International Research Centre for Infectious Diseases

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

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Like Ebola, influenza, and Nipah viruses, 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 Nipah viruses as models.

1. Architecture of ribonucleoprotein complexes in influenza A virus particles.

Noda T, Sagara H, Yen A, Takada A, Kida H, Cheng RH, Kawaoka Y

In viruses, as in eukaryotes, elaborate mechanisms have evolved to protect the genome and to ensure its timely replication and reliable transmission to progeny. Influenza A viruses are enveloped, spherical or filamentous structures, ranging from 80 to 120 nm in diameter. Inside each envelope is a viral genome consisting of eight single-stranded negative-sense RNA segments of 890 to 2,341 nucleotides each. These segments are associated with nucleoprotein and three polymerase subunits, designated PA, PB1 and PB2; the resultant ribonucleoprotein complexes (RNPs) resemble a twisted rod (10-15 nm in width and 30-120 nm in length) that is folded back and coiled on itself. Late in viral infection, newly synthesized RNPs are transported from the nucleus to the plasma membrane, where they are incorporated into progeny virions capable of infecting other cells. Here we show, by transmission electron microscopy of serially sectioned virions, that the RNPs of influenza A virus are organized in a distinct pattern (seven segments of different lengths surrounding a central segment). The individual RNPs are suspended from the interior of the viral envelope at the distal end of the budding virion and are oriented perpendicular to the budding tip. This finding argues against random incorporation of RNPs into virions, supporting instead a model in which each segment contains specific incorporation signals that enable the RNPs to be recruited and packaged as a complete set. A selective mechanism of RNP incorporation into virions and the unique organization of the eight RNP segments may be crucial to maintaining the integrity of the viral genome during repeated cycles of replication.

2. Molecular determinants of Ebola virus virulence in mice

Ebihara H, Takada A, Kobasa D, Jones S,

Neumann G, Theriault S, Bray M, Feldmann H, Kawaoka Y

Zaire ebolavirus (ZEBOV) causes severe hemorrhagic fever in humans and nonhuman primates, with fatality rates in humans of up to 90 %. The molecular basis for the extreme virulence of ZEBOV remains elusive. While adult mice resist ZEBOV infection, the Mayinga strain of the virus has been adapted to cause lethal infection in these animals. To understand the pathogenesis underlying the extreme virulence of Ebola virus (EBOV), here we identified the mutations responsible for the acquisition of the high virulence of the adapted Mayinga strain in mice, by using reverse genetics. We found that mutations in viral protein 24 and in the nucleoprotein were primarily responsible for the acquisition of high virulence. Moreover, the role of these proteins in virulence correlated with their ability to evade type I interferon-stimulated antiviral responses. These findings suggest a critical role for overcoming the interferon-induced antiviral state in the pathogenicity of EBOV and offer new insights into the pathogenesis of EBOV infection.

3. Assembly and budding of Ebolavirus

Noda T, Ebihara H, Muramoto Y, Fujii K, Takada A, Sagara H, Kim JH, Kida H, Feldmann H, Kawaoka Y

Ebolavirus is responsible for highly lethal hemorrhagic fever. Like all viruses, it must reproduce its various components and assemble them in cells in order to reproduce infectious virions and perpetuate itself. To generate infectious Ebolavirus, a viral genome-protein complex called the nucleocapsid (NC) must be produced and transported to the cell surface, incorporated into virions, and then released from cells. To further our understanding of the Ebolavirus life cycle, we expressed the various viral proteins in mammalian cells and examined them ultrastructurally and biochemically. Expression of nucleoprotein alone led to the formation of helical tubes, which likely serve as a core for the NC. The matrix protein VP40 was found to be critical for transport of NCs to the cell surface and for the incorporation of NCs into virions, where interaction between nucleoprotein and the matrix protein VP40 is likely essential for these processes. Examination of virus-infected cells revealed that virions containing NCs mainly emerge horizontally from the cell surface, whereas empty virions mainly bud vertically, suggesting that horizontal budding is the major mode of Ebolavirus budding. These data form a foundation for the identification and development of potential antiviral agents to combat the devastating disease caused by this virus.

