International Research Center for Infectious Diseases

Department of Special Pathogens
高病原性感染症研究部門

Professor	Chieko Kai, D.V.M., Ph.D.	教授(兼)	農学博士	甲斐智恵子
Professor	Yoshihiro Kawaoka, D.V.M., Ph.D.	教授(兼)	獣医学博士	河岡義裕
Project Assistant Professor	Takeshi Kuraishi, Ph.D.	特任助教	農学博士	倉石武
Project Assistant Professor	Akira Watanabe, Ph.D.	特任助教	薬学博士	渡邉彰
Project Assistant Professor	Takeshi Noda, D.V.M., Ph.D.	特任助教	獣医学博士	野田岳志
Project Assistant Professor	Makoto Ozawa, D.V.M., Ph.D.	特任助教	医学博士	小澤真

Highly pathogenic viral agents causing emerging infectious diseases are of concern not only as public health problems but also as possible biological weapons. The ultimate goal of our research is to unlock the secrets of their pathogenicity in human and to develop effective vaccines and antiviral compounds against these pathogens. We have been investigating the molecular basis for replication cycle and extreme virulence of special pathogens, using Ebola, influenza, and Nipa viruses as models.

1. Replication-Deficient Ebolavirus as a Vaccine Candidate.

Halfmann P, Ebihara H, Marzi A, Hatta Y, Watanabe S, Suresh M, Neumann G, Feldmann H, Kawaoka Y.

Ebolavirus causes severe hemorrhagic fever, with case fatality rates as high as 90%. Currently, no licensed vaccine is available against Ebolavirus. We previously generated a replication-deficient, biologically contained Ebolavirus, EbolaDeltaVP30, which lacks the essential VP30 gene, grows only in cells stably expressing this gene product, and is genetically stable. Here, we evaluated the vaccine potential of EbolaDeltaVP30. First, we demonstrated its safety in STAT-1-knockout mice, a susceptible animal model for Ebolavirus infection. We then tested its protective efficacy in two animal models, mice and guinea pigs. Mice immunized twice with EbolaDeltaVP30 were protected from a lethal infection of mouse-adapted Ebolavirus. Virus titers in the serum of vaccinated mice were significantly lower than those in nonvaccinated mice. Protection of mice immunized with EbolaDeltaVP30 was associated with a high antibody response to the Ebolavirus glycoprotein and the generation of an Ebolavirus NP-specific CD8(+) T-cell response. Guinea pigs immunized twice with EbolaDeltaVP30 were also protected from a lethal infection of guinea pig-adapted Ebolavirus. Our study demonstrates the potential of the EbolaDeltaVP30 virus as a new vaccine platform.

2. Characterization of Ebolavirus Regulatory Genomic Regions.

Neumann G, Watanabe S, Kawaoka Y.

For filoviruses, such as Ebolavirus and the closely related Marburgvirus, transcriptional regulation is poorly understood. The open reading frames (ORFs) that encode the viral proteins are separated by regulatory regions composed of the 3′ nontranslated region (NTR) of the upstream gene, highly conserved transcription stop

and start signals, and the 5′NTR of the downstream gene. The conserved transcription stop and start signals either overlap, or they are separated by intergenic regions (IGRs) of different lengths. To assess the contribution of the regulatory regions to transcription, we established bicistronic minireplicons in which these regions were flanked by upstream and downstream ORFs, the Ebolavirus leader and trailer regions, and by T7 RNA polymerase promoter and ribozyme sequences. We found that the individual viral regulatory regions differ in their ability to direct protein synthesis from the upstream or downstream ORFs. Deletion or modification of the NTRs, IGRs, or transcription stop and start signals affected protein expression levels to various extents; for example, 5′NTRs appear to affect efficient protein expression from the downstream ORF, whereas 3′NTRs seem to attenuate protein expression from the upstream ORF. Overall, our data suggest that the regulation of Ebolavirus protein levels is complex.

3. CD147/EMMPRIN acts as a functional entry receptor for measles virus on epithelial cells.

Watanabe, A., Yoneda, M., Ikeda, F., Terao-Muto, Y., Sato, H., Kai, C.

The wide tissue tropism of measles virus (MeV) suggests that it involves ubiquitously expressed molecules besides signaling lymphocytic activation molecule (SLAM) known as a cellular receptor for morbilliviruses, We identified cyclophilin B (CypB) as host factors binding to MeV nucleoprotein (N) by proteomic analysis. Western blot analysis of purified viral particles showed that CypB was incorporated in viral virions through the binding to N protein. As CypB is known as a ligand for CD147/EMMPRIN (extracellular matrix metalloproteinase inducer), a cell surface glycoprotein, we examined whether CD147 acts as a receptor for MeV. Anti-CD147 antibody or recombinant CypB inhibited MeV-infection on HEK293 cells which are SLAM-negative epithelial cells, while overexpression of human CD147 on CHO cells enhanced MeV-infection. Furthermore, prevention of CypB-incorporation in virions significantly reduced their infectivity to SLAM-negative HEK293 cells, while it had no effect on their infectivity to SLAM-positive HEK293-SLAM cells. These results indicated that CD147 is used as an entry receptor for MeV through incorporated CypB in the virions on SLAM-negative cells.

Publications

Takano R, Nidom CA, Kiso M, Muramoto Y, Yamada S, Sakai-Tagawa Y, Macken C, Kawaoka Y. Phylogenetic characterization of H5N1 avian influenza viruses isolated in Indonesia from 2003-2007. *Virology* 390: 13-21, 2009.

Makino A, Kawaoka Y. Generation of vero cells expressing ebola virus glycoprotein. *J Vet Med Sci* 71: 505-507, 2009.

Makino A, Horimoto T, Kawaoka Y. Binding properties of GP1 protein of Borna disease virus. *J Vet Med Sci* 71: 243-246, 2009.

Sakuma T, Noda T, Urata S, Kawaoka Y, Yasuda J. Inhibition of Lassa and Marburg virus production by tetherin. *J Virol* 83: 2382-2385, 2009.

Murakami S, Horimoto T, Mai le Q, Nidom CA, Chen H, Muramoto Y, Yamada S, Iwasa A, Iwatsuki-Horimoto K, Shimojima M, Iwata A, Kawaoka Y. Growth determinants for H5N1 influenza vaccine seed viruses in MDCK cells. *J Virol* 82: 10502-10509, 2009.

