International Research Center for Infectious Diseases

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

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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. Generation of biologically contained Ebolaviruses.

Halfmann P, Kim JH, Ebihara H, Noda T, Neumann G, Feldmann H, Kawaoka Y.

Ebola virus (EBOV), a public health concern in Africa and a potential biological weapon, is classified as a biosafety level-4 agent because of its high mortality rate and the lack of approved vaccines and antivirals. Basic research into the mechanisms of EBOV pathogenicity and the development of effective countermeasures are restricted by the current biosafety classification of EBOVs. We therefore developed biologically contained EBOV that express a reporter gene instead of the VP30 gene, which encodes an essential transcription factor. A Vero cell line that stably expresses VP30 provides this essential protein in trans and biologically confines the virus to its complete replication cycle in this cell line. This complementation approach is highly efficient because biologically contained EBOVs lacking the VP30 gene grow to titers similar to those obtained with wild-type virus. Moreover, EBOVs lacking the VP30 gene are indistinguishable in their morphology from wild-type virus and are genetically stable, as determined by sequence analysis after seven serial passages in VP 30-expressing Vero cells. We propose that this system provides a safe means to handle EBOV outside a biosafety level-4 facility and will stimulate critical studies on the EBOV life cycle as well as large-scale screening efforts for compounds with activity against this lethal virus.

2. Ebola virus matrix VP40 protein uses the COPII transport system for its intracellular transport.

Yamayoshi S, Noda T, Ebihara H, Goto H, Morikawa Y, Lukashevich IS, Neumann G, Feldmann H, Kawaoka Y.

The Ebola virus matrix protein VP40 plays an important role in virion formation and viral egress from cells. However, the host cell proteins and mechanisms responsible for intracellu-

lar transport of VP40 prior to its contribution to virion formation remain to be elucidated. Therefore we used coimmunoprecipitation and mass spectrometric analyses to identify host proteins interacting with VP40. We found that Sec24C, a component of the host COPII vesicular transport system, interacts specifically with VP40 via VP40 amino acids 303 to 307. Coimmunoprecipitation and dominant-negative mutant studies indicated that the COPII transport system plays a critical role in VP40 intracellular transport to the plasma membrane. Marburg virus VP40 was also shown to use the COPII transport system for intracellular transport. These findings identify a conserved intersection between a host pathway and filovirus replication, an intersection that can be targeted in the development of new antiviral drugs.

3. Heparin-like glycosaminoglycans prevent the infection of measles virus in SLAM-negative cell lines

Terao-Muto Y, Yoneda M, Seki T, Watanabe A, Tsukiyama-Kohara K, Fujita K, Kai C

The wide tissue tropism of measles virus (MV) suggests that it involves ubiquitously expressed molecules. We constructed a recombinant MV expressing an enhanced green fluorescent protein (EGFP) (rMV-EGFP) and demonstrated that the rMV-EGFP infected several cell types (HEK293, HepG2, Hep3B, Huh7 and WRL 68 cells) which do not express the human signaling lymphocyte activation molecule (SLAM) known as a cellular receptor for morbilliviruses. MV infection in HEK293 and HepG2 cells was not inhibited in an infectivity-inhibition assay using an anti-SLAM monoclonal antibody, indicating that MV could infect SLAM-negative cells. Soluble heparin (HP) inhibited rMV-EGFP infection in SLAM-negative cell lines in a dose-dependent manner. Direct interaction between purified virions and HP was detected by surface plasmon resonance analysis. We also demonstrated that the hemagglutinin (H) protein but not the fusion (F) protein is responsible for the interaction between virions and HP. Taken together, our results suggest that HP-like glycosaminoglycans bind to the H protein of MV and play a key role in MV infection in SLAM-negative cells.

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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
Project Research Associate	Takuya Maeda, M.D., Ph.D.		特任研究員	医学博士	前 田 卓 哉

Our research targets are HIV, AIDS-related opportunistic infections, parasitic diseases and other emerging infectious diseases. 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). We offer the diagnosis and treatment of infectious diseases including HIV/AIDS, malaria, toxoplasmosis, schistosomiasis, etc. in the IMSUT hospital.

1. Evaluation of the extraction solution for the reverse transcription loop- mediated isothermal amplification assay (RT-LAMP).

