International Research Center for Infectious Diseases

Department of Special Pathogens
高病原性感染症系

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

Identification of PB2 Mutations Responsible for the Efficient Replication of H5N1 Influenza Viruses in Human Lung Epithelial Cells

Yamaji, R., Yamada, S., Le, MQ.[1], Li, C.[2], Chen, H.[2], Qurnianingsih, E.[3], Nidom, CA.[3], Ito, M., Sakai-Tagawa, Y., and Kawaoka, Y.: [1]National Institute of Hygiene and Epidemiology, Hanoi, Vietnam [2]State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China [3]Avian Influenza Research Center, Airlangga University, Surabaya, Indonesia

Highly pathogenic H5N1 avian influenza viruses have caused outbreaks among poultry worldwide, resulting in sporadic infections in humans with approximately 60% mortality. However, efficient transmission of H5N1 viruses among humans has yet to occur, suggesting that further adaptation of H5N1 viruses to humans is required for their efficient transmission among humans. The viral determinants for efficient replication in humans are currently poorly understood. Here, we report that the polymerase PB2 protein of an H5N1 influenza virus isolated from a human in Vietnam (A/Vietnam/UT 36285/2010, virus 36285) increased the growth capability of an avian H5N1 virus (A/wild bird/Anhui/82/2005, virus Wb/AH82) in human lung epithelial A549 cells (however, the reassortant virus did not replicate more efficiently than human 36285 virus). Furthermore, we found that the amino acid residues at positions 249, 309, and 339 of the PB2 protein from this human isolate were responsible for its efficient replication in A549 cells. PB2 residues 249G and 339M, which are found in the human H5 N1 virus, are rare in H5N1 viruses from both human and avian sources. Interestingly, PB2-249G is found in over 30% of human seasonal H3N2 viruses, which suggests that H5N1 viruses may replicate well in human cells when they acquire this mutation. Our data are of value for H5N1 virus surveillance.

Efficacy of Recombinant Canine Distemper Virus Expressing Leishmania Antigen against Leishmania Challenge in Dogs.

Miura, R., Kooriyama, T., Yoneda, M., Takenaka, A., Doki, M., Goto, Y.[2], Sanjoba, C.[2], Endo, Y., Fujiyuki, T., Sugai, A., Tsukiyama-Kohara, K.[3], Matsumoto, Y.[2], Sato, H. and Kai, C.: [2]Department of Molecular Immunology, School of Agriculture and Life Sciences, The University of Tokyo. [3]Joint Faculty of Veterinary Medicine, Kagoshima University

Canine distemper virus (CDV) vaccination confers long-term protection against CDV reinfection. To investigate the utility of CDV as a polyvalent vaccine vector for Leishmania, we generated recombinant CDVs, based on an avirulent Yanaka strain, that expressed Leishmania antigens: LACK, TSA, or LmSTI1 (rCDV-LACK, rCDV-TSA, and rCDV-LmSTI1, respectively). Dogs immunized with rCDV-LACK were protected against challenge with lethal doses of virulent CDV, in the same way as the parental Yanaka strain. To evaluate the protective effects of the recombinant CDVs against cutaneous leishmaniasis in dogs, dogs were immunized with one recombinant CDV or a cocktail of three recombinant CDVs, before intradermal challenge (in the ears) with infective-stage promastigotes of Leishmania major. Unvaccinated dogs showed increased nodules with ulcer formation after 3 weeks, whereas dogs immunized with rCDV-LACK showed markedly smaller nodules without ulceration. Although the rCDV-TSA- and rCDV-LmSTI1-immunized dogs showed little protection against L. major, the cocktail of three recombinant CDVs more effectively suppressed the progression of nodule formation than immunization with rCDV-LACK alone. These results indicate that recombinant CDV is suitable for use as a polyvalent live attenuated vaccine for protection against both CDV and L. major infections in dogs.