Watanabe T, Watanabe S, Shinya K, Kim JH, Hatta M, Kawaoka Y. Viral RNA polymerase complex promotes optimal growth of 1918 virus in the lower respiratory tract of ferrets. *Proc Natl Acad Sci USA* 106: 588-592, 2009.

Marsolais D, Hahm B, Walsh KB, Edelmann KH, McGavern D, Hatta Y, Kawaoka Y, Rosen H, Oldstone MB. A critical role for the sphingosine analog AAL-R in dampening the cytokine response during influenza virus infection. *Proc Natl Acad Sci USA* 106: 1560-1565, 2009.

Ozawa M, Maeda J, Iwatsuki-Horimoto K, Watanabe S, Goto H, Horimoto T, Kawaoka Y. Nucleotide sequence requirements at the 5′ end of the influenza A virus M RNA segment for efficient virus replication. *J Virol* 83: 3384-3388, 2009.

Kakugawa S, Shimojima M, Goto H, Horimoto T, Oshimori N, Neumann G, Yamamoto T, Kawaoka Y. The Mitogen-activated protein kinase-activated kinase RSK2 plays a role in innate immune responses to influenza virus infection. *J Virol* 83: 2510-2517, 2009.

Fan S, Deng G, Song J, Tian G, Suo Y, Jiang Y, Guan Y, Bu Z, Kawaoka Y, Chen H. Two amino acid residues in the matrix protein M1 contribute to the virulence difference of H5N1 avian influenza viruses in mice. *Virology* 384: 28-322, 2009.

Halfmann P, Ebihara H, Marzi A, Hatta Y,

Watanabe S, Suresh M, Neumann G, Feldmann H, Kawaoka Y. Replication-Deficient Ebolavirus as a Vaccine Candidate. *J Virol* 83: 3810-3805, 2009.

Li Z, Watanabe T, Hatta M, Watanabe S, Nanbo A, Ozawa M, Kakugawa S, Shimojima M, Yamada S, Neumann G, Kawaoka Y. Mutational analysis of conserved amino acids in the influenza A virus nucleoprotein. *J Virol* 83: 4153-4162, 2009.

Neumann G, Watanabe S, Kawaoka Y. Characterization of Ebolavirus Regulatory Genomic Regions. *Virus Res* 144: 1-7, 2009.

Le QM, Sakai-Tagawa Y, Ozawa M, Ito M, Kawaoka Y. Selection of H5N1 influenza virus PB2 during replication in humans. *J Virol* 83: 5278-5281, 2009.

Tamura D, Mitamura K, Yamazaki M, Fujino M, Nirasawa M, Kimura K, Kiso M, Shimizu H, Kawakami C, Hiroi S, Takahashi K, Hatta M, Minagawa H, Kimura Y, Kaneda S, Sugita S, Horimoto T, Sugaya N, Kawaoka Y. Oseltamivir-Resistant Influenza A Viruses Circulating in Japan. *J Clin Micro* 47: 1424-1427, 2009.

Takano R, Nidom CA, Kiso M, Muramoto Y, Yamada S, Shinya K, Sakai-Tagawa Y, Kawaoka Y. A comparison of the pathogenicity of avian and swine H5N1 influenza viruses in Indonesia. *Arch Virol* 154: 677-681, 2009.

Fan S, Gao Y, Shinya K, Li CK, Li Y, Shi J, Jiang Y, Suo Y, Tong T, Zhong G, Song J, Zhang Y, Tian G, Guan Y, Xu XN, Bu Z, Kawaoka Y, Chen H. Immunogenicity and protective efficacy of a live attenuated H5N1 vaccine in nonhuman primates. *PLoS Pathogens* 5(5): 1-11, 2009.

Hatta M, Kohlmeier CK, Hatta Y, Ozawa M, Kawaoka Y. The region required for protein expression from the stop-start pentanucleotide in the M gene of influenza B virus. *J Virol* 83: 5939-5942, 2009.

Watanabe S, Watanabe T, Kawaoka Y. Influenza A virus lacking M2 protein as a live attenuated vaccine. *J Virol* 83: 5947-5950, 2009.

Fujii K, Ozawa M, Iwatsuki-Horimoto K, Horimoto T, Kawaoka Y. The incorporation of influenza A virus genome Segments Does Not Absolutely Require Wild-Type Sequences. *J Gen Virol* 90: 1734-1740, 2009.

Kakugawa S, Shimojima M, Neumann G, Goto H, Kawaoka Y. RuvB-like 2 protein is a suppressor of influenza A virus polymerases. *J Virol* 83: 6429-6434, 2009.

WHO/OIE/FAO H5N1 Evolution Working Group. Continuing progress towards a unified nomenclature for the highly pathogenic H5N1 avian influenza viruses: divergence of clade 2.2 viruses. *Influenza Other Respi Viruses* 3: 59-62, 2009.

Itoh Y, Shinya K, Kiso M, Watanabe T, Sakoda Y, Hatta M, Muramoto Y, Tamura D, Sakai-Tagawa Y, Noda T, Sakabe S, Imai M, Hatta Y, Watanabe S, Li C, Yamada S, Fujii K, Murakami S, Imai H, Kakugawa S, Ito M, Takano R, Iwatsuki-Horimoto K, Shimojima M, Horimoto T, Goto H, Takahashi K, Makino A, Ishigaki H, Nakayama M, Okamatsu M, Takahashi K, Warshauer D, Shult PA, Saito R, Suzuki H, Furuta Y, Yamashita M, Mitamura K, Nakano K, Nakamura M, Brockman-Schneider R, Mitamura H, Yamazaki M, Sugaya N, Suresh M, Ozawa M, Neumann G, Gern J, Kida H, Ogasawara K, Kawaoka Y. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. *Nature* 460: 1021-1025, 2009.

Zhou B, Donnelly ME, Scholes DT, St George K, Hatta M, Kawaoka Y, Wentworth DE. Single Reaction Genomic Amplification Accelerates Sequencing and Vaccine Production for Classical and Swine Origin Human Influenza A Viruses. *J Virol* 83: 10309-10313, 2009.

Victor ST, Lezhava A, Ishidao T, Endo R, Mitani Y, Kawaoka Y, Hayashizaki Y. Isothermal single nucleotide polymorphism genotyping and direct PCR from whole blood using a novel whole-blood lysis buffer. *Mol Diagn Ther* 13: 383-387, 2009.