Maeda T[1], Fujii T[2], Kikuchi T[2], Koibuchi T[3], Odawara T[3], Iwamoto A[1,2,3]: [1]Department of Infectious Disease Control, International Research Center for Infectious Diseases, [2]Department of Infectious Diseases and Applied Immunology, Research Hospital, [3]Division of Infectious Diseases, Advanced Clinical Research Center

The reverse transcription loop-mediated isothermal amplification (RT-LAMP) is capable of amplifying RNA under isothermal conditions with high specificity and efficiency. We previously reported novel primer sets for the detection of respiratory RNA viruses including influenza virus, respiratory syncytial virus (RSV) and human coronavirus. So far viral nucleic acids had to be purified before RT-LAMP procedure.

In the present study, we tested an extraction buffer manufactured by EIKEN chemical Co. Ltd. Clinical nasopharyngeal-swab specimens from the patients with flu like illness were subjected to RT-LAMP procedure without RNA extraction and purification ('Direct-LAMP'). Simply the clinical specimens were mixed with extraction buffer before RT-LAMP reaction.

Nasopharyngeal swab specimens were collected from 104 Japanese outpatients (age range 0- 13 years) presenting with flu-like illness. The samples were tested immediately with IC kits to detect the antigens of influenza A/B virus according to manufacturer's instructions. The remaining samples were sent to our laboratory. Although the conventional RT-LAMP had the best sensitivity in comparison with the real-time PCR and IC test, Direct LAMP could detect influenza genomes in 51 of 54 positive samples of conventional RT-LAMP (94.4%).

In conclusion, Direct LAMP method may be a simpler, less expensive and useful diagnostic tool in resource limited settings. This technique could be used for emerging viral diseases such as SARS.

2. First documented outbreak of trichinosis associated with soft-shelled turtles in Taiwan.

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ment of Infectious Diseases and Applied Immunology, Research Hospital, [3]Division of Infectious Diseases, Advanced Clinical Research Center

We experienced two Japanese cases of human trichinosis associated with ingestion of raw soft-shelled turtles in Taiwan. The municipal health department and Taiwan CDC subsequently investigated of our cases and concluded a unique outbreak of human trichinellosis consisting of 20 Taiwanese and 5 Japanese. Our report of reptile-associated human trichinosis is the first documented outbreak in Taiwan and gives the important information about the human risk of acquiring trichineosis from reptiles.

3. Unusual radiological findings of *Fasciola hepatica* infection with huge cystic and multilocular lesions

Maeda T[1], Fujii T[2], Koibuchi T[3], Odawara T[3], Iwamoto A[1,2,3]: [1]Department of Infectious Disease Control, International Research Center for Infectious Diseases, [2]Department of Infectious Diseases and Applied Immunology, Research Hospital, [3]Division of Infectious Diseases, Advanced Clinical Research Center

We reported a case of hepatic phase *Fasciola hepatica* infection presenting huge and multilocular lesions. The unique radiological findings mimicked hydatid diseases and also cystic liver neoplasm. Fascioliasis should be included in the differential diagnosis for cystic liver diseases.

Publications

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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

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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. Transmission of simian immunodeficiency virus carrying multiple cytotoxic T lymphocyte escape mutations with diminished replicative ability can result in AIDS progression in rhesus macaques

Sayuri Seki, Miki Kawada, Akiko Takeda, Hiroko Igarashi[1], Tetsutaro Sata[2], and Tetsuro Matano: [1]Department of Microbiology, Graduate School of Medicine, The University of Tokyo, [2]Department of Pathology, National Institute of Infectious Diseases

Cytotoxic T lymphocyte (CTL) responses frequently select for immunodeficiency virus mutations resulting in escape from CTL recognition with viral fitness costs. Replication *in vivo* of such viruses carrying not single but multiple escape mutations in the absence of the CTL pressure has remained undetermined. Here, we have examined replication of simian immunodeficiency virus (SIV) with 5 *gag* mutations selected in a macaque possessing a major histocompatibility complex (MHC) haplotype *90-120-Ia* after its transmission into *90-120-Ia* negative macaques. Our results showed that even such a crippled SIV infection can result in persistent viral replication, multiple reversions, and AIDS progression.