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Our research targets are HIV immune responses and viral tropism. In order to understand immunopathogenesis of HIV infection, we are focusing on T cell dysfunction during chronic HIV infection. On the other hands, co-receptor usage is one of the most fundamental steps in HIV-1 replication. Since the first CCR5 inhibitor, maraviroc, was commercialized, tropism assays have become a leading topic in HIV-1 research. For analyzing the viral tropism, we developed a novel HIV-1 phenotypic tropism assay based on the cell fusion. Our other special interest is focused upon searching for effective methods to protect or control viral infection by using accumulated knowledge based on molecular pathogenicity, and developing novel anti-viral drugs and attenuated strains for novel vaccines. The works have been conducted by close collaboration with Division of Infectious Diseases, Advanced Clinical Research Center and Division of Molecular Virology, Department of Microbiology and Immunology

1. Possible difference in virus-to-cell infection versus cell-to-cell infection of HIV-1

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Human immunodeficiency virus type 1 (HIV-1) uses a CD4 receptor and either CCR5 or CXCR4 as co-receptor for infection. HIV-1 may be categorized according to the co-receptor usage: CCR5-using virus as an R5 virus vs CXCR4-using virus as an X4 virus. The virus which can use both CCR5 and CXCR4 is designated as an R5X4 virus. Co-receptor usage can be estimated by a phenotypic assay or a genotypic assay. The gold standard of the phonotypic assay utilizes a recombinant pseudovirus and is carried out as follows: (1) HIV-1 envelope gene from patients' plasma is amplified by PCR and cloned into an expression vector. (2) Pseudovirus is produced by the transfection of the envelope gene to a producer cell carrying the HIV-1 genome cassette with a reporter gene. (3) The pseudovirus having the envelope from patient's origin and the reporter as the genome is recovered from the suopernatant. (4) The pseudovirus is infected to the test cells expressing CCR5 or CXCR4. (4) If the envelope is derived from the R5 virus, the produced pseudovirus would infect and express the reporter gene only in the CCR5-indicator cells, vice versa. (5) The result is obtained by measuring the reporter activity (i.e. the luciferase activity). The pseudovirus assay mimics the actual infection event but has shortcomings, such as biosafety issues and a long turnaround time (about a month). Our laboratory has reported a novel assay system (DSP-Pheno) based on the cell-cell fusion to detect tropism of HIV-1. DSP-Pheno does not need pseudoviruses and has a short turnaround time (5days).

Although DSP-Pheno assay is convenient, our unpublished results indicated that DSP-Pheno may overestimate CXCR4 usage compared to pseudovirus assay. Therefore, we isolated 25 clones of the envelope gene from the 6 patients and tested their co-receptor usage. According to the pseudovirus assay, 21 clones were R5 while 4 clones were R5X4 viruses. On the contrary, DSP-Pheno predicted 20 clones as R5X4 while only 5 clones were R5 viruses. Sequencing of the whole envelope showed that the amino acid mutation correlating the R5 to R5X4 change were scattered in C1, V1, C3, V4, and V5 region of the envelope gene. Our results suggested that the membrane fusion caused by X4 envelope may be different when X4 is expressed on the virion or cell surface. It may be speculated that cell-to-cell infection of HIV-1 may be more robust than virus-to-cell infection.

2. Role of Host Cell p32 in Herpes Simplex Virus 1 De-envelopment During Viral Nuclear Egress

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To clarify the function(s) of the herpes simplex virus 1 (HSV-1) major virion structural protein UL47 (also designated VP13/14), we screened cells overexpressing UL47 for UL47-binding cellular proteins. Tandem affinity purification of transiently expressed UL47 coupled with mass spectrometry-based proteomics technology and subsequent analyses showed that UL47 interacted with cell protein p32 in HSV-1-infected cells. Unlike in mock-infected cells, p32 accumulated at the nuclear rim in HSV-1-infected cells and this p32 recruitment to the nuclear rim required UL47. p32 formed a complex(es) with HSV-1 proteins UL31, UL34, Us3, UL47 and/or ICP22 in HSV-1-infected cells. All these HSV-1 proteins were previously reported to be important for HSV-1 nuclear egress, in which nucleocapsids bud through the inner nuclear membrane (primary envelopment) and the enveloped nucleocapsids then fuse with the outer nuclear membrane (de-envelopment). Like viral proteins UL31, UL34, Us3 and UL47, p32 was detected in primary enveloped virions. p32 knock-down reduced viral replication and induced membranous invaginations adjacent to the nuclear rim containing primary enveloped virions and aberrant localization of UL31 and UL34 in punctate structures at the nuclear rim. These effects of p32 knock-down were reduced in the absence of UL47. Therefore, the effects of p32 knock-down in HSV-1 nuclear egress were similar to those of the previously reported mutation(s) in HSV-1 regulatory proteins for HSV-1 de-envelopment during viral nuclear egress. Collectively, these results suggested that p32 regulated HSV-1 de-envelopment and replication, in a UL47-dependent manner.