Shinya K, Makino A, Ozawa M, Kim JH, Sakai-Tagawa Y, Ito M, Le QM, Kawaoka Y. Ostrich involvement in the selection of H5N1 influenza virus possessing mammalian-type amino acids in the PB2 protein. *J Virol* 83: 13015-13018, 2009.

Takahashi T, Hashimoto A, Maruyama M, Ishida H, Kiso M, Kawaoka Y, Suzuki Y, Suzuki T. Identification of amino acid residues of influenza A virus H3 HA contributing to the recognition of molecular species of sialic acid. *FEBS Lett* 583: 3171-31714, 2009.

Kashima Y, Ikeda M, Itoh Y, Sakoda Y, Nagata T, Miyake T, Soda K, Ozaki H, Nakayama M, Shibuya H, Okamatsu M, Ishigaki H, Ishida H, Sawai T, Kawaoka Y, Kida H, Ogasawara K. Intranasal administration of a live nonpathogenic avian H5N1 influenza virus from a virus library confers protective immunity against H5N1 highly pathogenic avian influenza virus infection in mice: comparison of formulations and administration routes of vaccines. *Vaccine* 27: 7402-7408, 2009.

Cillóniz C, Shinya K, Peng X, Korth MJ, Proll SC, Aicher LD, Carter VS, Chang JH, Kobasa D, Feldmann F, Strong JE, Feldmann H, Kawaoka Y, Katze MG. Lethal influenza virus infection in macaques is associated with early

dysregulation of inflammatory related genes. *PLoS Pathog* 5(10): e1000604, 2009.

Gao Y, Zhang Y, Shinya K, Deng G, Jiang Y, Li Z, Guan Y, Tian G, Li Y, Shi J, Liu L, Zeng X, Bu Z, Xia X, Kawaoka Y, Chen H. Identification of amino acids in HA and PB2 critical for the transmission of H5N1 avian influenza viruses in a mammalian host. *PLoS Pathog* 5: e 1000709, 2009.

Kim JH, Hatta M, Watanabe S, Neumann G, Watanabe T, Kawaoka Y. Role of host-specific amino acids in the pathogenicity of avian H5 N1 influenza viruses in mice. *J Gen Virol*. 2009 [Epub ahead of print]

Neumann G, Noda T, Kawaoka Y. Emergence and pandemic potential of H1N1 influenza virus. *Nature* 459: 931-939, 2009.

Horimoto T, Kawaoka Y. Designing vaccines for pandemic influenza. *Curr Top Microbiol Immunol* 333: 165-176, 2009.

Watanabe, A., Yoneda, M., Ikeda, F., Terao-Muto, Y., Sato, H., Kai, C. CD147/EMMPRIN acts as a functional entry receptor for measles virus on epithelial cells. *J. Virol*. (in press)

Imai, C., Fujita, K., Shimizu, F., Sugai, A., Yoneda, M., Kai, C. Comparative and mutational analyses of promoter regions of rinderpest virus. *Virology* 396: 169-177, 2010.

Sugai A, Kooriyama T, Sato H, Yoneda M, Kai C. Epitope mapping of Canine Distemper Virus phosphoprotein by monoclonal antibodies. *Microbiol Immunol*. 53(12): 667-74, 2009.

Yoneda M, Fujita K, Sato H, Kai C. Reverse genetics of Nipah virus to probe viral pathogenicity. *Methods Mol Biol*. 515: 329-37, 2009.

Takayama I, Kubo M, Takenaka A, Fujita K, Sugiyama T, Arai T, Yoneda M, Sato H, Yanai T, Kai C. Pathological and phylogenetic features of prevalent canine distemper viruses in wild masked palm civets in Japan. *Comp Immunol Microbiol Infect Dis*. 32(6): 539-49, 2009.

Inoue Y, Tsukiyama-Kohara K, Yoneda M, Sato H, Kai C. Inhibition of host protein synthesis in B95a cells infected with the HL strain of measles virus. *Comp Immunol Microbiol*. 32(1): 29-41, 2009.

佐藤宏樹・甲斐知恵子　ジステンパーウイルス（特集：動物のウイルス感染症）*Virus Report*, 6(1)：72–80，2009.

International Research Center for Infectious Diseases

Department of Infectious Disease Control
感染制御部門

Professor	Aikichi Iwamoto, M.D., D.M.Sc.		教授(兼)	医学博士	岩本愛吉
Research Associate	Dayong Zhu, Ph.D.		特任研究員	医学博士	朱大勇
Research Associate	Jun-ichi Nunoya. Ph.D.		特任研究員	医学博士	布矢純一

Our research targets are HIV pathogenesis and immune control of the virus. In order to analyze the HIV-antigen presentation to the cytotoxic T lymphocytes (CTLs), we established a monoclonal antibodies to the CTL epitope. We also studies the role of cell surface molecule on HIV-infection. We have been working closely with Division of Infectious Diseases (DID) in the Advanced Clinical Research Center and Department of Infectious Diseases and Applied Immunology (DIDAI).

1. Generation of recombinant monoclonal antibodies against an immunodominant HLA-A*2402-restricted HIV-1 CTL epitope

Jun-ichi Nunoya, Toshihiro Nakashima[1], Ai Kawana-Tachikawa, Katsuhiro Kiyotani[2], Yuji Ito[3], Kazuhisa Sugimura[3], Aikichi Iwamoto

1. Division 2, First Research Department, Kikuchi Research Center, The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan
2. Department of Virology, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan
3. Department of Bioengineering, Faculty of Engineering , Kagoshima University , Kagoshima, Japan.

Cytotoxic T lymphocytes (CTLs) play a crucial role in the immune control of immunodeficiency virus type 1 (HIV-1). HIV-specific CTLs recognize HIV-derived peptides bound to the Major Histocompatibility Complex class I molecules (pMHCs). Antibodies against pMHCs could be a useful tool to analyze antigen presentation both qualitatively and quantitatively, and the molecular interaction between pMHC and their ligands. It has been known to be notoriously difficult to isolate monoclonal antibodies against pMHCs by the conventional hybridoma technique.

To isolate monoclonal antibodies against an immunodominant HIV-1-derived CTL epitope in the nef gene, we used a human scFv phage display library. We panned phage clones with biotinylated Nef138-10/HLA-A*24(A24) and streptavidin-coated magnetic beads. We isolated eight Nef138-10/A24-specific scFv clones and two of them (scFv#3 and scFv#27) were selected for further analysis. The clones specifically stained A24-positive cells pulsed with Nef138-10 peptides. We tried to reconstitute humanized immunoglobulin Gs (IgGs) using a Baculovirus expression system. All of the reconstituted IgGs kept the original specificities of the parental scFvs. The dissociation constants were 23 μM and 20 μM by Biacore, respectively. Unfortunately, the antibodies we cloned could not stain the cells infected HIV-1. He have been continuing our effort to isolate antibodies with higher affinity.

