucleotides in the ORF represses its promoter in somatic cells and that demethylation is necessary for gene expression in spermatogenic cells. We are analyzing the molecular basis of the epigenetic modifications, including DNA methylation and histone methylation, which occur in a distinct genomic region in germ cells.

(2) Unique structure of sperm chromatin.
In the mammalian sperm nucleus, the haploid genome is packaged into a highly compact structure that contains protamines and some remaining histones. We are analyzing the physiological importance of the somatic-like, histone-containing regions of sperm chromatin.

(3) Establishment of an in vitro germ cell differentiation system
To examine the genetic requirements that are needed for germ cell formation and epigenetic reprogramming, we are in the process of establishing an in vitro developmental system based on ES cell differentiation.

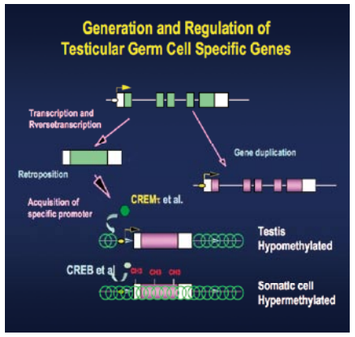


Figure legend. Generation and regulation of testicular germ cell-specific genes. Testicular isoform genes are generated by conventional gene duplication or retroposition. In the case of retroposons, the inserted cDNA, which is reverse-transcribed from mRNA, cannot be expressed in any tissue because the mRNAs lack a promoter in the 5'-flanking sequence of the genomic DNA. This implies that testicular germ cells provide an appropriate environment for retroposon transcription and facilitate gene expression from promoter-like sequences. This in turn suggests that the rules governing gene transcription during the later stages of spermatogenesis differ drastically from those in other cell types.

Recent publications

Kato Y, Kaneda M, Hata K, Kumaki K, Hisano M, Kohara Y, Okano M, Li E, Nozaki M, Sasaki H. Role of the Dnmt3 family in de novo methylation of imprinted and repetitive sequences during male germ cell development in the mouse. Hum Mol Genet. 2007 Oct1; 16(19): 2272-80.

Animal Resource Center for Infectious Diseases

Research Group

Head, Professor	Masaru Okabe,Ph.D.
Associate Professor	Masahito Ikawa,Ph.D.
Assistant Professor	Ayako Isotani,Ph.D.
Assistant Professor	Hidetoshi Hasuwa,Ph.D.
Assistant Professor	Naokazu Inoue,Ph.D.
Specially Appointed Assistant Professor	Yuhkoh Satouh,Ph.D.

To study microbial diseases, it is important to analyze the interactions between the host and pathogenic organisms. Animal models are indispensable in current microbiological and immunological research, particularly since through the use of molecular biology and biotechnology, we can generate gene-manipulated mice that can aid our understanding of the mechanisms of infection. For these purposes, experimentally infected animals should be managed in a suitable, safe and controlled manner. Animal Resource Center for Infectious Diseases is a unique facility that was established in 1967 to meet these requirements. The center is separated into three areas: one for animal experiment for microbial disease model (P2 and P3 level), an SPF area, and a conventional area. The section for animal experiment for disease model is completely air-conditioned and maintained at a negative air pressure to minimize the risk of contamination. Each sub-area has an individual pass-through type autoclave to sterilize all materials before their removal. The exhaust air is filtered to avoid exterior dissemination of pathogenic microbes. These measures ensure that disease model animals can be handled safely without accidental cross-contamination.
Before gaining access to this restricted facility, researchers must take an official orientation and submit a research plan for committee review. The condition of the animals is regularly inspected.

Services such as the generation of gene-manipulated animals, in vitro fertilization, and cryopreservation of mouse strains are available at our facility. (Table 1)

Figure 1: Biosafety level 3 room (Building A, 1st floor)
The room for disease model animal and experimentation at biosafety level 3. Hemorrhageic fever with renal syndrome-causing virus (HFRSV) was isolated in this area. In addition, animal experiments for Creutzfeldt-Jakob Disease (CJD), severe acute respiratory syndrome (SARS) and Acquired Immune Deficiency Syndrome (AIDS) can be handled in this facility.

Table 1) No. of mouse lines produced/preserved at the facility

period	TG mice	KO mice	Cryopreservation
1995-1997	92	14	83
1998-2000	116	23	178
2001-2003	101	49	443
2004-2006	43	76	331
2007-2009	21	69	216
2010	21	57	56

TG, transgenic; KO, knock-out

DNA-chip Development Center for Infectious Diseases

Research Group

Head, Professor (SUP)	Hiroshi Nojima, Ph. D.
Assistant Professor	Daisuke Okuzaki, Ph. D.
Assistant Professor (SUP)	Norikazu Yabuta, Ph. D.