4. Establishment of a Nipah virus rescue system

Yoneda M, Guillaume V, Ikeda F, Sakuma Y, Sato H, Wild TF, Kai C

A paramyxovirus, the Nipah virus (NiV), was rescued from a full-length cDNA clone. The plasmid (pNiV6+) contained the T7 promoter which enabled the transcrition of full-length antigenomic RNA by a T7 RNA polymerase, provided by the infection of the cells with the vaccinia recombinant MVA-T7. Support plasmids required for the rescue, expressing the nucleocapsid protein (N), phosphoprotein (P) and viral RNA dependent RNA polymerase (L) from a T7 promoter, were also generated. Infection of CV-1 cells with MVA-T7, followed by transfection of pNiV6+ and the rescue plasmids, led to the recovery of infectious virus. In a second construction, the gene coding for enhanced green fluorescent protein was inserted between the N and P NiV genes. Comparison of the growth cycles and virus yields in tissue culture of the two rescued viruses (rNiV and rNiV-EGFP) with the parental virus revealed similar properties. The rNiV-EGFP virus was used to infect a number of cell lines of different origins and was compared with the known cellular receptor for NiV, ephrin B2. Interestingly, one cell line expressing ephrin B2 was not susceptible for rNiV-EGFP. In conclusion, additional factors may control NiV replication in rat cells.

Publications

1. Noda T, Sagara H, Yen A, Takada A, Kida H, Cheng RH, Kawaoka Y. Architecture of ribonucleoprotein complexes in influenza A virus particles. Nature, 439, 490-2, 2006
2. Ebihara H, Takada A, Kobasa D, Jones S, Neumann G, Theriault S, Bray M, Feldmann H, Kawaoka Y. Molecular determinants of Ebola virus virulence in mice. PLoS Pathog. 2, 705-11, 2006
3. Noda T, Ebihara H, Muramoto Y, Fujii K, Takada A, Sagara H, Kim JH, Kida H, Feldmann H, Kawaoka Y. Assembly and budding of Ebolavirus. PLoS Pathog. 2, 864-72, 2006
4. Yoneda M, Guillaume V, Ikeda F, Sakuma Y,

Sato H, Wild TF, Kai C. Establishment of a Nipah virus rescue system. Proc. Natl. Acad. Sci. USA, 103, 16508-13, 2006
5. Sato H, Masuda M, Miura R, Yoneda M, Kai C. Morbillivirus nucleoprotein possesses a novel nuclear localization signal and a CRM1 -independent nuclear export signal. Virology 352, 121-30, 2006

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

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 clarification 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. Follow-up of SIVmac239-controllers: involvement of multiple CTL responses in vaccine-based SIV control.

Miki Kawada, Tetsuo Tsukamoto, Hiroyuki Yamamoto, Sayuri Seki, Chikaya Moriya, Akiko Takeda, Hiroko Igarashi[1], Sachi Dohki[2], Masafumi Takiguchi[2], and Tetsuro Matano: [1]Department of Microbiology, Graduate School of Medicine, The University of Tokyo, [2]Division of Viral Immunology, Center for AIDS Research, Kumamoto University.