2. Influence of polymorphism in dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin-related (DC-SIGNR) gene on HIV-1 trans-infection.

Dayong Zhu, Ai Kawana-Tachikawa, Aikichi Iwamoto, Yoshihiro Kitamura[1]

1. Research Center for Asian Infectious Diseases, the Institute of Medical Science, the University of Tokyo, Tokyo, Japan

The dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) and DC-SIGN-related (DC-SIGNR) molecules on the cell surface are known to enhance human immunodeficiency virus type 1 (HIV-1) infection by capturing the virions and transmitting them to CD4+ T-cell, a process termed trans-infection. The neck region and carbohydrate recognition domain of the two proteins are important for efficient binding to the HIV-1 envelope protein. DC-SIGNR is polymorphic in Exons 4 and 5 that encode the neck region and carbohydrate recognition domain, respectively; the former contains a variable number of tandem repeats, and the latter the SNP (rs2277998). Since it remains unclear whether the DC-SIGNR polymorphism is related to the risk of HIV-1 infection, we tested possible effects of the polymorphism on HIV-1 trans-infection efficiency, by constructing six kinds of cDNAs encoding DC-SIGNR variants with various numbers of repeat units and various SNP. We were able to express the variants on the surface of Raji cells, a human B cell line. Flow cytometry showed that all the tested DC-SIGNR molecules were efficiently expressed on the cell surface at various levels; the assay for HIV trans-infection efficacy showed that all the tested variants had that activity with different efficacy levels. We found a correlation between the HIV trans-infection efficiency and the mean fluorescent intensity of DC-SIGNR expression ($R(2)=0.95$). Our results suggest that the variation of the tested DC-SIGNR genotypes affects the efficacy of trans-infection by affecting the amounts of the protein expressed on the cell surface.

Publications

1. Nunoya, J., T. Nakashima, A. Kawana-Tachikawa, K. Kiyotani, Y. Ito, K. Sugimura, and A. Iwamoto. 2009. Short communication: generation of recombinant monoclonal antibodies against an immunodominant HLA-A* 2402-restricted HIV type 1 CTL epitope. AIDS Res Hum Retroviruses 25: 897-904.
2. Zhu D, Kawana-Tachikawa A, Iwamoto A, Kitamura Y. Influence of polymorphism in dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin-related (DC-SIGNR) gene on HIV-1 trans-infection. Biochem Biophys Res Commun. 2010 Feb 10. [Epub ahead of print]
3. Miyazaki, E., A. Kawana-Tachikawa, M. Tomizawa, J. Nunoya, T. Odawara, T. Fujii, Y. Shi, G. F. Gao, and A. Iwamoto. 2009. Highly restricted T-cell receptor repertoire in the CD8+ T-cell response against an HIV-1 epitope with a stereotypic amino acid substitution. AIDS 23: 651-60.
4. Koga, M., Tachikawa, A., Heckerman, D., Odawara,T., Nakamura, H., Koibuchi, T., Fujii, T., Miura, T., Iwamoto, A. Transition of impact of HLA class I allele expression on HIV-1 plasma virus loads at a population level over time. Microbiology and Immunology, In press

International Research Center for Infectious Diseases

Department of Infectious Disease Control Division of Microbial Infection

感染制御部門　微生物学分野

Professor	Tetsuro Matano, M.D., D.M.Sc.		教　授	医学博士	俣　野　哲　朗
Project Assistant Professor	Hiroaki Takeuchi, D.M.Sc.		特任助教	医学博士	武　内　寛　明

We are working on Microbiology and Immunology to elucidate the molecular mechanism of viral replication in vivo. We focus on HIV, a representative virus inducing chronic persistent infection. Our current projects are elucidation of AIDS pathogenesis and development of an AIDS vaccine. For clarifying the mechanism of persistent HIV replication and developing an effective AIDS vaccine interfering with its establishment, we are studying acquired immune responses in non-human primate AIDS models.

1. Polyfunctional CD4$^+$ T-cell induction in neutralizing antibody-triggered control of simian immunodeficiency virus infection

Takuya Yamamoto[1], Nami Iwamoto, Hiroyuki Yamamoto, Tetsuo Tsukamoto, Tetsuya Kuwano, Akiko Takeda, Miki Kawada, Yasuko Tsunetsugu-Yokota[1], and Tetsuro Matano: [1]Department of Immunology, National Institute of Infectious Diseases

Rapid depletion of memory CD4$^+$ T cells and delayed induction of neutralizing antibody (NAb) responses are characteristics of HIV and simian immunodeficiency virus (SIV) infections. Although it was speculated that post-infection NAb induction could have only a limited suppressive effect on primary HIV replication, a recent study has shown that a single passive NAb immunization of rhesus macaques one week after SIV challenge can result in reduction of viral loads at the setpoint, indicating possible contribution of post-infection NAb responses to viral control. However, the mechanism accounting for this NAb-triggered SIV control has remained unclear. Here we report rapid induction of virus-specific polyfunctional T-cell responses after the passive NAb immunization post-infection. Analysis of SIV Gag-specific responses of interferon-γ, tumor necrosis factor-α, interleukin-2, macrophage inflammatory protein-1β, and CD 107a revealed that the polyfunctionality of Gag-specific CD4$^+$ T cells, as defined by their multiplicity of these responses, was markedly elevated in the acute phase in NAb-immunized animals. In the chronic phase, despite the absence of detectable NAbs, viral control was maintained, accompanied by polyfunctional Gag-specific T-cell responses. These results implicate virus-specific polyfunctional CD4$^+$ T-cell responses in this NAb-triggered viral control, suggesting possible synergism between NAbs and T cells for control of HIV/SIV replication.