2. Gag specific cytotoxic T lymphocyte based control of primary simian immunodeficiency virus replication in a vaccine trial

Miki Kawada, Tetsuo Tsukamoto, Hiroyuki Yamamoto, Nami Iwamoto, Kyoko Kurihara, Akiko Takeda, Chikaya Moriya, Hiroaki Takeuchi, Hirofumi Akari[3], and Tetsuro Matano: [3]Tsukuba Primate Research Center, National Institute of Biomedical Innovation

Gag specific CTLs exert strong suppressive pressure on HIV and SIV replication. However, it has remained unclear whether they can actually contain primary viral replication. Recent trials of prophylactic vaccines inducing virus-specific T cell responses have indicated their potential to confer resistance against primary SIV replication in rhesus macaques, while the immu-

nological determinant for this vaccine-based viral control has not been elucidated thus far. Here, we present evidence implicating Gag-specific CTLs as responsible for the vaccine-based primary SIV control. Prophylactic vaccination using a Gag expressing Sendai virus (SeV) vector resulted in containment of SIVmac239 challenge in all rhesus macaques possessing the MHC haplotype *90-120-Ia*. In contrast, *90-120-Ia* positive vaccinees failed to contain SIVs carrying multiple *gag* CTL escape mutations that had been selected, at the cost of viral fitness, in SIVmac239-infected *90-120-Ia* positive macaques. These results show that Gag-specific CTL responses do play a crucial role in the control of wild-type SIVmac239 replication in the vaccinees. This study implies the possibility of Gag specific CTL-based primary HIV containment by prophylactic vaccination, although it also suggests that CTL-based AIDS vaccine efficacy may be abrogated in viral transmission between MHC-matched individuals.

3. Abrogation of AIDS vaccine-induced cytotoxic T lymphocyte efficacy in vivo due to a change in viral epitope flanking sequences

Chikaya Moriya, Hiroko Igarashi, Akiko Takeda, Tetsuo Tsukamoto, Miki Kawada, Hiroyuki Yamamoto, Makoto Inoue[4], Akihiro Iida[4], Tsugumine Shu[4], Mamoru Hasegawa[4], Yoshiyuki Nagai[5], and Tetsuro Matano: [4]DNAVEC Corp., [5]Center of Research Network for Infectious Diseases, RIKEN

A current promising AIDS vaccine strategy is to elicit $CD8^+$ CTL responses that broadly recognize highly-diversified HIVs. In our previous vaccine trial eliciting SIVmac239 Gag specific CTL responses, a group of Burmese rhesus macaques possessing a MHC haplotype *90-120-Ia* have shown vaccine-based viral control against a homologous SIVmac239 challenge. Vaccine-induced $Gag_{206-216}$ epitope specific CTL responses exerted strong selective pressure on the virus in this control. Here, we have evaluated in vivo efficacy of vaccine-induced $Gag_{206-216}$ specific CTL responses in two *90-120-Ia* positive macaques against challenge with a heterologous SIVsmE 543-3 that has the same $Gag_{206-216}$ epitope sequence with SIVmac239. Despite efficient $Gag_{206-216}$ specific CTL induction by vaccination, both vaccinees failed to control SIVsmE543-3 replication and neither of them showed mutations within the $Gag_{206-216}$ epitope. Further analysis indicated that $Gag_{206-216}$ specific CTLs failed to show responses against SIVsmE543-3 infection due to a change from aspartate to glutamate at Gag residue 205 immediately preceding the amino terminus of $Gag_{206-216}$ epitope. Our results suggest that even vaccine-induced CTL efficacy can be abrogated by a single amino acid change in viral epitope flanking region, underlining the influence of viral epitope flanking sequences on CTL-based AIDS vaccine efficacy.

4. Antigen-specific T-cell induction by vaccination with a recombinant Sendai virus vector even in the presence of vector-specific neutralizing antibodies in rhesus macaques

Chikaya Moriya, Satoshi Horiba, Makoto Inoue, Akihiro Iida, Hiroto Hara[4], Tsugumine Shu, Mamoru Hasegawa, and Tetsuro Matano

Recombinant viral vectors are promising vaccine tools for eliciting potent cellular immune responses against immunodeficiency virus infection, but pre-existing anti-vector antibodies can be an obstacle to their clinical use in humans. We have previously vaccinated rhesus macaques with a recombinant SeV vector twice at an interval of more than 1 year and have shown efficient antigen-specific T-cell induction by the second as well as the first vaccination. Here, we have established the method for measurement of SeV-specific neutralizing titers and have found efficient SeV-specific neutralizing antibody responses just before the second SeV vaccination in these macaques. This suggests the feasibility of inducing antigen-specific T-cell responses by SeV vaccination even in the host with pre-existing anti-SeV neutralizing antibodies.