Cytotoxic T lymphocyte (CTL) responses are crucial for the control of HIV and simian immunodeficiency virus (SIV) replication. Possible involvement of a dominant single epitope-specific CTL in control of viral replication has recently been indicated in preclinical AIDS vaccine trials, but it has remained unclear if multiple epitope-specific CTLs can be involved in the vaccine-based control. In this study, by following up five rhesus macaques that showed vaccine-based control of primary SIVmac239 replication, we have presented evidence indicating involvement of multiple epitope-specific CTL responses in this control. Three macaques maintained control for more than 2 years without additional mutations in the provirus. However, in the other two that shared a major histocompatibility complex (MHC) haplotype, viral mutations were accumulated in a similar order leading to viral evasion from three epitope-specific CTL responses with viral fitness costs. Accumulation of these multiple escape mutations resulted in the reappearance of plasma viremia around week 60 after challenge. Our results implicate multiple epitope-specific CTL responses in control of immunodeficiency virus replication, and furthermore, suggest that sequential accumulation of multiple CTL escape mutations, if allowed, can result in viral evasion from this control.

2. Follow-up of SHIV89.6P-controllers: vaccine-based long-term stable control of SHIV 89.6P replication.

Hiroyuki Yamamoto, Miki Kawada, Tetsuo Tsukamoto, Mitsuhiro Yuasa, Akiko Takeda, Hiroko Igarashi, Masaaki Miyazawa[3], Taeko Naruse[4], Michio Yasunami[4], Akinori Kimura[4], and Tetsuro Matano: [3]Department of Immu-

nology, Kinki University School of Medicine, [4]Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University.

An X4-tropic SHIV89.6P causes rapid $CD4^+$ T-cell depletion leading to an acute crash of the host immune system, whereas pathogenic R5-tropic SIV infection, like HIV-1 infection in humans, results in chronic disease progression in macaques. Recent preclinical vaccine trials inducing CTL responses have succeeded in controlling the former replication but shown difficulty in control of the latter. In this study, we followed up rhesus macaques that showed vaccine-based control of primary SHIV89.6P replication and found that all these controllers maintained viremia control for more than 2 years. SHIV89.6P control was observed in vaccinees of diverse MHC haplotypes and maintained without a sign of particular CTL pressure, rapid selection of CTL escape mutations. Despite the vaccine regimen not targeting Env, all the SHIV-controllers showed efficient induction of *de novo* neutralizing antibodies by 6 weeks after challenge. These results contrast with our previous observation in particular MHC-associated control of SIV replication without involvement of neutralizing antibodies and suggest that vaccine-based control of SHIV89.6P replication can be maintained stably in the presence of multiple functional immune effectors.

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.

Publications

Kawada, M., Igarashi, H., Takeda, A., Tsukamoto, T., Yamamoto, H., Dohki, S., Takiguchi, M., and Matano, T. Involvement of multiple epitope-specific cytotoxic T-lymphocyte responses in vaccine-based control of simian immunodeficiency virus replication in rhesus macaques. J. Virol. 80: 1949-1958, 2006.

Takahashi-Tanaka, Y., Yasunami, M., Naruse, T., Hinohara, K., Matano, T., Mori, K., Miysazawa, M., Honda, M., Yasutomi, Y., Nagai, Y., and Kimura, A. Reference strand-mediated conformation analysis (RSCA)-based typing of multiple alleles in the rhesus macaque MHC class I Mamu-A and Mamu-B loci. Electrophoresis, in press.

Yamamoto, H., Kawada, M., Tsukamoto, T., Takeda, A., Igarashi, H., Miyazawa, M., Naruse, T., Yasunami, M., Kimura, A., and Matano, T. Vaccine-based long-term stable control of simian-human immunodeficiency virus 89.6PD replication in rhesus macaques. J. Gen. Virol., in press.

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. | 助教授 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. Herpes Simplex Virus 1-Encoded Protein Kinase UL13 Phosphorylates the Viral Us3 Protein Kinase and Regulates Nuclear Localization of Viral Envelopment Factors UL 34 and UL31.