3. Herpes simplex virus 1 recruits CD98 heavy chain and β1 integrin to the nuclear membrane for viral de-envelopment

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Herpesviruses have evolved a unique mechanism for nucleocytoplasmic transport of nascent nucleocapsids: the nucleocapsids bud through the inner nuclear membrane (INM) (primary envelopment), and the enveloped nucleocapsids then fuse with the outer nuclear membrane (de-envelopment). Little is known about the molecular mechanism of herpesviral de-envelopment. Here we showed that knockdown of both CD98 heavy chain (CD98hc) and its binding partner β1 integrin induced membranous structures containing enveloped herpes simplex virus 1 (HSV-1) virions that are invaginations of the INM into the nucleoplasm, and induced aberrant accumulation of enveloped virions in the perinuclear space and in the invagination structures. These effects were similar to those of the previously reported mutation(s) in HSV-1 proteins gB, gH, UL31 and/or Us3, which were shown here to form a complex(es) with CD98hc in HSV-1-infected cells. These results suggested that cellular proteins CD98hc and β1 integrin synergistically or independently regulated HSV-1 de-envelopment, probably by interacting directly and/or indirectly with these HSV-1 proteins.

4. Non-Muscle Myosin Heavy Chain IIB Mediates Herpes Simplex Virus 1 Entry

Jun Arii, Yoshitaka Hirohata, Akihisa Kato and Yasushi Kawaguchi

Non-muscle myosin heavy chain IIA (NMHC-IIA) has been reported to function as a herpes simplex virus 1 (HSV-1) entry co-receptor by interacting with viral envelope glycoprotein B (gB). Vertebrates have three genetically distinct isoforms of the NMHC-II, designated NMHC-IIA, NMHC-IIB and NMHC-IIC. COS cells, which are readily infected by HSV-1, do not express NMHC-IIA but do express NMHC-IIB. This observation prompted us to investigate whether NMHC-IIB might associate with HSV-1 gB and be involved in an HSV-1 entry like NMHC-IIA. In these studies, we show that: (i)

NMHC-IIB co-precipitated with gB in COS-1 cells upon HSV-1 entry; (ii) a specific inhibitor of myosin light chain kinase inhibited both cell-surface expression of NMHC-IIB in COS-1 cells upon HSV-1 entry as well as HSV-1 infection as reported with NMHC-IIA; (iii) overexpression of mouse NMHC-IIB in IC21 cells significantly increased their susceptibility to HSV-1 infection; (iv) knock-down of NMHC-IIB in COS-1 cells inhibited HSV-1 infection as well as cell-cell fusion mediated by HSV-1 envelope glycoproteins. These results supported the hypothesis that, like NMHC-IIA, NMHC-IIB associated with HSV-1 gB and mediated HSV-1 entry.

5. The Function of the Herpes Simplex Virus 1 Small Capsid Protein VP26 is Regulated by Phosphorylation at a Specific Site.

Ryosuke Kobayashi, Akihisa Kato, Shinya Oda, Naoto Koyanagi, Masaaki Oyama, Hiroko Kozuka-Hata, Jun Arii and Yasushi Kawaguchi

Replacement of the herpes simplex virus 1 small capsid protein VP26 phosphorylation site Thr-111 with alanine reduced viral replication and neurovirulence to levels observed with the VP26 null-mutation. This mutation reduced VP26 expression and mis-localized VP26 and its binding partner, the major capsid protein VP5, in the nucleus. VP5 mis-localization was also observed with the VP26 null-mutation. Thus we postulate that phosphorylation of VP26 at Thr-111 regulates VP26 function in vitro and in vivo.

