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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 Nipa viruses as models.

1. Involvement of heparin-like molecule in CDV infection

Fujita K, Miura R, Yoneda M, Shimizu F, Sato H, Muto Y, Endo Y, Tsukiyama-Kohara K, Kai C.

As a model for analyzing the mechanism of mononegavirus infection, we constructed recombinant canine distemper viruses expressing enhanced green fluorescent protein (EGFP) or firefly luciferase. Using these viruses, we examined susceptibilities of different cell lines to CDV infection. It was shown that the recombinant CDVs can infect a broad range of cell lines including cells not only of canine origin but also from human, monkey, cat, seal and rodents. Infection of B95a cells (a marmoset B cell line) with the recombinant CDV was inhibited by a monoclonal antibody specific to SLAM (signaling lymphocyte activation molecule), a receptor for morbilliviruses, whereas the infection of 293 cell lines was not, implying the presence of one or more alternative receptors for CDV in non-lymphoid tissue. We also showed that the infection of 293 cells with the recombinant CDV was inhibited by soluble heparin, and the recombinant virus bound to immobilized heparin. Both F and H proteins of CDV have ability to bind to immobilized heparin. These results suggest that heparin-like molecules are involved in CDV infection.

2. Membrane Topography of the Hydrophobic Anchor Sequence of Poliovirus 3A and 3 AB Proteins and the Functional Effect of 3 A/3AB Membrane Association upon RNA Replication

Fujita K, Krishnakumar SS, Franco D, Paul AV, London E, Wimmer E

Replication of poliovirus RNA takes place on the cytoplasmic surface of membranous vesicles that form after infection of the host cell. It is generally accepted that RNA polymerase 3Dpol interacts with membranes in a complex with viral protein 3AB, which binds to membranes by

means of a hydrophobic anchor sequence that is located near the C-terminus of the 3A domain. In this study, we used fluorescence and fluorescence quenching methods to define the topography of the anchor sequence in the context of 3A and 3AB proteins inserted in model membranes. Mutants with a single tryptophan near the center of the anchor sequence but lacking Trp elsewhere in 3A/3AB were constructed which, after the emergence of suppressor mutations, replicated well in HeLa cells. When a peptide containing the mutant anchor sequence was incorporated in model membrane vesicles, measurements of Trp depth within the lipid bilayer indicated formation of a transmembrane topography. However, rather than the 22-residue length predicted from hydrophobicity considerations, the transmembrane segment had an effective length of 16 residues, such that Gln64 likely formed the N-terminal boundary. Analogous experiments using full-length proteins bound to preformed model membrane vesicles showed that the anchor sequence formed a mixture of transmembrane and nontransmembrane topographies in the 3A protein but adopted only the nontransmembrane configuration in the context of 3AB protein. Studies of the function of 3A/3 AB inserted into model membrane vesicles showed that membrane-bound 3AB is highly efficient in stimulating the activity of 3Dpol in vitro while membrane-bound 3A totally lacks this activity. Moreover, in vitro uridylylation reactions showed that membrane-bound 3AB is not a substrate for 3Dpol, but free VPg released by cleavage of 3AB with proteinase 3CDpro could be uridylylated.

3. Mapping of theVP40-binding regions of the Nucleoprotein of Ebola virus.

Noda T, Watanabe S, Sagara H, Kawaoka Y.