Akihisa Kato, Mayuko Yamamoto[1], Takashi Ohno, Michiko Tanaka[2], Tetsutaro Sata[2], Yukihiro Nishiyama[1], and Yasushi Kawaguchi: [1]Department of Virology, Nagoya University Graduate School of Medicine, [2]Department of Pathology, National Institute of Infectious Disease

UL13 and Us3 are protein kinases encoded by herpes simplex virus 1. We report here that Us3 is a physiological substrate for UL13 in infected cells, based on the following observations. (i) The electrophoretic mobility, in denaturing gels, of Us3 isoforms from wild-type virus-infected Vero cells was slower than isoforms from cells infected with a UL13 deletion mutant virus (DUL13). After treatment with phosphatase, the electrophoretic mobility of the Us3 isoforms from wild-type virus-infected cells changed, with one isoform migrating as fast as one of the Us3 isoforms from DUL13-infected cells. (ii) A recombinant protein containing a domain of Us3 was phosphorylated by UL13 *in vitro*. (iii) The phenotype of DUL13 resembles that of a recombinant virus lacking the Us3 gene (DUs3) with respect to localization of the viral envelopment factors UL34 and UL31, whose localization has been shown to be regulated by Us3. UL34 and UL31 are localized in a smooth pattern throughout the nucleus of wild-type virus-infected cells, whereas their localization in DUL13- and DUs3-infected cells appeared as nuclear punctate patterns. These results indicate that UL13 phosphorylates Us3 in infected cells and regulates UL34 and UL31 localization, either by phosphorylating Us3 or by a Us3-independent mechanism.

2. Epstein-Barr Virus Protein Kinase BGLF4 Is a Virion Tegument Protein That Dissociates From Virions in a Phosphorylation Dependent Process and Phosphorylates the Viral Immediate-Early Protein BZLF1.

Risa Asai, Ai Kato[1], Kentaro Kato[3], Mikiko Kanamori-Koyama[1], Ken Sugimoto, Takeshi Sairenji[4], Yukihiro Nishiyama, and Yasushi Kawaguchi: [3]Department of Cell Regulation,

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Epstein-Barr virus (EBV) BGLF4 is a viral protein kinase that is expressed in the lytic phase of infection and is packaged in virions. We report here that BGLF4 is a tegument protein that dissociates from the virion in a phosphorylation dependent process. We also present data that BGLF4 interacts with and phosphorylates BZLF 1, a key viral regulator of lytic infection. These conclusions are based on the following observations. (i) In *in vitro* tegument release assays, a significant fraction of BGLF4 was released from virions in the presence of physiological NaCl concentrations. (ii) Addition of physiological concentrations of ATP and $MgCl_2$ to virions enhanced BGLF4 release, but phosphatase treatment of virions significantly reduced BGLF4 release. (iii) A recombinant protein containing a domain of BZLF1 was specifically phosphorylated by purified recombinant BGLF4 *in vitro* and BGLF4 altered BZLF1 post-translational modification *in vivo*. (iv) BZLF1 was specifically co-immunoprecipitated with BGLF4 in TPA-treated B95-8 cells and in COS-1 cells transiently expressing both of these viral proteins. (v) BGLF 4 and BZLF1 were co-localized in intranuclear globular structures, resembling the viral replication compartment, in Akata cells treated with anti-human IgG. Our results suggest that BGLF4 functions, not only in lytically infected cells by phosphorylating viral and cellular targets, but also immediately after viral penetration like other herpesvirus tegument proteins.

Publications

R. Asai, A. Kato, K. Kato, M. Kanamori-Koyama, K. Sugimoto, T. Sairenji, Y. Nishiyama, and Y. Kawaguchi. (2006) Epstein-Barr Virus Protein Kinase BGLF4 Is a Virion Tegument Protein That Dissociates From Virions in a Phosphorylation Dependent Process and Phosphorylates the Viral Immediate-Early Protein BZLF1. J. Virol. 80: 5125-5134.

A. Kato, M. Yamamoto, T. Ohno, M. Tanaka, T. Sata, Y. Nishiyama, and Y. Kawaguchi. (2006) Herpes Simplex Virus 1-Encoded Protein Kinase UL13 Phosphorylates the Viral Us3 Protein Kinase and Regulates nuclear Localization of Viral Envelopment Factors UL34 and UL31. J. Virol. 80: 1476-1486.