6. Phosphorylation of Herpes Simplex Virus 1 dUTPase Regulates Viral Virulence and Genome Integrity by Compensating for Low Cellular dUTPase Activity in the Central Nervous System

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A mutation in herpes simplex virus 1 dUTPase (vdUTPase), which precluded its phosphorylation at Ser-187, decreased viral neurovirulence and increased mutation frequency in progeny virus genomes in the brains of mice where endogenous cellular dUTPase activity was relatively low, and overexpression of cellular dUTPase restored viral neurovirulence and mutation frequency altered by the mutation. Thus, phosphorylation of vdUTPase appeared to regulate viral virulence and genome integrity by compensating for low cellular dUTPase activity *in vivo*.

7. Interactome Analysis of Herpes Simplex Virus 1 Envelope Glycoprotein H

Yoshitaka Hirohata, Akihisa Kato, Masaaki Oyama, Hiroko Kozuka-Hata, Naoto Koyanagi, Jun Arii and Yasushi Kawaguchi

Herpes simplex virus 1 (HSV-1) envelope glycoprotein H (gH) is important for viral entry into cells and nuclear egress of nucleocapsids. To clarify additional, novel roles of gH during HSV-1 replication, we screened host cell proteins interacting with gH by tandem affinity purification coupled with mass spectrometry-based proteomics in 293T cells transiently expressing gH. This screen identified 123 host cell proteins as potential gH interactors. Of these proteins, general control nonderepressive-1 (GCN1), a *trans*-acting positive effector of GCN2 kinase, which regulates the phosphorylation of the α subunit of translation initiation factor 2 (eIF2α), was subsequently confirmed to interact with gH in HSV-1-infected cells. eIF2α phosphorylation is known to downregulate protein synthesis, and various viruses have evolved mechanisms to prevent the accumulation of phosphorylated eIF2α in infected cells. Here, we showed that GCN1 knockdown reduced the phosphorylation of eIF2α in HSV-1-infected cells and that the *gH*-null mutation increased eIF2α in HSV-1-infected cells, whereas gH overexpression in the absence of other HSV-1 proteins reduced eIF2α phosphorylation. These results suggest that GCN1 can regulate eIF2α phosphorylation in HSV-1-infected cells and that the GCN1-binding viral partner gH is necessary and sufficient to prevent the accumulation of phosphorylated eIF2α. Our database of 123 host cell proteins potentially interacting with gH will be useful for future studies to unveil further novel functions of gH and the roles of the cellular proteins in HSV-1-infected cells.

Publications

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International Research Center for Infectious Diseases

Department of Infectious Disease Control Division of Viral Infection

感染制御系　ウイルス学分野

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We focus on understanding how viruses are recognized by NLRP3 inflammasome and how the innate recognition receptor controls antigen-specific adaptive immune responses. We study immune responses to influenza viruses in the lung. Our recent focus also includes the study of how microbiota regulates adaptive immune responses to these pathogens. Our ultimate goal is to utilize the knowledge we gain through these areas of research in the rational design of effective vaccines for the prevention of infectious diseases.

1. Response of host inflammasomes to viral infection.

Chen IY and Ichinohe T

Inflammasomes are multiprotein complexes that induce downstream immune responses to specific pathogens, environmental stimuli, and host cell damage. Components of specific viruses activate different inflammasomes; for example, the influenza A virus M2 protein and encephalomyocarditis virus (EMCV) 2B protein activate the nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain (PYD)-containing 3 (NLRP3) inflammasome, whereas viral double-stranded RNA (dsRNA) activates the retinoic acid inducible gene-I (RIG-I) inflammasome. Once activated in response to viral infection, inflammasomes induce the activation of caspases and the release of mature forms of interleukin-1β (IL-1β) and IL-18. Here we review the association between viral infection and inflammasome activation. Identifying the mechanisms underlying virus-induced inflammasome activation is important if we are to develop novel therapeutic strategies to target viruses.