Expression of Ebola virus nucleoprotein (NP) in mammalian cells leads to the formation of helical structures, which serve as a scaffold for the nucleocapsid. We recently found that NP binding with the matrix protein VP40 is important for nucleocapsid incorporation into virions (T. Noda, H. Ebihara, Y. Muramoto, K. Fujii, A. Takada, H. Sagara, J.H. Kim, H. Kida, H. Feldmann, and Y. Kawaoka, PLoS Pathog. 2: e99, 2006). To identify the region(s) on the NP molecule required for VP40 binding, we examined the interaction of a series of NP deletion mutants with VP40 biochemically and ultrastructurally. We found that both termini of NP (amino acids 2 to 150 and 601 to 739) are essential for its interaction with VP40 and for its incorporation into virus-like particles (VLPs). We also found that the C terminus of NP is important for nucleocapsid incorporation into virions. Of interest is that the formation of NP helices, which involves the N-terminal 450 amino acids of NP, is dispensable for NP incorporation into VLPs. These findings enhance our understanding of Ebola virus assembly and in so doing move us closer to the identification of targets for the development of antiviral compounds to combat Ebola virus infection.

4. Regions in Ebola virus VP24 that are important for nucleocapsid formation.

Noda T, Halfmann P, Sagara H, Kawaoka Y.

Ebola virus (EBOV) VP24, together with nucleoprotein and VP35, is an essential component of viral RNA-protein complexes called "nucleocapsids". In this study, using a series of deletion mutants of VP24, we identified regions within VP24 that are important for the formation of nucleocapsid-like structures and determined that both termini of VP24 are essential for nucleocapsid formation. This finding advances our knowledge of both EBOV morphogenesis and the nature of VP24 molecules in nucleocapsid formation, which will be useful for the development of antiviral compounds.

5. In vitro and in vivo characterization of recombinant Ebola viruses expressing enhanced green fluorescent protein.

Ebihara H, Theriault S, Neumann G, Alimonti JB, Geisbert JB, Hensley LE, Groseth A, Jones SM, Geisbert TW, Kawaoka Y, Feldmann H.

To facilitate an understanding of the molecular aspects of the pathogenesis of Zaire ebolavirus (ZEBOV) infection, we generated 2 different recombinant viruses expressing enhanced green fluorescent protein (eGFP) from additional transcription units inserted at different positions in the virus genome. These viruses showed in vitro phenotypes similar to that of wild-type ZEBOV (wt-ZEBOV) and were stable over multiple passages. Infection with one of the viruses expressing eGFP produced only mild disease in rhesus macaques, demonstrating a marked attenuation in this animal model. However, in mice lacking signal transducer and activator of transcription 1, both viruses expressing eGFP caused lethal cases of disease that were moderately attenuated, compared with that caused by wt-ZEBOV. In mice, viral replication could be easily tracked by the detection of eGFP-positive cells in tissues, by use of flow cytometry. These findings demonstrate that the incorporation of a foreign gene

will attenuate ZEBOV in vivo but that these viruses still have potential for in vitro and in vivo research applications.

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We are working hard to improve the diagnostic methods for infectious diseases including HIV and its related infections and parasitic 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) in the IMSUT Hospital. We offer diagnostic services for parasitic and tropical diseases including malaria, toxoplasmosis, schistosomiasis and some international infections, working together for the patients' care in the IMSUT hospital.

1. Association analysis of *N*-acetyltransferase 2 gene polymorphisms as a possible risk factor for adverse events of co-trimoxazole in HIV-positive patients.

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

Co-trimoxazole (trimethoprim- sulphamethoxazole) is an effective drug for prevention and treatment of *Pneumocystis jirovecii* pneumonia that occurs in immunodeficiency patients such as HIV infected patients. However, the usage of co-trimoxazole in HIV-positive patients is associated with very high frequency of adverse events including hypersensitivity and hepatotoxicity, which is reported to be between 40% and 80%. Of the two chemical components of co-trimoxazole, sulphamethoxazole is thought to be responsible for hypersensitivity. The major metabolic pathway for sulphamethoxazole is catalyzed by *N*-acetyltransferase 2 (NAT-2). The pathogenetic mechanisms of this hypersensitivity is not clarified yet, but it is suggested that *NAT-2* gene polymorphisms might be involved.

We investigated the relationship between the genetic polymorphisms of the *NAT-2* gene and the incidence of adverse events in a Japanese HIV-positive population.