Facility Management: The establishment of infectious diseases is driven by the gene expression of pathogenic organisms within the infected host cells. To understand parasite pathogenesis and pathophysiology, and to develop new methods to prevent and treat infectious diseases, it is necessary to identify the pathogenic genes that are expressed in the infected host cells and to determine how they induce disease at the genetic level. This requires the analysis of the transcriptional patterns of both the genes of the pathogenic organism and the responsive genes of the host genome.

The DNA-chip Development Center for Infectious Diseases is a unique facility that was established in 2004 to analyze the transcriptional dynamics and variations involved in infectious diseases. Two research approaches are employed in this facility:

(1) Transcriptome analysis using DNA-chip analyzers.

The high density DNA microarray system in this facility permits comprehensive transcriptional analysis of gene expression in the human or mouse host, and in various pathogenic organisms. Two DNA microarray systems, namely the Agilent-type and the Affymetrix-type, are available in this center. Our real-time PCR analysis system (ABI, PRISM7900HT-2) is also useful for more accurate quantitative analysis of the transcriptional levels of particular genes. In addition, a novel DNA microarray system (Genopal of Mitsubishi Rayon Co. Ltd.) is currently being used in this center. An example of its practical use is the establishment of a blood RNA-based system for the diagnosis of autoimmune diseases. This system employs a "focused microarray" that examines the expression of ~200 blood cell-specific and disease-related genes.

(2) Proteome analysis using mass spectrometry.

Comprehensive translational analyses are also very important in furthering our understanding of infectious diseases. The MS/MS spectrometer installed in this facility enables the analysis of the expression, interactions and modifications of proteins from humans, mice, and pathogenic organisms. This center is also capable of recent technical innovations, such as the mass spectrometric detection of pathogenic organisms that facilitates the development of novel diagnostic systems for infectious diseases.

Fig. 1 : High density DNA microarray system.

Fig. 2 : MS/MS spectrometer.

Recent publications

1. Okuzaki D, Fukushima T, Tougan T, Ishii T., Kobayashi S, Yoshizaki K, Akita T, and Nojima H. Genopal™: a novel hollow fiber array for focused microarray analysis. DNA Res., 2010 Dec;17(6):369-79.
2. Tougan T, Okuzaki D, Nojima H. Chum-RNA allows preparation of a high-quality cDNA library from a single-cell quantity of mRNA without PCR amplification.Nucleic Acids Res. 2008 Sep;36(15):e92.
3. Kobayashi S, Ito A, Okuzaki D, Onda H, Yabuta N, Nagamori I, Suzuki K, Hashimoto H, Nojima H. Expression profiling of PBMC-based diagnostic gene markers isolated from vasculitis patients. DNA Res. 2008 Aug;15(4):253-65.
4. Tougan T, Onda H, Okuzaki D, Kobayashi S, Hashimoto H, Nojima H. Focused microarray analysis of peripheral mononuclear blood cells from Churg-Strauss syndrome patients. DNA Res. 2008 Apr 30;15(2):103-14.
5. Nakamura N, Shimaoka Y, Tougan T, Onda H, Okuzaki D, Zhao H, Fujimori A, Yabuta N, Nagamori I, Tanigawa A, Sato J, Oda T, Hayashida K, Suzuki R, Yukioka M, Nojima H, Ochi T. Isolation and expression profiling of genes upregulated in bone marrow-derived mononuclear cells of rheumatoid arthritis patients. DNA Res. 2006 Aug 31;13(4):169-83.

Center for genetic analysis of biological responses

Research Group

<Production laboratory for genetically-manipulated animals>

Head, Professor	Masaru Okabe, Ph. D.
SA Associate Professor	Kazuo Yamagata, Ph. D.
Assistant Professor	Hidetoshi Hasuwa, Ph. D.
SA Assistant Professor	Jun Ueda, Ph. D.

<Resource laboratory for genetically-manipulated animals>

Visiting Professor	Kenichi Yamamura, M. D., Ph. D.
Associate Professor	Masahito Ikawa, Ph. D.
Assistant Professor	Ayako Isotani, Ph. D

<Laboratory for promotion of collaborative research>

Visiting Professor	Yoichiro Iwakura, Ph. D.
Assistant Professor	Naohisa Goto, Ph. D.
SA Assistant Professor	Yuhkoh Satouh, Ph. D.