A. Kudoh, T. Daikoku, Y. Ishimi, Y. Kawaguchi, N. Shirata, S. Iwahori, H. Isomura, and T. Tsurumi. (2006) Phosphorylation of MCM4 at Sites Inactivating DNA Helicase Activity of the MCM4-6-7 Complex during Epstein-Barr Virus Productive Replication. J. Virol. 80: 10064-10072.

J. Arii, O. Hushur, K. Kato, Y. Kawaguchi, Y. Tohya and H. Akashi. (2006) Construction of an infectious clone of canine herpesvirus genome as a bacterial artificial chromosome. Microbes and Infection 8: 1054-1063.

T. Koshizuka, Y. Kawaguchi, F. Goshima, I. Mori, and Y. Nishiyama. (2006) Association of two membrane proteins encoded by herpes simplex virus type2, UL11 and UL56. Virus Genes 32: 157-167.

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

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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

Atsuo Sakurai & 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.

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 thought to be related to the diversity of pathogenic bacterial phenotypes. Only comparative genomic analyses based on whole genome sequences can provide useful in-

formation 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 are trying to analyze the genomic organization during the evolution how the pathogenic bacteria acquire the virulence genes to evade from the host defense systems.

Publications

Nakano N, Lapirattanakul J, Nomura R, Nemoto H, Alaluusua S, Groroos L., Vaara M, Hamada S, Ooshima T, Nakagawa I., *Streptocccus mutans* exhibits clonal variations as revealed by multilocus sequence typing, J. Clin. Microbiol., in press (2007)

Kato T, Kawai S, Nakano K, Inaba H, Kuboniwa M, Nakagawa I, Tsuda K, Omori H, Ooshima T, Yoshimori T, Amano A., Virulence of *Porphyromonas gingivalis* is altered by substitution of fimbria gene with different genotype, Cell. Microbiol., in press (2007)

Amano A, Nakagawa I, Yoshimori T., Autophagy in innate immunity against intracellular bacteria., J. Biochem. (Tokyo), 140, 161-166 (2006)

Nakagawa I, Inaba H, Yamamura T, Kato T, Kawai S, Ooshima T, Amano A., Invasion of epithelial cells and proteolysis of cellular focal adhesion components by distinct types of *Porphyromonas gingivalis* fimbriae, Infect. Immun., 74, 3773-3782 (2006)

International Research Center for Infectious Diseases

Pathogenic Microbes Repository Unit
病原微生物資源室

Professor	Chihiro Sasakawa, D.M.Sc.		教　授	医学博士	笹　川　千　尋
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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 has 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.

Participation with The National BioResource Project (NBRP) "Pathogenic Microorganisms (Bacteria, Fungi and Protozoa)"

The National BioResource Project (NBRP) aims to enable Japan to structurally provide the systematic accumulation, storage and provision of nationally recognized bioresources, which are used widely in life science researches as materials. This project started in July 2002 as part of the "Research Revolution 2002 (RR2002)" project by the Ministry of Education, Culture, Sports, Science and Technology. In addition to our institute, pathogenic bacteria are collected, stored and provided by the Research Institute for Microbial Diseases of Osaka University and the Graduate School of Medicine of Gifu University, pathogenic fungi and actinomycetes by Chiba University Research Center for Pathogenic Fungi and Microbial Toxicoses, and protozoan parasites by the Institute of Tropical Medicine of Nagasaki University. The border between pathogenic and non-pathogenic microbes is vague, such that taxonomic studies can be conducted using both types of microorganisms. Therefore, the project is performed in cooperation with the Japan Collection of Microorganisms of the Riken Bioresource Center. The National Institute of Genetics (NIG) has built a database for microbial strains and bacterial toxins.