2. Impact of cytotoxic-T-lymphocyte memory induction without virus-specific CD4$^+$ T-cell help on control of a simian immunodeficiency virus challenge in rhesus macaques

Tetsuo Tsukamoto, Akiko Takeda, Takuya Yamamoto, Hiroyuki Yamamoto, Miki Kawada, and Tetsuro Matano

Despite many efforts to develop AIDS vaccines eliciting virus-specific T-cell responses, whether induction of these memory T cells by vaccination before HIV exposure can actually contribute to effective T-cell responses post-infection remains unclear. In particular, induction of HIV-specific memory $CD4^+$ T cells may increase the target cell pool for HIV infection because the virus preferentially infects HIV-specific $CD4^+$ T cells. However, virus-specific $CD4^+$ helper T-cell responses are thought to be important for functional $CD8^+$ cytotoxic T lymphocyte (CTL) induction in HIV infection, and it has remained unknown whether HIV-specific memory $CD8^+$ T cells induced by vaccination without HIV-specific $CD4^+$ T-cell help can exert effective responses after virus exposure. Here we show the impact of $CD8^+$ T-cell memory induction without virus-specific $CD4^+$ T-cell help on control of a SIV challenge in rhesus macaques. We developed a prophylactic vaccine using a Sendai virus (SeV) vector expressing a single SIV $Gag_{241-249}$ CTL epitope fused with EGFP protein. The vaccination resulted in induction of SeV-EGFP-specific $CD4^+$ T-cell and $Gag_{241-249}$-specific $CD8^+$ T-cell responses. After SIV challenge, the vaccinees showed dominant $Gag_{241-249}$-specific $CD8^+$ T-cell responses with higher effector memory frequencies in the acute phase and exhibited significantly reduced viral loads. These results demonstrate that virus-specific memory $CD8^+$ T cells induced by vaccination without virus-specific $CD4^+$ T-cell help could indeed facilitate SIV control after virus exposure, indicating the benefit of prophylactic vaccination eliciting virus-specific CTL memory with non-virus-specific $CD4^+$ T-cell responses for HIV control.

These studies were performed with the help of DNAVEC Corp., National Institute of Infectious Diseases, and Tsukuba Primate Research Center, National Institute of Biomedical Innovation. A project for a clinical trial of an AIDS vaccine using Sendai virus vectors is proceeding in collaboration with DNAVEC Corp. and International AIDS Vaccine Initiative (IAVI).

Publications

1. Yamamoto, T., Iwamoto, N., Yamamoto, H., Tsukamoto, T., Kuwano, T., Takeda, A., Kawada, M., Tsunetsugu-Yokota, Y., and Matano, T. Polyfunctional $CD4^+$ T-cell induction in neutralizing antibody-triggered control of simian immunodeficiency virus infection. J. Virol. 83: 5514-5524, 2009.
2. Tsukamoto, T., Takeda, A., Yamamoto, T., Yamamoto, H., Kawada, M., and Matano, T. Impact of cytotoxic-T-lymphocyte memory induction without virus-specific $CD4^+$ T-cell help on control of a simian immunodeficiency virus challenge in rhesus macaques. J. Virol. 83: 9339-9346, 2009.
3. Yamamoto, H., and Matano, T. Neutralizing antibodies in SIV control: co-impact with T cells. Vaccine, in press.
4. Takeuchi, H. Contribution of cyclophilin A to determination of simian immunodeficiency virus tropism: a progress update. Vaccine, in press.
5. 野村拓志，俣野哲朗．エイズワクチン開発の論理．ウイルス．日本ウイルス学会．59：267–276，2009.

International Research Center for Infectious Diseases

Department of Infectious Disease Control Division of Viral Infection

感染制御部門・ウイルス学分野

Associate Professor Yasushi Kawaguchi, D.V.M., Ph.D. | 准教授 獣医学博士 川口 寧

To date, approximately 130 herpesviruses have been identified, affecting most animal species. These viruses are associated with a variety of diseases such as encephalitis, malignancy and mucocutaneous diseases in human and animals. The objective of our research is to understand the mechanisms by which herpesviruses replicate in cells, survive and manifest diseases in their hosts. Our goal is to apply our fundamental findings for control of herpesvirus infections and development of viral vectors and manipulated viruses in human therapy.

1. Regulation of the Catalytic Activity of Herpes Simplex Virus 1 Protein Kinase Us3 by Auto-Phosphorylation and Its Role in Pathogenesis.

K. Sagou, T. Imai, H. Sagara, M. Uema, and Y. Kawaguchi

Us3 is a serine-threonine protein kinase encoded by herpes simplex virus 1 (HSV-1). We recently identified serine at Us3 position 147 (Ser-147) as a physiological phosphorylation site of Us3 (A. Kato et al. 2008. J. Virol. 82: 6172-6189). In the present study, we investigated the effects of phosphorylation of Us3 Ser-147 on regulation of Us3 catalytic activity in infected cells and on HSV-1 pathogenesis. Our results were as follows. (i) Only a small fraction of Us3 purified from infected cells was phosphorylated at Ser-147. (ii) Us3 phosphorylated at Ser-147 purified from infected cells had significantly higher kinase activity than Us3 unphosphorylated at Ser-147. (iii) Phosphorylation of Us3 Ser-147 in infected cells was dependent on Us3 kinase activity. (iv) Replacement of Us3 Ser-147 by alanine significantly reduced viral replication in the mouse cornea and development of herpes stroma keratitis and periocular skin disease in mice. These results indicated that Us3 catalytic activity is tightly regulated by auto-phosphorylation of Ser-147 in infected cells and that regulation of Us3 activity by auto-phosphorylation appeared to play a critical role in viral replication in vivo and in HSV-1 pathogenesis.

2. Herpes Simplex Virus 1 Protein Kinase Us 3 Phosphorylates Viral Envelope Glycoprotein B and Regulates Its Expression on the Cell Surface.

Akihisa Kato, Jun Arii, Ikuo Shiratori, Hiroomi Akashi, Hisashi Arase, and Yasushi Kawaguchi

Us3 is a serine-threonine protein kinase encoded by herpes simplex virus 1 (HSV-1). As reported here, we attempted to identify the previously unreported physiological substrate of Us3 in HSV-1-infected cells. Our results were as follows. (i) Bioinformatics analysis predicted two putative Us3 phosphorylation sites in the viral envelope glycoprotein B (gB) at codons 557-562

(RRVSAR) and codons 884-889 (RRNTNY). (ii) In *in vitro* kinase assays, the threonine residue at position 887 (Thr-887) in the gB domain was specifically phosphorylated by Us3, while the serine residue at position 560 was not. (iii) Phosphorylation of gB Thr-887 in Vero cells infected with wild-type HSV-1 was specifically detected using an antibody that recognized phosphorylated serine or threonine residues with arginine at the -3 and -2 positions. (iv) Phosphorylation of gB Thr-887 in infected cells was dependent on the kinase activity of Us3. (v) Substitution of Thr-887 with alanine markedly up-regulated cell surface expression of gB in infected cells, whereas substitution with aspartic acid, which sometimes mimics constitutive phosphorylation, restored the wild-type phenotype. Up-regulation of gB expression on the cell surface was also observed in cells infected with a recombinant HSV-1 encoding catalytically inactive Us3. These results supported the hypothesis that Us3 phosphorylates gB and down-regulates cell surface expression of gB in HSV-1-infected cells.