43 patients receiving primary prophylaxis had median CD4 count of 97.4 cells/μl and adverse events were occurred in 13 patients (30.2%) with median CD4 count of 91.8 cells/μl. 15 patients with median CD4 count of 61.3 cells/μl were administrated oral co-trimoxazole for the treatments of *pneumocystis jirovecii* pneumonia and

only 4 patients (26.7%) of them with median CD 4 count of 13.3 cells/μl were free from adverse events. Adverse events occurred less frequently in those without the *NAT-2**4 haplotype (1/7 [14.3%]) than in those with at least one *NAT-2**4 haplotype (23/51 [45.1%]). Although all patients were evaluated with a clinical assessment and laboratory monitoring that including age, sex, steroids usage, CD4 count, HIV-RNA levels and genotyping of *NAT-2* gene, CD4 count was the only associated parameter with adverse events with co-trimoxazole in Japanese HIV-positive population.

A low CD4 count (<200/μl) with HIV infection is a risk factor for adverse event of co-trimoxazole, but the patients with profoundly low CD4 count (<50/μl) were relatively free from adverse event by co-trimoxazole.

2. A BACTERIAL EFFECTOR TARGETS MAD 2L2, AN APC INHIBITOR, TO MODULATE HOST CELL CYCLING.

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The gut epithelium self-renews every several days, providing an important innate defense system that limits bacterial colonization. Nevertheless, many bacterial pathogens, including Shigella, efficiently colonize the intestinal epithelium. Here, we show that the *Shigella* effector IpaB, when delivered into epithelial cells, causes cell-cycle arrest by targeting Mad2L2, an anaphase-promoting complex/cyclosome (APC) inhibitor. Cyclin B1 ubiquitination assays revealed that APC undergoes unscheduled activation due to IpaB interaction with the APC inhibitor Mad2L2. Synchronized HeLa cells infected with *Shigella* failed to accumulate Cyclin B1, Cdc20, and Plk1, causing cell-cycle arrest at the G2/M phase in an IpaB/Mad2L2-dependent manner. IpaB/Mad2L2-dependent cell-cycle arrest by *Shigella* infection was also demonstrated in rabbit intestinal crypt progenitors, and the IpaB-mediated arrest contributed to efficient colonization of the host cells. These results strongly indicate that *Shigella* employ special tactics to influence epithelial renewal in order to promote bacterial colonization of intestinal epithelium.

3. Assessments of clinical diagnosis for infectious deseases

a. *Pneumocystis jiroveci* pneumonia in an AIDS patient: Unusual manifestation of multiple nodules with multiloculated cavities

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We reported a case of *Pneumocystis jiroveci* pneumonia (PCP) in an acquired immune deficiency syndrome (AIDS) patient with multiloculated cavitary lesions. Time-course analysis of chest computed tomography (CT) showed spontaneous ballooning of cavities and their disappearance after completion of PCP treatment. The characteristic radiological findings as well as histological features of cavitary lesions suggested that check-valve phenomenon in small airways might explain the pathogenesis of cavities in this case.

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

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We repoerted 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.

c. Neurocysticercosis case with tuberculoma-like epithelial granuloma strongly suspected by serology and confirmed by mitochondrial DNA

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

An Indian woman of 44 years old with multiple and nodular cerebral lesions was diagnosed as suspected to have malignant tumors and a biospy was carried at local hospital in 2005 in Japan. The specimen was pathologically diagnosed as tuberculoma. However, due to her nationality, we suspected a possibility of neurocysticercosis (NCC). We carried out serologic studies using highly specific glycoproteins and recombinant antigens of *Taenia solium* cysticerci. We also tried to detect mitochondrial DNA of *T. solium* in the lesion. These efforts allowed us that the patient had NCC instead of central nervous tuberculomas. Molecular confirmation of NCC in the biopsy specimens is strongly recommended in patients with a possibility.

d. Evaluation of the Rapid Diagnostic Tests for Malaria

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

Microscopic detection of malaria parasites on a blood smear is the gold standard for the diagnosis of malaria. However, it is still difficult for persons with a little experience in most non-endemic nations and rapid malaria diagnosing kits such as OptiMAL-IT™ (DiaMed, USA), NOW Malaria™ (Binax, USA), and pan-R MALARIA™ (Panbio, Australia) should be usuful.