2. Influenza A virus NS1 protein inhibits NLRP3 inflammasome-mediated IL-1β secretion

Moriyama M, Chen IY, Kawaguchi A, Koshiba T, Nagata K, Takeyama H, Hasegawa H and Ichinohe T

Innate immune sensing of influenza virus via pattern-recognition receptors not only plays a key role in generating type I interferons but also triggers inflammatory responses. We previously demonstrated that influenza A virus M2 protein activates the NLRP3 inflammasome, leading to the secretion of interleukin 1 beta (IL-1β) and IL-18 following the activation of caspase-1. Although the nonstructural protein 1 (NS1) of influenza virus inhibits IL-1β secretion, the precise mechanism by which it achieves this remains to be defined. Here, we demonstrate that the NS1 protein interacts with NLRP3 to suppress the NLRP3 inflammasome activation. J774A.1 macrophages stably expressing the NS1 protein suppressed NLRP3 inflammasome-mediated IL-1β secretion. The NS1 RNA-binding domain (basic residues 38 and 41) and TRIM25-binding domain (acidic residues 96 and 97) are important for suppression of NLRP3 inflammasome-me-

diated IL-1β secretion. These results shed light on a mechanism by which the NS1 protein of influenza virus suppresses NLRP3 inflammasome-mediated IL-1β secretion.

3. The activation of NLRP3 inflammasome induced by SARS-CoV

Chen IY, Moriyama M and Ichinohe T

Severe acute respiratory syndrome associated coronavirus (SARS-CoV), the causative agent of SARS, can induce various cytokines release in the lung tissue of SARS's patients and in SARS-CoV-infected culture cells. The structural protein envelope (E) and the accessory protein 3a of SARS-CoV polymerize to form ion channels with its specificity of ionic conductance, such as sodium and potassium ion, and these viroporin properties have been suggested to be important for virus multiplication cycle. Viroporins, such as influenza virus M2 protein and EMCV 2B protein, can induce the activation of the NLR family, pyrin domain-containing 3 (NLRP3) inflammasome to induce the maturation of pro-caspase-1 and the release of mature interleukin IL-1beta (IL-1β and IL-18 in virus infection. Here, we demonstrated that the expression of E and 3a proteins stimulated secretion of IL-1β and the E protein partially colocalized with NLRP3. The ion channel activity-loss mutant of E protein, EV25F, and 3a protein, 3aCS, reduced IL-1β release induced by E and 3a protein, respectively. These results suggested the E and 3a viroporins are important in SARS-CoV-induced inflammasome activation.

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International Research Center for Infectious Diseases

Department of Infectious Disease Control Division of Bacteriology

感染制御系　細菌学分野

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Bacteria-gut interplay and the host immune response are the most critical issues in determining the fate of bacterial infection and severity of the diseases. Our group has been studying pathogenesis of mucosal infectious bacteria, such as Helicobacter pylori, Shigella, enteropathogenic E. coli, and Streptococcus pyogenes, by defining the molecular and cellular mechanisms of infection and the roles of factors of pathogens and host in infection. The expected output of our research will not only shed further light into understanding bacterial pathogenesis, but also provide new paradigm in microbiology, cell biology, immunity, and pathology, and strengthen the molecular basis in developing diagnostic products, vaccines, animal models, and therapeutic agents.

1. Bacterial infection activates HIF-1α/miR-210 axis to control *IL8* stability.

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MicroRNAs (miRNAs) are small non-coding RNAs functioning as negative regulators of gene expression and associated with various biological phenomena. Here, we show that bacterial infection upregulates miRNA (miR)-210 expression, which is important for Interleukin-8 (IL-8) production *in vitro*. MiRNA expression profiling revealed that miR-210 was the most increased miRNAs by *Helicobacter pylori* infection in AGS gastric epithelial cells. Up-regulation of miR-210 was also observed in cells infected with *Staphylococcus aureus, Shigella flexneri, Salmonella* Typhimurium, *Vibrio parahaemolyticus, Listeria monocytogenes* and *Mycobacterium tuberculosis*. We have defined bacterial infection-induced HIF-1α as an indispensable factor for miR-210 expression, by binding with its binding site in the promoter region of primary miR-210. Additionally, overexpression of miR-210 increased expression levels of *IL8* in ERK phosphorylation-dependent manner. Since inhibition of miR-210 attenuated *IL8* expression levels during bacterial infection in AGS cells, miR-210 may play an important role in host defense mechanism.