<Laboratory for analysis of genetically-manipulated animals>

Professor	Shizuo Akira, M. D., Ph. D.
Professor	Taroh Kinoshita, Ph. D.
Professor	Atsushi Kumanogoh, M. D., Ph. D.
Professor	Hisashi Arase, M. D., Ph. D.
Professor	Hitoshi Kikutani, M. D., Ph. D.
Professor	Masato Okada, Ph. D.
Professor	Nobuyuki Takakura, M. D., Ph. D.
Professor	Hiroshi Nojima, Ph. D.
Assistant Professor	Naokazu Inoue, Ph. D.

Our bodies are kept homeostatically stable by functions of proteins produced from many genes. In other words, our health is basically maintained in accordance with the balance of our gene products. Many diseases can therefore be traced to a defect in, or malfunction of, various genes. In order to find and develop new drugs or new therapy, it is very important to identify the function of each gene in vivo. However, at present, we do not have enough information about the function of various genes to clarify their relationships to each other, or to analyze the relationships to specific diseases systematically.

Gene-disrupted animals can be a powerful tool in helping us understand the role of certain genes in vivo. Such animals can be produced and used as a model for various human diseases as well as to screen new drugs. With this in mind, preparation of gene-disrupted mouse lines of entire genes is planned and progressing as research projects on a national level in many countries. The production of gene-manipulated animals may have another aspect. These animals are considered to be variable animal resources protected by patents to develop new drugs and therapeutic methods. It is highly important for Japan to make a reasonable contribution in this area. Under these circumstances, we created a consortium of three research institutes from three universities (The Research Institute for Microbial Diseases, Osaka University: The Institute of Medical Science, University of Tokyo: Center for Animal Resources and Development, Kumamoto University), placing the headquarters at Kumamoto University. With this consortium, we are sharing our specialties with each other and aiming to produce many gene-manipulated animals closely focused on human diseases.?In our center, we are mainly focusing on genes related to reproduction, infection and allergy, taking advantage of an existing disease screening system in our university, including features such as fluorescent colored sperm and eggs (Figure 1). Through these gene-manipulated animals, we aim to perform translational research for the establishment of new therapeutic systems and to aid in the discovery of new drugs.

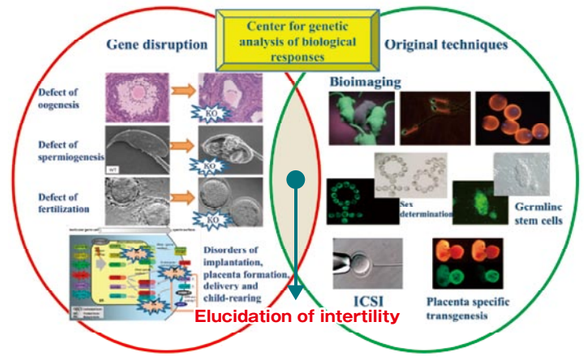


Figure 1. Strategy for elucidating sterility

Recent publications

1. Isotani A, Hatayama H, Kaseda K, Ikawa M, Okabe M. Formation of a thymus from rat ES cells in xenogeneic nude mouse↔rat ES chimeras. Genes Cells. 2011 Apr;16(4):397-405.
2. Kumasawa K, Ikawa M, Kidoya H, Hasuwa H, Saito-Fujita T, Morioka Y, Takakura N, Kimura T, Okabe M. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. Proc Natl Acad Sci U S A. 2011 Jan 25;108(4):1451-5.
3. Ikawa M, Tokuhiro K, Yamaguchi R, Benham AM, Tamura T, Wada I, Satouh Y, Inoue N, Okabe M. Calsperin is a testis-specific chaperone required for sperm fertility. J Biol Chem. 2011 Feb 18;286(7):5639-46.
4. Inoue N, Kasahara T, Ikawa M, Okabe M. Identification and disruption of sperm-specific angiotensin converting enzyme-3 (ACE3) in mouse. PLoS ONE. 2010 Apr 22;5(4):e10301.
5. Fujihara Y, Murakami M, Inoue N, Satouh Y, Kaseda K, Ikawa M, Okabe M. Sperm equatorial segment protein 1, SPESP1, is required for fully fertile sperm in mouse. J Cell Sci. 2010 May 1;123(Pt 9):1531-6.

Biken History Museum

Head — Head, Professor Hiroshi Nojima, Ph.D.