We prospectively evaluated the effectiveness of pan-R MALARIA™ kit on 11 blood specimens derived from febrile returnees from malaria endemic areas. In all cases, microscopic examinations, pan-R MALARIA™ kit and OptiMAL-IT™ kit were performed. One of the eleven cases (9.1%) was microscopically confirmed to have falciparum malaria and also 2 rapid malaria kits gave the compatible result. Three of 11 cases (27.2%), who were microscopically negative for the malaria parasites, were p. f. positive by the pan-R MALARIA™ kit. They were simultaneously evaluated by OptiMAL-IT™ kit and PCR method for the detection of plasmodial DNA fragments. They were negative by both methods, and we finally excluded the the diagnosis of malaria. There were 3 false positive results with pan-R MALARIA™ kit. Although Panbio Inc. declares that the sensitivity of pan-R MALARIA™ kit is 96.0% and the specificity of 99.7%, our experience showed the sensitivity of 100% (1/1) and a specificity of 71.4% (8/11).

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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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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. Long-term SIV control with central memory CD4$^+$ T-cell preservation after non-sterile protection by a CTL-based vaccine

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Induction of virus-specific CD8$^+$ cytotoxic T lymphocyte (CTL) responses is a promising strategy for AIDS vaccine development. However, it remained unclear if or how long-term viral containment and disease control are attainable by CTL-based non-sterile protection. In this study, we have presented three rhesus macaques that successfully maintained Env-independent vaccine-based control of simian immunodeficiency virus (SIV) mac239 replication without disease progression for more than 3 years. SIV-specific neutralizing antibody induction was inefficient in these controllers. Vaccine-induced Gag-specific CTLs were crucial for the chronic as well as the primary viral control in one of them, whereas those Gag-specific CTL responses became undetectable and CTLs specific for SIV antigens other than Gag, instead, became predominant in the chronic phase in the other two controllers. A transient CD8$^+$ cell depletion experiment 3 years post-infection resulted in transient reappearance of plasma viremia in these two animals, suggesting involvement of the SIV non-Gag-specific CTLs in the chronic SIV control. This sustained, neutralizing antibody-independent viral control was accompanied with preservation of central memory CD 4$^+$ T cells in the chronic phase. Our results suggest that prophylactic CTL vaccine-based non-sterile protection can result in long-term viral containment by adapted CTL responses for AIDS prevention.

2. Post-infection HIV/SIV control by neutralizing antibodies.

Hiroyuki Yamamoto, Miki Kawada, Akiko Takeda, Hiroko Igarashi, and Tetsuro Matano