5. Evaluation of the immunogenicity of replication competent V knocked-out and replication defective F-deleted Sendai virus vector-based vaccines in macaques

Akiko Takeda, Hiroko Igarashi, Miki Kawada[a], Tetsuo Tsukamoto, Hiroyuki Yamamoto, Makoto Inoue, Akihiro Iida, Tsugumine Shu, Mamoru Hasegawa, and Tetsuro Matano

Viral vectors are promising vaccine tools for eliciting antigen specific T cell responses. We previously showed the potential of recombinant SeV vectors to induce virus-specific T-cell responses in macaque AIDS models. Here, we have evaluated the immunogenicity of replication competent V knocked-out and replication defective F-deleted SeV vectors in macaques. Intranasal replication competent and replication defective SeV immunizations both elicited robust systemic antigen specific T cell responses, whereas the responses induced by the former

were more durable than those by the latter. However, even the latter-induced T cell responses remained detectable in a local, retropharyngeal lymph node two months after the immunization. These findings are useful for establishment of a vaccine protocol using SeV vectors.

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

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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

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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. Simultaneous Tracking of Capsid, Tegument and Envelope Protein Localization in Living Cells Infected with Triple Fluorescent Herpes Simplex Virus 1.

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The dynamic processes of the herpes simplex virus 1 (HSV-1) maturation pathway and the cellular site(s) for virion assembly are poorly defined. We report here the construction of a triple fluorescent-tagged herpes simplex virus 1 (HSV-1) expressing capsid protein VP26, tegument protein VP22 and envelope protein gB as fusion proteins with monomeric yellow, red and cyan fluorescent proteins, respectively. The recombinant virus enabled us to monitor the dynamics of these capsid, tegument and envelope proteins simultaneously in the same live HSV-1-infected cells and to visualize single extracellular virions with three different fluorescent emissions. In Vero cells infected by the triple fluorescent virus, multiple cytoplasmic compartments were found to be induced close to the basal surfaces of the infected cells (the adhesion surfaces of the infected cells on the solid growth substrate). Major capsid, tegument and envelope proteins accumulated and co-localized in the compartments, as did marker proteins for the *trans*-Golgi network (TGN) which has been implicated to be the site of HSV-1 secondary envelopment. Moreover, formation of the compartments was correlated with the dynamic redistribution of the TGN proteins induced by HSV-1 infection. These results suggest that HSV-1 infection causes redistribution of TGN membranes to form multiple cytoplasmic compartments, possibly, for optimal secondary envelopment. This is the first real evidence for the assembly of all three types of herpesvirus proteins; capsid, tegument and envelope membrane proteins in TGN.

2. Identification of a physiological phosphorylation site of the Herpes simplex virus 1-encoded protein kinase Us3 which regulates its optimal catalytic activity in vitro and influences its function in infected cells.

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HSV-1 Us3 is a viral protein kinase that plays important roles in viral replication and pathogenesis. Here, we report the identification of a physiological Us3 phosphorylation site on serine at position 147 (Ser-147) which regulates its protein kinase activity *in vitro*. Moreover, mutation of this site influences Us3 function, including correct localization of the enzyme and induction of the usual morphological changes in HSV-1-infected cells. These conclusions are based on the following observations: (i) in *in vitro* kinase assays, a domain of Us3 containing Ser-147 was specifically phosphorylated by Us3 and protein kinase A, while a mutant domain in which Ser-147 was replaced with alanine was not; (ii) *in vitro*, alanine replacement of Ser-147 (S147A) in Us3 resulted in significant impairment of the kinase activity of the purified molecule expressed in a baculovirus system; (iii) phosphorylation of Ser-147 in Us3 tagged with the monomeric fluorescent protein VenusA206K (VenusA206K-Us3) from Vero cells infected with a recombinant HSV-1 encoding VenusA206K-Us 3 was specifically detected using an antibody that recognizes phosphorylated serine or threonine residues with arginine at the -3 and -2 positions; and (iv) the S147A mutation influenced some but not all Us3 functions including the ability of the protein to localize itself properly and to induce wild-type cytopathic effects in infected cells. Our results suggest that some of the regulatory activities of Us3 in infected cells are controlled by phosphorylation at Ser-147.