The Research Institute for Microbial Diseases (RIMD) was established in 1934 by combining two institutions, namely the Takeo Tuberculosis Institute and the Osaka Leprosy Institute. To commemorate the 70th anniversary of its establishment, a plan of the Biken History Museum was proposed, and it was opened on December 17, 2010. The opening ceremony was started with the ribbon cutting by Professor Hitoshi Kikutani (Head of RIMD, middle in Fig. 1), Dr. Yasushi Higashi (Chairman of BIKEN) and Mr. Tokuji Takeo (Adviser of AKUA).

Fig.1. A picture of Biken History Museum before the opening ceremony.

Fig. 2. The ribbon cutting ceremony.

Inside the museum, you will find the portrait bust of Mr. Jiemon Takeo, 10th (left) and 11th (right), Koch's microscope donated by the German government, Influenza and SARS virus model made by Kaiyodo Co. Ltd. (Kadoma, Osaka).
Both members and non-members of Osaka University can visit the Museum free of charge from 9:00 a.m. to 5 p.m. on working days. A gorgeous pamphlet will be presented by writing the visitors name at the information desk of RIMD which is located near the Museum entrance.

Fig. 3. The portrait bust of Mr. Jiemon Takeo, 10th (left) and 11th (right).

Fig. 4. Koch's microscope.

Fig. 5. A plastic model of Influenza virus made by Kaiyodo Co. Ltd

Fig. 6. A plastic model of Severe Acute Respiratory Syndrome (SARS) virus made by Kaiyodo Co. Ltd.

Central Instrumentation Laboratory

Head — Professor Masato Okada, Ph. D.
Associate Professor Masuo Yutsudo, Ph. D.
Assistant Professor Shinji Higashiyama, Ph. D.

This laboratory was established around the late 1950's. Since then, it has grown to possess a variety of high-performance instruments, including ultracentrifuges, electron microscopes, a laser microdissection system, cell sorters, automatic plasmid purification systems, DNA sequencers, and a mass spectrum analyzer. This laboratory also provides a room installed with large liquid nitrogen tanks for the preservation of living materials such as cells and viruses, and a room for treating specified injurious chemicals. Several technicians are employed to keep the instruments in proper working condition as well as to provide advice to beginners and ongoing support for researchers. In addition, they execute cell sorting, nucleotide sequencing, observation by electron microscope, and mass spectrometric analyses on samples upon request from Institute researchers. These kinds of services will become more and more important in the future since many instruments are becoming increasingly precise and complicated and require extensive training. Plans to accommodate such changes are currently in progress.

Radioisotope Laboratory

Head — Head, Professor Masato Okada, Ph.D.
Assistant Professor Shinji Higashiyama, Ph.D.

The Radioisotope (RI) Laboratory is adjacent to the main building of the Institute in 1967, and was extended by branch laboratories with a combined space of about 600 sq. m. during the establishment of the North building in 1979 and the Central Laboratory for Biological Hazardous Microbes in 1983. In 1998, a radiation exposure room was established on the first basement level of the South building. The Genome Information Research Center radioisotope laboratory joined in 2007. The main RI Laboratory was closed in 2010, and the new RI Laboratory will be established in the Immunology Frontier Research Center building. The RI Laboratory is designed for biomedical experiments with radioisotopes and plays an important role in the Institute. Its facilities include an RI stockroom, a distribution room, a tissue culture room, and an area for RI measuring equipment. Safety requirements are met by a stringent security system that involves the use of ID cards and the computerized management of radioisotopes. About 200 researchers use this laboratory every year.

Central Laboratory for Biological Hazardous Microbes

Head — Professor Tatsuo Shioda, D. Med. Sc.

This laboratory was set up in 1983 to ensure the safe handling of biologically hazardous microbes such as the HFRS (hemorrhagic fever with renal syndrome) virus. Since then, all experimental studies using such microbes, including the human immunodeficiency virus (HIV), have been carried out in this laboratory. The laboratory is a 3-story building that is 550 sq. m. in area. The first floor is reserved for experiments using radioisotopes. The facilities are designed to protect researchers from getting infected with pathogens and to prevent the spread of biohazardous pathogens outside the building.

The supply of fresh air is regulated to keep room interiors at negative pressure. Air is released from the facility through high-quality outlet filters to minimize contamination of the outside environment. Furthermore, each room is equipped with safety cabinets and autoclaves for the sterilization of used materials before their disposal. The entire laboratory has been renovated from 2005 to 2007 to increase numbers of pathogens simultaneously used in this laboratory.

In 2008 and 2009, 64 and 66 researchers, respectively, were approved by the Biosafety Committee to use this laboratory. The microbes used included HIV, human and avian influenza viruses, SARS corona virus, and scrapie agent.