Unlike most acute viral infections controlled with the appearance of virus-specific neutralizing antibodies (NAbs), primary HIV infections are not met with such potent and early Ab responses. This brings into question if or how the presence of potent Abs can contribute to primary HIV control, but protective efficacies of antiviral Abs in primary HIV infections remain elusive and it was speculated that even NAb induction could have only a limited suppressive effect on primary HIV replication once infection is established. In this study, in an attempt to answer this question, we have examined the effect of passive NAb immunization post-infection on primary viral replication in a macaque AIDS model. The inoculums for passive immunization with SIVmac239-specific neutralizing activity were prepared by purifying polyclonal immunoglobulin G from pooled plasma of six SIVmac 239-infected rhesus macaques with NAb induction in the chronic phase. Passive immunization of rhesus macaques with the NAbs at day 7 after SIVmac239 challenge resulted in significant reduction of set-point plasma viral loads and preservation of central memory CD4 T lymphocyte counts, despite the limited detection period of the administered NAb responses. Peripheral lymph node dendritic cell (DC)-associated viral RNA loads showed a remarkable peak with the NAb administration, and DCs stimulated in vitro with NAb-preincubated SIV activated virus-specific CD4 T lymphocytes in an Fc-dependent manner, implying Ab-mediated virion uptake by DCs and enhanced T cell priming. Our results present evidence indicating that potent Ab induction post-infection can result in primary immunodeficiency virus control and suggest direct and indirect contribution of its absence to initial control failure in HIV infections. Although difficulty in achieving requisite neutralizing titers for sterile HIV protection by prophylactic vaccination has been suggested, this study points out a possibility of non-sterile HIV control by prophylactic vaccine-induced, sub-sterile titers of NAbs post-infection, providing a rationale of vaccine-based NAb induction for primary HIV control.

3. Induction of $CD8^+$ cells able to suppress CCR5-tropic SIVmac239 replication by controlled infection of CXCR4-tropic SHIV in vaccinated rhesus macaques

Tetsuo Tsukamoto, Mitsuhiro Yuasa, Hiroyuki Yamamoto, Miki Kawada, Akiko Takeda, Hiroko Igarashi, and Tetsuro Matano

Recent recombinant viral vector-based AIDS vaccine trials inducing cellular immune responses have shown control of CXCR4-tropic simian-human immunodeficiency virus (SHIV) replication but difficulty in containment of pathogenic CCR5-tropic SIV in rhesus macaques. In contrast, controlled infection of live attenuated SIV/SHIV can confer the ability to contain SIV superchallenge on macaques. The specific immune responses responsible for this control may be induced by live virus infection but not consistently by viral vector vaccination, although those responses have not been determined. In this study, we have examined *in vitro* anti-SIV efficacy of $CD8^+$ cells in rhesus macaques that showed prophylactic viral vector vaccine-based control of CXCR4-tropic SHIV89.6 PD replication. Analysis of the effect of $CD8^+$ cells obtained at several time points from these macaques on CCR5-tropic SIVmac239 replication *in vitro* revealed that $CD8^+$ cells in the chronic phase after SHIV challenge suppressed SIV replication more efficiently compared to those before challenge. SIVmac239 superchallenge of two of these macaques at 3 or 4 years post-SHIV challenge was contained, and the following anti-CD8 antibody administration resulted in transient $CD8^+$ T-cell depletion and appearance of plasma SIVmac239 viremia in both of them. Our results indicate that $CD8^+$ cells acquired the ability to efficiently suppress SIV replication by controlled SHIV infection, suggesting contribution of $CD8^+$ cell responses induced by controlled live virus infection to containment of HIV/SIV superinfection.

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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. Novel virus factory-like compartments in herpes simplex virus 1-infected cells.

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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 expressing capsid protein VP26, tegument protein VP22 and envelope protein gB as fusion proteins with 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 cells infected by the triple fluorescent virus, we identified novel virus factory-like cellular compartments at the bottom of HSV-1-infected cells growing on a solid substrate. Major capsid, tegument and envelope proteins accumulated and co-localized in these compartments, as did marker proteins for the *trans*-Golgi network which is though to be the site of final HSV-1 envelopment. Ultrastructural analysis revealed that, in cells infected with wild-type HSV-1, progeny virions also accumulated in the compartments induced at the bottom of the infected cells. Time lapse analyses demonstrated that the dynamics of tegument and envelope protein accumulation in the compartments was identical and these proteins start accumulating in the compartments before capsid proteins. Monensin treatment of infected cells inhibited formation of the compartments and reduced progeny virus yield. These results suggest that the cellular compartments identified in this study play a role in optimal virus replication and, possibly, virus assembly.