2. *Helicobacter pylori* regulates BabA adhesion function by modulating Lewis b expression on the bacterial surface.

Kuroda E, Ishijima I[1], Kiga K, Hirukawa S, Sanada T, Park T[1], Suzuki M[1], Ashida H[2], Ichikawa Y[1], Bóren T[3], Haas R[4], Sasakawa C[1,2,5,6], and Mimuro H: [1]Department of Microbiology and Immunology, [2]Division of Bacterial Infection Biology, Institute of Medical Science, The University of Tokyo, [3]Medical Biochemistry and Biophysics, Umeå universitet, Sweden, [4]Max von Pettenkofer-Institut für Hygiene und Medizinische Mikrobiologie, Department Bakteriologie, Ludwig-Maximilians-Universität, Germany, [5]Nippon Institute for Biological Science, [6]Medical Mycology Research Center, Chiba University.

The persistent colonization of *Helicobacter pylori* (Hp) in the gastric pits of the stomach as well as the translocation of the bacterial cytotoxin-associated gene A (CagA) effector protein via the *cag*-Type IV secretion system (TFSS) into host epithelial cells are major risk factors for gastritis, gastric ulcers, and cancer. The blood group antigen-binding adhesin BabA mediates the adherence of Hp to Lewis b (Le^b) blood group antigens in the human stomach and triggers TFSS-dependent host cell signaling to induce the transcription of genes that enhance inflammation and intestinal metaplasia. Modification of BabA expression during Hp infection is thought to be a mechanism to adapt to changing conditions of inflammation and glycan expression at the epithelial surface. However, how Hp modulates BabA binding activity to the host for the long-term adaptation remains unclear. Here, we show *in vivo* that Hp binding activity to the host cell surface Le^b is diminished during long-term colonization of stomach. Challenge of Mongolian gerbils with Hp for 8 weeks yielded output strains that lost BabA-binding ability to Le^b, but expressed complete BabA protein. We found that the output strains expressed increased levels of Le^b on the bacterial surface polysaccharides. Breakdown of the bacterial surface Le^b structures by periodate oxidation or fucosidase treatment resulted in increased levels of bacterial binding to Le^b. This suggests that Hp in the stomach modulates Le^b expression levels of its own surface to mask BabA adhesion to regulate adhesion function during infection.

3. Identification of sRNAs controlling respiratory chain in *Helicobacter pylori*.

Kiga K, Zhu B, Mimuro H.

Helicobacter pylori (Hp) is a spiral-shaped, microaerophilic bacterium that is mainly found in the stomach. Chronic infection of Hp can lead to variety of gastric disorders, such as chronic gastritis, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma. In microaerophilic condition such as in the stomach, Hp forms an actively dividing helical form, but it turns into coccoid form when it is in a state of hostile conditions such as anaerobic condition in the intestine. Bacterial small RNAs (sRNA) are small (50-250 nucleotide) non-coding RNA molecules produced by bacteria. In 2010, about 60 sRNAs were firstly identified in Hp. Although high conservation of sRNA in Hp between species indicates the importance of the sRNA in Hp, there are few reports about the function of sRNA until now. In this study we identified sRNA important for regulation in response to anaerobic condition. We cultured Hp in either microaerophillic or anaerobic condition and identified one sRNA, which is significantly induced in anaerobic conditions, by comprehensive sRNA expression analysis. When gene expression pattern in the sRNA-deletion mutant was examined by deep sequencing, one gene cluster, which is important for respiratory chain, was increased in the deletion mutant compared with wild type. Together, our results indicate that hypoxia-induced sRNA controls energy metabolism by regulating respiratory chain in Hp.

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International Research Center for Infectious Diseases

Pathogenic Microbes Repository Unit
病原微生物資源室

Associate Professor Hitomi Mimuro, Ph.D.

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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.

Publication

Ogawa, H., Fujikura, D., Ohnuma, M., Ohnishi, N., Hang'ombe, B.M., Mimuro, H., Ezaki, T., Mweene, A.S., and Higashi, H. A novel multiplex PCR discriminates Bacillus anthracis and its genetically related strains from other Bacillus cereus group species. PLoS One, 10: e0122004, 2015.