Library

Head — Professor Yoshiharu Matsuura, D.V.M., Ph.D.

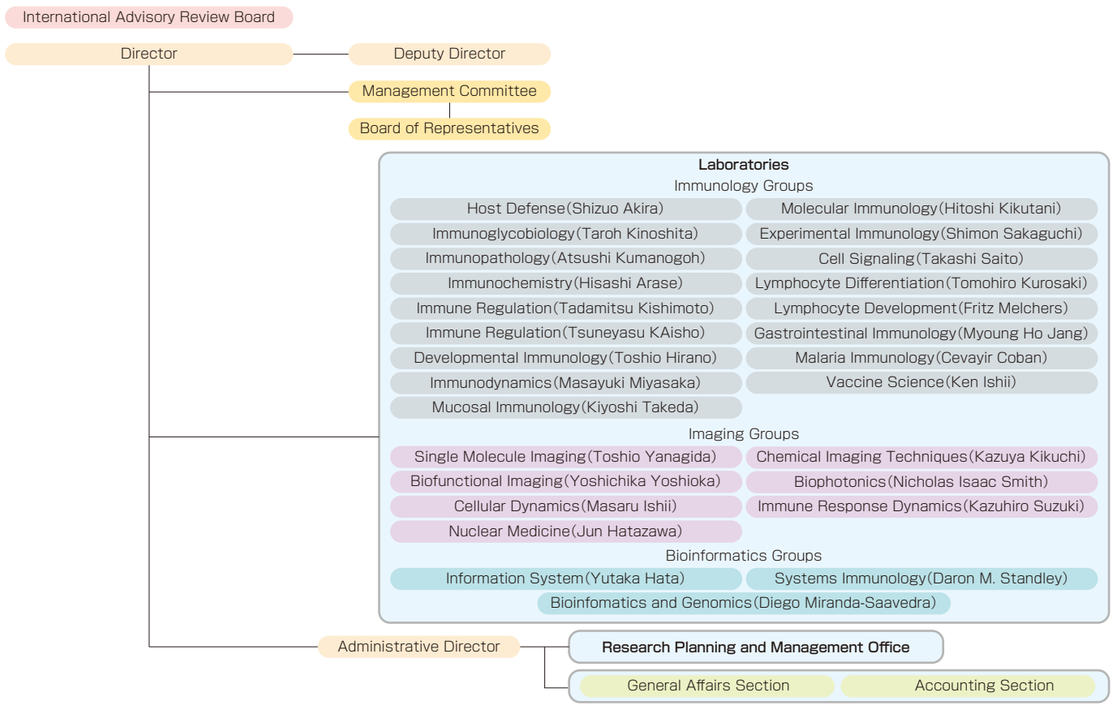
The RIMD library collects academic books and journals on microbiology and immunology, as well as work on related scientific fields such as cell biology, genetics, histology, developmental biology, biochemistry, pharmacology pathology, microbiology and oncology. In particular, we have collected rare books on parasitology that cannot be found at other institutes. These books are frequently accessed by visitors to the RIMD library. Due to the construction of a new building for the Integrated Life Science Building, the RIMD library was moved to a temporary library room set up on the 1st floor of the south building of RIMD on December 2007. At July 2010, the RIMD library was moved to a new space located at the 1st floor of the new RIMD main building. Since the temporary library space was quite small, we were forced to discard most of the old books and journals. For this reason, we now only hold journals published after 1991, all of the back issues of Biken Journal, and 13,000 books. We now purchase 70 and 20 journals published in English and Japanese, respectively. Most of the books are kept on the bookshelves in the stock room, but textbooks and newly arrived journals are arranged on the front shelf of the bureau. Most of the materials in the RIMD library are registered on the online index at the main Library of Osaka University, which can also be accessed by libraries throughout Japan via the Inter Library Loan (ILL) system. Three librarians handle the RIMD library together with two professors, two associate professors and one assistant professor who act as members of RIMD library committee. These members also take care of the publication of the "Annual Reports of the Research Institute for Microbial Diseases Osaka University" (online only from 2003).