2. Identification of Proteins Directly Phosphorylated by UL13 Protein Kinase from Herpes Simplex Virus 1.

Risa Asai, Takashi Ohno, Akihisa Kato, Yasushi Kawaguchi

Herpes simplex virus 1 (HSV-1) UL13 is a viral protein kinase that regulates optimal viral replication in cell cultures. Identification of substrates of protein kinases is a crucial step to elucidate the mechanism by which they function. Using our developed system to analyze the specific protein kinase activity of UL13, we have shown that UL13 protein kinase directly phosphorylates the viral proteins ICP22 and UL49 previously reported to be putative substrates. We also identified UL41 as a previously unreported and novel substrate of UL13. These data will serve as a basis to clarify the mechanism by which UL13 influences viral replication.

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International Research Center for Infectious Diseases

Department of Infectious Disease Control Division of Bacteriology

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 defense mechanism, and to maximize their virulence for surviving. For counterattacking to bacterial infection, our immune system has also acquired the various defense mechanisms in the evolution. Our research team, Division of Bacteriology, aims to resolve the mechanism of infection of gram-positive bacteria in humans. Our approaches include; i) comparative genomics of pathogenic bacteria, ii) gene expression analysis of pathogenic bacteria with bioinformatics, iii) analysis of dynamics of the pathogens inside host cells, iv) interaction analysis of the pathogen and host in infection state, and v) activation mechanism of immune systems via host factors through in vitro and in vivo experiments.

Whole-genome transcriptome analysis of group A streptococci.

Group A streptococci is a major pathogen of pharyngitis and tonsillitis, and sometimes causes fatal cases with invasive infection. Although 16 *Streptococcus pyogenes* genomes have been completely sequenced, the difference of pathogenicities among these strains remain to be unknown with analysis based on their sequences. One of the important factors for genome transcriptome analysis of these pathogens is the existence of specific pattern in genome sequence. In the case of *E. coli* and *B. subtilis* in which GC content of the genome is approximately 50%, prediction of transcription start site related to gene expression has been studied intensively based on accumulated database. In contrast, it is difficult for low GC content genome such as *S. pyogenes* (38%) to predict even promoter sequence in the gene expression analysis. To appreciate the pathogenicity of *S. pyogenes* comprehensively, whole genome tiling array with overlapping probes per 20-30 intervals was designed and used in our study. While conventional microarray for bacteria usually have less than 10,000 probes which target only known ORF, in the tiling array, about 400,000 probes in 50-75 mer length were aligned without bias and are able to hybridize to non-protein-coding regions. Therefore, the tiling array can apply to analysis of the operon unit and promoter region, detection of non-coding RNA at the whole genome level leading to comprehensive understanding of phathogenicity of *S. pyogenes*.

Comparative genomics of genus *Streptococcus*.

Bacteria belonging to genus *Streptococcus* can attach and infect mucosal surface of human such as oral cavity, gastrointestinal tract, and upper-air passage for their early infection sites leading to significant infection. To clarify the

history of their gene loss and gain for adaptation to existing native habitat or ecological niche should bring elucidation of mechanism of streptococcal infection. Therefore, we developed multi-locus sequencing typing method for *Streptococcus mutans* which inhabits oral cavity and is closely related to group A or B streptococci, and genome analysis of *S. mutans* NN2025 and comparative genome analysis of genus *Streptococcus* were carried out. In addition, genome of bacterium, which causes periodontal disease is also analyzed to reveal the lateral gene transfer in oral environments.

Host recognition of intracellular bacteria and exclusion mechanism by host immune system.

The endocytic degradation pathway of phagocytic cells 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 pathogenic bacteria that have invaded non-phagocytic cells. Gram-positive bacteria such as genus *Streptococcus* in oral cavity and pharynges that have ability to invade host cell are also degraded by the autophagic system. 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.

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International Research Center for Infectious Diseases

Pathogenic Microbes Repository Unit
病原微生物資源室

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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.