3. Differences in the Regulatory and Functional Effects of the Us3 Protein Kinase Activities of Herpes Simplex Virus 1 and 2.

Tomomi Morimoto, Jun Arii, Michiko Tanaka, Tetsutaro Sata, Hiroomi Akashi, Masao Yamada, Yukihiro Nishiyama, Masashi Uema and Yasushi Kawaguchi

Us3 protein kinases encoded by herpes simplex virus 1 (HSV-1) and 2 (HSV-2) are serine/threonine protein kinases and play critical roles in viral replication and pathogenicity in vivo. In the present study, we investigated differences in the biological properties of HSV-1 and HSV-2 Us3 protein kinases and demonstrated that HSV-2 Us3 did not have some of the HSV-1 Us3 kinase functions, including control of nuclear egress of nucleocapsids, localization of UL31 and UL34, and cell surface expression of viral envelope glycoprotein B. In agreement with the observations that HSV-2 Us3 was less important for these functions, the effect of HSV-2 Us3 kinase activity on virulence in mice following intracerebral inoculation was much lower than that of HSV-1 Us3. Furthermore, we showed that alanine substitution in HSV-2 Us3 at a site (aspartic acid at position 147) corresponding to one that can be autophosphorylated in HSV-1 Us3 abolished HSV-2 Us3 kinase activity. Thus, the regulatory and functional effects of Us3 kinase activity are different between HSV-1 and HSV-2.

4. Entry of herpes simplex virus 1 and other alphaherpesviruses via the paired immunoglobulin-like type 2 receptor α

Jun Arii, Masashi Uema, Tomomi Morimoto, Hiroshi Sagara, Hiroomi Akashi, Etsuro Ono, Hisashi Arase, and Yasushi Kawaguchi

Herpes simplex virus 1 (HSV-1) enters cells either via fusion of the virion envelope and host cell plasma membrane or via endocytosis, depending on the cell type. In the study reported here, we investigated a viral entry pathway dependent on the paired immunoglobulin-like type 2 receptor (PILR)α, a recently identified entry co-receptor for HSV-1 that associates with viral envelope glycoprotein B (gB). Experiments using inhibitors of endocytic pathways and ultrastructural analyses of chinese hamster ovary (CHO) cells transduced with PILRα showed that HSV-1 entry into these cells was via virus-cell fusion at the cell surface. Together with earlier observations that HSV-1 uptake into normal CHO cells and those transduced with a receptor for HSV-1 envelope gD is mediated by endocytosis, these results indicated that expression of PILRα produced an alternative HSV-1 entry pathway in CHO cells. We also showed that human and murine PILRα were able to mediate entry of pseudorabies virus, a porcine alphaherpesvirus, but not of HSV-2. These results indicated that viral entry via PILRα appears to be conserved, but there is a PILRα preference among alphaherpesviruses.

5. Epstein-Barr Virus Protein Kinase BGLF4 Interacts with Viral Transactivator BZLF1 and Regulates Its Transactivation Activity.

Risa Asai, Ai Kato, and Yasushi Kawaguchi

BGLF4 is a serine-threonine protein kinase encoded by Epstein-Barr virus and one of the physiological BGLF4 substrates is viral transactivator BZLF1. In the present study, we demonstrated that alanine substitution of serine residue at position 209 (S209A) in the BZLF1 abrogated phosphorylation of the protein by BGLF4 in vitro. The S209A mutation in BZLF1 as well as K102I mutation in BGLF4, which inactivated catalytic activity of the viral kinase, also inhibited formation of a stable BGLF4-BZLF1 complex and down-regulation of BZLF1 auto-transactivation activity mediated by BGLF4. Our results indicated that formation of a stable complex with BGLF4-BZLF1 enabled down-regulation of BZLF1 auto-regulation activity and it appeared that BGLF4 phosphorylation of BZLF1 might be involved in these processes.

6. Identification of multiple sites in herpes simplex virus genomes suitable for insertion of foreign genes.

Tomomi Morimoto, Jun Arii, Hiroomi Akashi and Yasushi Kawaguchi

Information on sites in herpes simplex virus (HSV) genomes at which foreign gene(s) can be inserted without disrupting viral genes or affecting parental virus properties are important for basic research on HSV and development of HSV-based vectors for human therapy. The intergenic region between HSV-1 UL3 and UL4 genes has been reported to satisfy the requirements for such an insertion site. The UL3 and UL4 genes are oriented toward the intergenic region and, therefore, insertion of a foreign gene (s) into the region between the UL3 and UL4 polyadenylation signals should not disrupt any viral genes or transcriptional units. HSV-1 and HSV-2 each have more than 10 additional regions structurally similar to the intergenic region between UL3 and UL4. In the studies reported here, we demonstrated that insertion of a reporter gene expression cassette into several of the HSV-1 and HSV-2 intergenic regions had no effect on viral growth in cell culture or virulence in mice, suggesting that these multiple intergenic regions can be used as suitable HSV insertion sites for foreign genes.

Publications

K. Sagou, M. Uema and Y. Kawaguchi. Nucleolin is required for efficient nuclear egress of herpes simplex virus 1 nucleocapsids. J. Virol. (in press).

T. Imai, K. Sagou, J. Arii and Y. Kawaguchi. Effects of Phosphorylation of Herpes Simplex Virus 1 Envelope Glycoprotein B by Us3 Kinase In Vivo and In Vitro. J. Virol. (in press).

K. Gotoh, Y. Tanaka, A. Nishikimi, R. Nakamura, H. Yamada, N. Maeda, T. Ishikawa, K. Hoshino, T. Uruno, Q. Cao, S. Higashi, Y. Kawaguchi, M. Enjoji, R. Takayanagi, T. Kaisho, Y. Yoshikai, and Y. Fukui. Selective control of type I IFN induction by the Rac activator DOCK2 during TLR-mediated plasmacytoid dendritic cell activation. J. Exp. Med. (in press)

T. Suenaga, T. Satoh, P. Somboonthum, Y. Kawaguchi, Y. Mori, and H. Arase. Myelin-associated glycoprotein mediates membrane fusion and entry of neurotropic herpesviruses. Proc. Natl. Acad. Sci. USA (in press).