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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

The group A streptococcus (GAS) *Streptococcus pyogenes* is a Gram-positive pathogen that causes a wide range of human diseases, such as pharyngitis, bacteremia, necrotizing fasciitis, and streptococcal toxic shock-like syndrome. Streptococcal infection can also induce bacterial septic arthritis, which occurs by bacterial invasion into joint cavity through blood circulation. Septic arthritis is the most rapidly progressing joint disease and permanent joint damage occurs in most cases. Atlthough a wide variety of both Gram-positive and Gram-negative bacteria induce septic arthritis, GAS is one of the most common bacteria isolated from patients with septic arthritis. The pathogenesis of arthritis is characterized by synovial proliferation and the destruction of cartilage and subchondral bone in joints. We report here that GAS strain JRS4 invaded a chondrogenic cell line ATDC5 and induced the degradation of the extracellular matrix (ECM), whereas an isogenic mutant of JRS4 lacking a fibronectin-binding protein, SAM1, failed to invade the chondrocytes or degrade the ECM. Reverse transcription-PCR and western blot analysis revealed that the expression of matrix metalloproteinase (MMP)-13 was strongly elevated during the infection with GAS. A reporter assay revealed that the activation of the AP-1 transcription factor and the phosphorylation of c-Jun terminal kinase participated in MMP-13 expression. These results suggest that MMP-13 plays an important role in the destruction of infected joints during the development of septic arthritis.

Autophagy is originally reported as self-digestion system and is involved in a number of clinical conditions and diseases such as neurodegenerative disease, cardiomyopathy, immunologic responses, the maintenance of liver function, and cell death. Our group previously reported intracellular group A streptococcus (GAS) was sequestered and degraded effectively by autophagic machinery. A cholesterol-dependent cytolysin of GAS, streptolysin O (SLO), was supposed to be associated with bacterial escape from endosomes in non-phagocytic

epithelial cells. However, details of both the intracellular behavior of GAS and the host cell process leading to autophagic degradation remain largely unknown. We show here that accumulation of SLO induced release of GAS into cytoplasm from the endosome, followed by its sequesteration by autophagosomes. A SLO-deficient mutant of GAS fails to escape but was viable in host cells longer than wild-type GAS strain. In addition, dynamics of two host small G proteins, Rab5 and Rab7 involved in membrane trafficking and key factors in the formation and maturation of endosomes, were analyzed during GAS infection. Rab5 was involved in bacterial invasion and the fusion of homotypic endosomes. Rab7 exerted the multifunctionality clearly because of its involvement in bacterial invasion, the maturation of endosomes and autophagosomes, and the formation of autophagosomes. Because the intracellular behavior of GAS was shown to be characteristic and was different from those known in other bacteria, new roles of Rab5 and Rab7 could be elucidated using epithelial cells infected with GAS. These data provide that more detailed picture of GAS infection and host cell response to eliminate it in addition to the mechanism of how GAS escape from endosome and new functions of Rab5 or Rab7.

2. Comparative genomic analysis for pathogenic bacterial evolution.

Fumito Maruyama and Ichiro Nakagawa

Streptococcus mutans is the major pathogen of dental caries, and occasionally causes infective endocarditis. Whereas the pathogenicity of this species is characteristic from other human pathogenic streptococci, the species-specific strategy of the evolution of genus *Streptococcus* as well as the genomic diversity of this species is poorly understood.

We have determined the complete genome sequence 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 contains a highly conserved core-genome between two strains. However, a large genomic inversion across the replication axis produces a characteristic X-shaped symmetrical diagram between *S. mutans* strains. This phenomenon was also observed in comparison with other streptococcal species, indicating that streptococcal genetic rearrangements across the replication axis play an important role for producing genetic shuffling in the genus *Streptococcus*. We further confirmed the genomic diversity among 95 clinical isolates using long-PCR analyses. Genomic diversities of *S. mutans* are between IS elements and transposons, and these regions consist of restriction-modification systems, antimicrobial peptide synthesis, and transporters. *S. mutans* may preferentially reject the phage infection by clustered regularly interspaced short palindromic repeats (CRISPR). Especially, CRISPR-2 region, highly divergent among strains, has long repeated spacer sequences corresponding the streptococcal phage genome in NN2025.

These observations suggest that *S. mutans* strains are evolving with chromosomal shuffling and the evolving force of *S. mutans* is not dependent on phage infection whereas other streptococcal species tolerate them for acquisition of virulent determinants for niche acquisition.

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International Research Center for Infectious Diseases

Pathogenic Microbes Repository Unit
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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 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.