World Premier International Research Center

Immunology Frontier Research Center

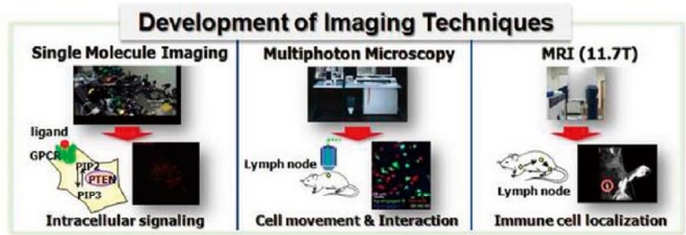
●Uniqueness and Objectives

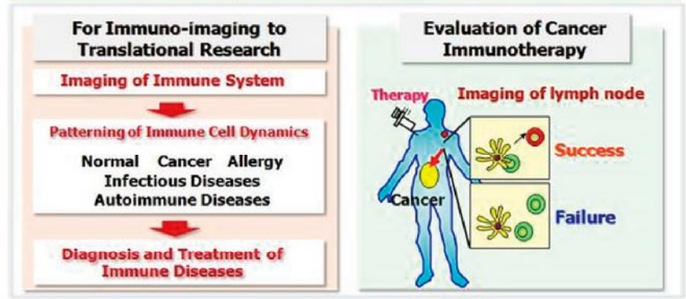
Immunology has always been a scientific strength of Japan. In particular, Osaka University has been historically known for its leading immunology researchers including Prof. Shizuo Akira.
The Osaka University Immunology Frontier Research Center (IFReC), directed by Prof. Akira, is an example of this reputation. IFReC was selected by the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) as one of the nation's exclusive World Premier International (WPI) Research Center Initiative Programs. Established on October 1st of 2007, the center is expected to engage in high level research that will make it an international leader in the field of immunology. Immunology investigates the mechanisms that protect the body against microbial infection. Because the immune system is essential for eliminating infectious pathogens from the body, its malfunction gives rise to various disorders such as autoimmune diseases, allograft rejection during transplantation, and allergies.
The scientific aim of IFReC is to unveil a comprehensive understanding of the dynamic immune system by employing a variety of imaging technologies and Bioinformatics to immunology.

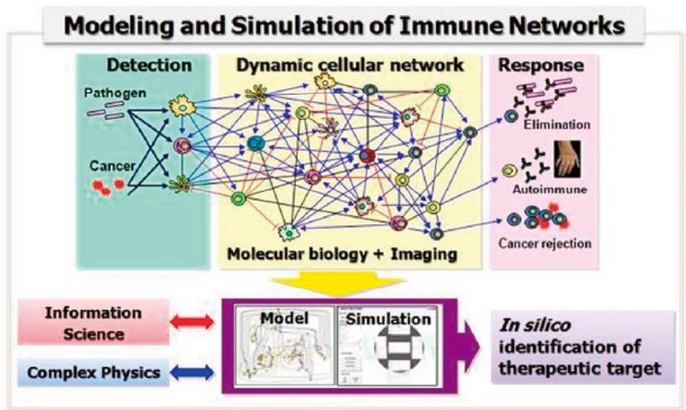


●Research fields／Expected Achievements

To date, research in immunology has either been carried out by isolating immune cells from the body and examining these cells in vitro or by using in vitro cultured cell lines. Although such studies have provided many new insights on the immunology system, we still do not understand the system well enough to predict how it will respond when a certain pathogen invades. One approach to resolving this problem is to study immune responses in a spatiotemporal manner. Given the importance of the spatiotemporal organization of the lymphoid organs in an immune response and the importance of understanding how single immune cells behave, combining immunology techniques with imaging techniques is vital for advancing immunology to a new frontier.
To meet our needs and expectations, new imaging techniques will need to be developed by applying an interdisciplinary effort that combines people from disparate fields like physics, computer science, and immunology.
By integrating the immunology and imaging fields, we will be able to understand the dynamic interactions of immune cells and their activation. This will lead to new and more efficient development strategies for vaccines and immune therapies when combating infectious diseases, cancers and autoimmunity disorders.







Management Expenses Grants

(unit : thousand yen)

Classification	2006	2007	2008	2009	2010
Personnel	943,574	917,415	905,437	859,673	887,150
Non-Personnel	643,140	495,488	513,073	548,947	704,408
Total	1,586,714	1,412,903	1,418,510	1,408,620	1,591,558

Other Grants

(unit : thousand yen)