W. Azab, K. Tsujimura, K. Kato, J. Arii, T. Morimoto, Y. Kawaguchi, Y. Tohya, T. Matsumura and H. Akashi. Characterization of a thymidine kinase-deficient mutant of equine herpesvirus 4 and in vitro susceptibility of the virus to antiviral agents. Antiviral Res. (in press).

T. Morimoto, J. Arii, M. Tanaka, T. Sata, H. Akashi, M. Yamada, Y. Nishiyama, M. Uema and Y. Kawaguchi. (2009) Differences in the Regulatory and Functional Effects of the Us3 Protein Kinase Activities of Herpes Simplex Virus 1 and 2. J. Virol. 83: 11624-11634.

K. Sagou, T. Imai, H. Sagara, M. Uema, and Y. Kawaguchi. (2009) Regulation of the Catalytic Activity of Herpes Simplex Virus 1 Protein Kinase Us3 by Auto-Phosphorylation and Its Role in Pathogenesis. J. Virol. 83: 5773-5783.

J. Arii, M. Uema, T. Morimoto, H. Sagara, H. Akashi, E. Ono, H. Arase, and Y. Kawaguchi. (2009) Entry of herpes simplex virus 1 and other alphaherpesviruses via the paired immunoglobulin-like type 2 receptor α. J. Virol. 83: 4520-4527.

A. Kato, J. Arii, I. Shiratori, H. Akashi, H. Arase and Y. Kawaguchi. (2009) Herpes simplex virus 1 protein kinase Us3 phosphorylates viral envelope glycoprotein B and regulates its expression on the cell surface. J. Virol. 83: 250-261.

R. Asai, A. Kato and Y. Kawaguchi. (2009) Epstein-Barr virus protein kinase BGLF4 interacts with viral transactivator BZLF1 and regulates its transactivation activity. J. Gen. Virol. 90: 1575-1581.

T. Morimoto, J. Arii, H. Akashi and Y. Kawaguchi. (2009) Identification of multiple sites in herpes simplex virus genomes suitable for insertion of foreign genes. Microbiol. Immunol. 53: 155-161.

H. Konno, T. Yamamoto, K. Yamazaki, J. Gohda, T. Akiyama, K. Semba, H. Goto, A. Kato, T. Yujiri, T. Imai, Y. Kawaguchi, B. Su, O. Takeuchi, S. Akira, Y. Tsunetsugu-Yokota, and J. Inoue. (2009) TRAF6 establishes innate immune responses by activating NF-kB and IRF7 upon sensing cytosolic viral RNA and DNA. PLoS ONE 4: e5674.

J. Wang, F. Fan, T. Satoh, J. Arii, L.L. Lanier, P. G. Spear, Y. Kawaguchi, H. Arase. (2009) Binding of herpes simplex virus glycoprotein B (gB) to PILRα depends on specific sialylated *O*-linked glycans on gB. J. Virol. 83: 13042-13045.

T. W. Wisner, C.C. Wright, A. Kato, Y. Kawa-

guchi, F. Mou, J.D. Baines, R.J. Roller, and D. C. Johnson. (2009) Herpesvirus gB-induced fusion between the virion envelope and outer nuclear membrane during virus egress is regulated by the viral US3 kinase. J. Virol. 83: 3115-3126.

T. Nojima, T. Oshiro-Ideue, H. Nakanoya, H. Kawamura, T. Morimoto, Y. Kawaguchi, N. Kataoka, M. Hagiwara. (2009) Herpesvirus protein ICP27 switches PML isoform by altering mRNA splicing. Nucleic Acids Res. 37: 6515-6527.

J. Arii, K. Kato, Y. Kawaguchi, Y. Tohya, H. Akashi. (2009) Analysis of herpesvirus host specificity determinants using herpesvirus genomes as bacterial artificial chromosomes. Microbiol. Immunol. 53: 433-441.

International Research Center for Infectious Diseases

Department of Infectious Disease Control Division of Microbiology

感染制御部門　細菌学分野

Associate Professor　Ichiro Nakagawa, D.D.S., Ph.D.　准教授　歯学博士　中　川　一　路

A number of pathogenic bacterial pathogens have been developing a variety of mechanisms to evade from the host defence mechanism, and to maximize their virulence for surviving. For counterattacking to bacterial infection, our immune system has also acquired the various defence mechanisms in the evolution. Our major research interests are to elucidate the bacterial evolution to escape from the host immune responses, and cellular defence mechanisms against the bacterial pathogens. Especially, we focus the analysis of recognition molecules and the cellular defence mechanism against the intracellularly invading pathogens.

1. Intracellular host defense mechanism

Ichiro Nakagawa

Elimination of pathogenic bacteria harboured within host cells is crucial for host defence. The endocytic degradation pathway has been thought to be the only system against such intracellular pathogens. We demonstrated that the autophagic machinery, a bulk degradation system for cellular components, effectively eliminates the pathogenic group A *Streptococcus* (GAS) that has invaded non-phagocytic cells. Macroautophagy, usually referred to simply as autophagy, is a physiologically important cellular process for the bulk degradation of organelles and cytosolic proteins. The cytoplasm-derived contents of the autophagosomes are degraded by lysosomal hydrolases. This lysosomal degradation system is thought to be required for the non-selective degradation and recycling of cellular proteins. However, the recognition mechanism of the intracellular bacteria by the autophagic degradation system has not well understood. We are investigating the intracellular recognition molecules to induce autophagy, and the bacterial factors recognized by this new surveillance system, especially targeting major gram-positive bacterial pathogens such as streptococci and staphylococci. We have identified some intracellular bacterial pattarn recognition molecules, which related to the recognition of intracellular group A streptococci and analyzed the function of these molecules.