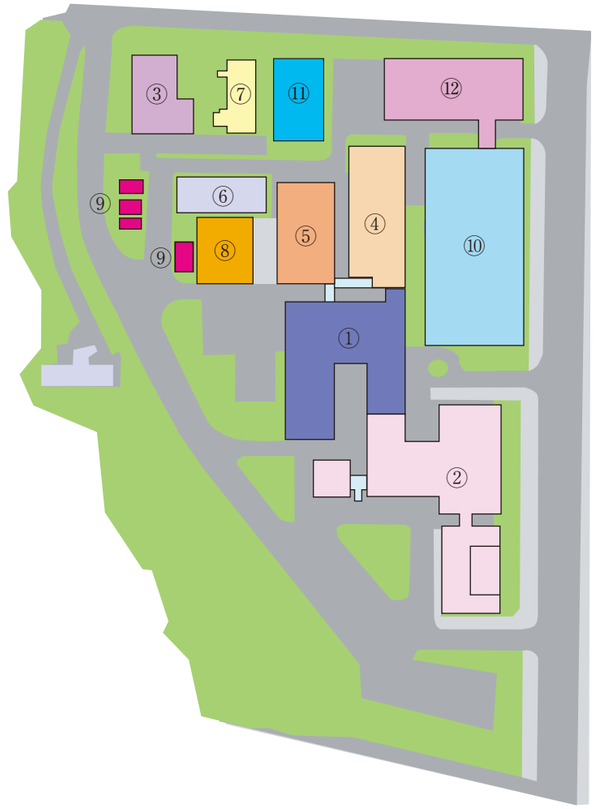
Classification	2006	2007	2008	2009	2010
Contract Research	997,753	1,175,396	1,022,353	1,040,180	908,861
Donations for Research	252,863	1,215,677	187,969	343,772	689,654
Miscellaneous	7,499	4,591	3,406	2,090	4,506
Total	1,258,115	2,395,664	1,213,728	1,386,042	1,603,021

Grants-in-Aid for Scientific Research

(unit : thousand yen)

Classification	2006	2007	2008	2009	2010
MEXT Research Grants	578,559	613,870	863,592	688,999	453,744
Health and Labor Sciences Research Grants	156,049	237,575	163,278	118,789	107,632
Health and Labor other Research Grants -	-	-	18,000	13,988	0
21st Century COE Program Grants	192,500	196,900	-	-	0
Global COE Program Grants -	-	-	149,599	120,037	85,441
Total	927,108	1,048,345	1,194,469	941,813	646,817

Site Area ····· **36,197**㎡ | Building Area ····· **7,349**㎡ | Gross Floor Area **27,005**㎡



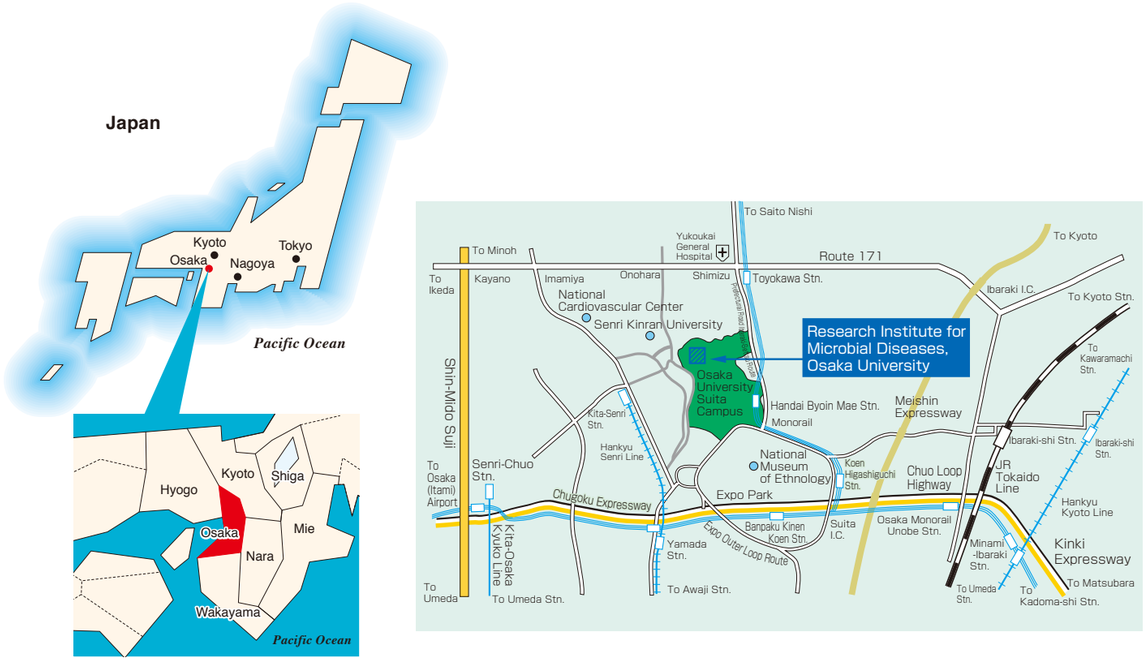
① Main building(left) and
⑩ Integrated Life Science building(right)