2. Comparative genomic analysis for pathogenic bacterial evolution.

Fumito Maruyama and Ichiro Nakagawa

Comparative genomic analyses indicate that the genes within closely related bacterial species are highly conserved, with the exception of inversions, translocations, phage integrations, and the mobile genetic elements. In particular, the genomic arrangement regions by inversion and translocation form a specific genetic segment called the “plasticity zone”. The genes in plasticity zones have undergone genetic reorganization to a much higher degree than the rest of the

chromosome, and this is related to the diversity of pathogenic bacterial phenotypes. Only comparative genomic analyses based on whole genome sequences can provide useful information about genomic organization. To develop effective prevention strategies or new therapeutic methods for bacterial infection, it is necessary to understand the biology of this organism at the genomic level. We have sequenced the complete genome of S. *mutans* serotype c strain NN2025, and compared it with the genome of UA159. The NN2025 genome is composed of 2,013,587 bp, and the two strains show highly conserved core-genome. However, comparison of the two *S. mutans* strains showed a large genomic inversion across the replication axis producing an X-shaped symmetrical DNA dot plot. This phenomenon was also observed between other streptococcal species, indicating that streptococcal genetic rearrangements across the replication axis play an important role in *Streptococcus* genetic shuffling. We further confirmed the genomic diversity among 95 clinical isolates using long-PCR analysis. Genomic diversity in *S. mutans* appears to occur frequently between insertion sequence (IS) elements and transposons, and these diversity regions consist of restriction/modification systems, antimicrobial peptide synthesis systems, and transporters. *S. mutans* may preferentially reject the phage infection by clustered regularly interspaced short palindromic repeats (CRISPRs). In particular, the CRISPR-2 region, which is highly divergent between strains, in NN2025 has long repeated spacer sequences corresponding to the streptococcal phage genome.

Publications

Yamaguchi H, Nakagawa I, Yamamoto A, Amano A, Noda T, and Yoshimori T, An initial step of GAS-containing autophagosome-like vacuoles formation requires Rab7, *PLoS Pathog* 5: e1000670, 2009.

Yoshikawa Y, Ogawa M, Hain T, Yoshida M, Fukumatsu M, Kim M, Mimuro H, Nakagawa I, Yanagawa T, Ishii T, Kakizuka A, Sztul E, Chakraborty T and Sasakawa C, *Listeria monocytogenes* ActA-mediated escape from autophagic recognition, *Nat Cell Biol* 11: 1233-1240, 2009

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*, 10: 358

Maruyama F, Nozawa T, Aikawa C, Sakurai A, Nakagawa I, Cost effective DNA sequencing and template preparation from bacterial colonies and plasmids, *J Biosci Bioeng*, 107: 471-473, 2009

Ogawa M, Nakagawa I, Yoshikawa Y, Hain T, Chakraborty T, Sasakawa C, *Streptococcus*-, *Shigella*-, and *Listeria*-induced autophagy, *Methods Enzymol*, 452: 363-381, 2009

International Research Center for Infectious Diseases

Pathogenic Microbes Repository Unit
病原微生物資源室

Professor	Chihiro Sasakawa, Ph.D.		教　授	医学博士	笹　川　千　尋
Project Assistant Professor	Minsoo Kim, Ph.D.		特任助教	医学博士	金　　玟　秀

This unit is collecting standardized bacterial strains and distributing to research organizations, hospital laboratories, and medical educational institutions throughout the country. In addition, under cooperation with the Japanese Society for Bacteriology, we are distributing authorized bacterial strains for microbiology course for medical school.

Our society is always threatened by emerging and reemerging infectious diseases with various kinds of altitude pathogenic microbes owing to increased foreign tourism, import increase including food, food poisoning such as the O-157 epidemic, and bioterrorism. In addition, by advanced medical developments, the aging society, and increased HIV infection, the quick identification of and therapy for opportunistic infection causative agents and multiple drug resistance bacteria have become important in the medical field.

The need for researchers and clinical practitioners specialized in bacteriology and infectious diseases have risen remarkably, and the substantial study and education required is an emergent problem. For thorough study and education, knowledge of bacteriology, a system of collecting pathogenic microorganism strains of reliable origin, to maintain and save them appropriately, and to provide them to cutting-edge researchers or educational establishments is indispensable. However, in Japan, research into pathogenic microorganisms and infectious diseases is performed mainly in universities, where there is no system for conservation and supply. Therefore, valuable bacterial strains have faced disappearance. Furthermore, under the CARTAGENA PROTOCOL ON BIOSAFETY for conventions of biological diversity, the provision and purchase of pathogenic microorganisms from foreign countries has become difficult.

In such circumstances, we are collecting, saving, and analyzing the pathogenicity of microorganisms and distributing pathogenic bacteria to 1) offer type cultures as a positive control in research, education and examinations, 2) prepare pathogenic bacterial strains that have socially high importance, and 3) offer microbes to universities or public research organizations for training or research. We possess about 1,500 strains that almost cover the main pathogenic microbes, including strains valuable internationally such as pathogenic *E. coli* of Orskov's collection, which is stored only in our laboratory in Japan. Furthermore, it is important to secure their utility as type cultures by preparing genomic and genetic information about the pathogenicity of our bacterial collection based on the researches of the Division of Bacterial Infection. Thus, our laboratory is expected to contribute to countermeasures against infectious disease, and to the education and research of medical microbiology in our country.

Collection, preservation and data management of bacterial strains

It is necessary for us to collect representative type strains and the derivatives of pathogenic microbes corresponding to the following six items.

a) Comprehensive collection of genome sequencing strains.
b) The causative agents of hospital-acquired (nosocomial) infection, such as opportunistic infectious bacteria and antibiotic-resistant bacteria.
c) Pathogenic Escherichia coli associated with the intestinal and urinary tract or meningeal infections, including *Shigella*, EPEC and EHEC O-157.
d) Intracellular bacterial pathogens such as *Mycobacterium avium* and obligate intracellular bacteria.
e) Zoonotic agents causing brucellosis (*Brucella*), leptospirosis (*Leptospira*), and so on.
f) Pathogens causing newly emerging infections and outbreaks, such as *Helicobacter pylori, Salmonella* spp. and *Clostridium* perfringens.

We dissect the biochemical properties of bacterial strains collected by deposition, and maintain them appropriately. We are also opening the database of our collection to the public.

Distribution of bacterial strains

We are distributing standardized bacterial strains to research organizations, hospital laboratories, and medical educational institutions throughout the country. In addition, under cooperation with the Japanese Society for Bacteriology, we are distributing authorized bacterial strains for microbiology course for medical school.

Value-added creation of a bacterial strain collection by pathogenic analysis

We are analyzing the pathogenicity of pathogenic microorganisms, especially pathogenic *E. coli*, the pathogenicity of new bacterial infection causative agents in cooperation with the Division of Bacterial Infection. Our collection has original added value by offering this information to users.