② South building

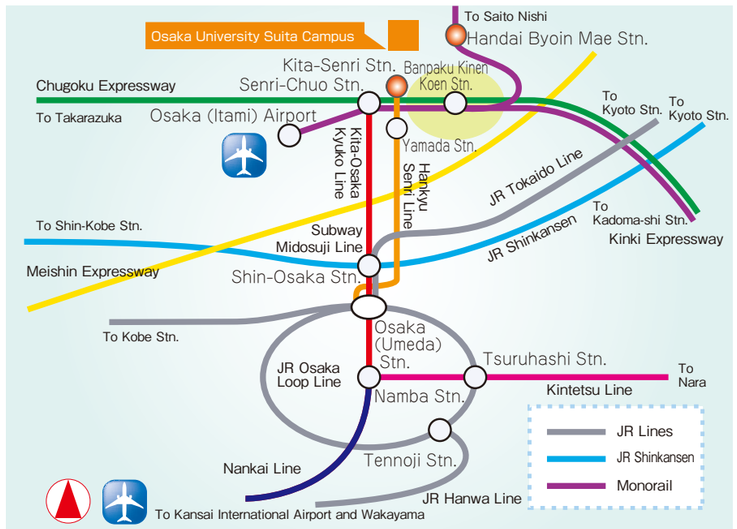
⑦ Central Laboratory for Biological Hazardous Microbes
and
⑤⑥ Animal Resource Center

Building name	Total floor numbers	Building area(㎡)	Total floor area(㎡)
①Main building	7	1,518	6,059
②South building	3(1basement)	1,712	4,941
	DNA-chip Developmet Center and Genome Information Research Center are included		
③North building	3	499	1,259
④Annex	2	771	1,548
⑤Animal Resource Center A	2	640	1,293
⑥Animal Resource Center B	4	354	1,430
⑦Central Laboratory for Biological Hazardous Microbes	3	242	550
⑧Central Instrumentation Laboratory	2	378	504
⑨Depository for dangerous chemicals	1	163	163
⑩Integrated Life Science building	10	1,072	9,258
⑪Animal Resource Center C(belonging to IFReC)	3 (1 basement)	600	2,400
⑫New IFReC building(tentative name)	9	770	6,592

Location



Transportation access



●Train : 12-minute walk from "Kita-Senri" Station on Hankyu Senri Line.
●Monorail : 20-minute walk from "Handai Byoin Mae" Station on Osaka Monorail Saito Line.
●Bus : From Senri-Chuo Station :
5-minute walk from "Handai-Guchi" Bus Stop on Hankyu Buses heading to "Onohara Higashi", "Toyokawa-Eki", "Fujikasai".
12-minute walk from "Handai Honbu Mae" Bus Stop on Hankyu Buses heading to "Handai Honbu Mae" or "Ibaraki Mihogaoka".
●Bus : From Hankyu Ibaraki-shi Station:
12-minute walk from "Handai Honbu Mae" Bus Stop on buses heading to "Handai Honbu Mae" (via JR Ibaraki Station).

Suita Campus

Open Laboratories for Advanced Bioscience and Biotechnology

1 Administration Bureau	17 International Student Center
2 Graduate School/School of Human Scienses	18 International Center for Biotechnology
3 Graduate School/Faculty of Medicine	19 Center for Advanced Science and Innovation
4 Faculty of Medicine (Dept. of Allied Health Sciences)	20 The Center for Advanced Medical Engineering and Informatics
5 Osaka University Hospital	21 Global Collaboration Center
6 OSaka University Dental Hospital	22 Sustainability Design Center
7 Graduate School/School of Pharmaceutical Scienses	23 Institute of Laser Engineering
8 Graduate School/ School of Engineering	24 Immunology Frontier Research Center
9 Graduate School of Frontier Biosciences	25 Institute of Scientific and Industrual Research
10 Graduate School of Information Science and Technology	26 Institute of Social and Economic Reseach
11 United Graduate School of Child Development	27 Joining and Welding Research Institute
12 Research Institute for Microbial Diseases	28 Research Center for Ultra-high Voltage
13 Institute for Protein Research	29 Cybermedia Center
14 Low Temperature Center	30 Research Center for Nuclear Physics
15 Radioisotope Research Center	
16 Research Center for Environmental Preservation	

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RIMD, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan
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