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 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.

Novel residues in avian influenza virus PB2 protein affect virulence in mammalian hosts.

Fan S, Hatta M, Kim JH, Halfmann P, Imai M, Macken CA, Le MQ, Nguyen T, Neumann G, Kawaoka Y.

Highly pathogenic avian H5N1 influenza viruses have sporadically transmitted to humans causing high mortality. The mechanistic basis for adaptation is still poorly understood, although several residues in viral protein PB2 are known to be important for this event. Here, we demonstrate that three residues, 147T, 339T and 588T, in PB2 play critical roles in the virulence of avian H5N1 influenza viruses in a mammalian host in vitro and in vivo and, together, result in a phenotype comparable to that conferred by the previously known PB2-627K mutation with respect to virus polymerase activity. A virus with the three residues and 627K in PB2, as has been isolated from a lethal human case, is more pathogenic than viruses with only the three residues or 627K in PB2. Importantly, H5N1 viruses bearing the former three PB2 residues have circulated widely in recent years in avian species in nature.

Newly identified minor phosphorylation site threonine-279 of measles virus nucleoprotein is a prerequisite for nucleocapsid formation.

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Measles virus nucleoprotein is the most abundant viral protein and tightly encapsidates viral genomic RNA to support viral transcription and replication. Major phosphorylation sites of nucleoprotein include the serine residues at locations 479 and 510. Minor phosphorylation residues have yet to be identified, and their functions are poorly understood. In our present study, we identified nine putative phosphorylation sites by mass spectrometry and demonstrated that threonine residue 279 (T279) is functionally significant. Minigenome expression assays revealed that a mutation at the T279 site caused a loss of activity. Limited proteolysis and electron microscopy suggested that a T279A mutant lacked the ability to encapsidate viral RNA but was not denatured. Furthermore, dephosphorylation of the T279 site by alkaline phosphatase treatment

caused deficiencies in nucleocapsid formation. Taken together, these results indicate that phosphorylation at T279 is a prerequisite for successful nucleocapsid formation.

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International Research Center for Infectious Diseases

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高病原性感染症系　微細構造ウイルス学分野

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Virus infections are accompanied by numerous morphological changes in viral and cellular components. Our group has been investigating the replication mechanism of influenza and Ebola viruses from the ultrastructural point of view, by using different microscopic analytical methods such as electron microscopy and high-speed atomic force microscopy. Visualization and characterization of the virus life cycle at the nano-mesoscopic level give us unique knowledge and novel paradigms, which will advance our understanding of molecular basis for the replication mechanism.

Disease Severity Is Associated with Differential Gene Expression at the Early and Late Phases of Infection in Nonhuman Primates Infected with Different H5N1 Highly Pathogenic Avian Influenza Viruses.

Muramoto Y, Shoemaker JE, Le MQ, Itoh Y, Tamura D, Sakai-Tagawa Y, Imai H, Uraki R, Takano R, Kawakami E, Ito M, Okamoto K, Ishigaki H, Mimuro H, Sasakawa C, Matsuoka Y, Noda T, Fukuyama S, Ogasawara K, Kitano H, Kawaoka Y.

Occasional transmission of highly pathogenic avian H5N1 influenza viruses to humans causes severe pneumonia with high mortality. To better understand the mechanisms via which H5N1 viruses induce severe disease in humans, we infected cynomolgus macaques with six different H5N1 strains isolated from human patients and compared their pathogenicity and the global host responses to the virus infection. Although all H5N1 viruses replicated in the respiratory tract, there was substantial heterogeneity in their replicative ability and in the disease severity induced, which ranged from asymptomatic to fatal. A comparison of global gene expression between severe and mild disease cases indicated that interferon-induced upregulation of genes related to innate immunity, apoptosis, and antigen processing/presentation in the early phase of infection was limited in severe disease cases, although interferon expression was upregulated in both severe and mild cases. Furthermore, coexpression analysis of microarray data, which reveals the dynamics of host responses during the infection, demonstrated that the limited expression of these genes early in infection led to a failure to suppress virus replication and to the hyperinduction of genes related to immunity, inflammation, coagulation, and homeostasis in the late phase of infection, resulting in a more severe disease. Our data suggest that the attenuated interferon-induced activation of innate immunity, apoptosis, and antigen presentation in the early phase of H5N1 virus infection leads to subsequent severe disease outcome.

Publications

Muramoto Y, Shoemaker JE, Le MQ, Itoh Y, Tamura D, Sakai-Tagawa Y, Imai H, Uraki R, Takano R, Kawakami E, Ito M, Okamoto K, Ishigaki H, Mimuro H, Sasakawa C, Matsuoka Y, Noda T, Fukuyama S, Ogasawara K, Kitano H, Kawaoka Y. Disease Severity Is Associated with Differential Gene Expression at the Early and Late Phases of Infection in Nonhuman Primates Infected with Different H5N1 Highly Pathogenic Avian Influenza Viruses. J Virol 88: 8981-8997.

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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. Epigenetic Repression of Interleukin 2 Expression in Senescent CD4+ T Cells During Chronic HIV Type 1 Infection.

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The molecular mechanisms for IL2 gene-specific dysregulation during chronic human immunodeficiency virus type 1 (HIV-1) infection are unknown. Here, we investigated the role of DNA methylation in suppressing interleukin 2 (IL-2) expression in memory CD4(+) T cells during chronic HIV-1 infection. We observed that CpG sites in the IL2 promoter of CD4(+) T cells were fully methylated in naive CD4(+) T cells and significantly demethylated in the memory populations. Interestingly, we found that the memory cells that had a terminally differentiated phenotype and expressed CD57 had increased IL2 promoter methylation relative to less differentiated memory cells in healthy individuals. Importantly, early effector memory subsets from HIV-1-infected subjects expressed high levels of CD57 and were highly methylated at the IL2 locus. Furthermore, the increased CD57 expression on memory CD4(+) T cells was inversely correlated with IL-2 production. These data suggest that DNA methylation at the IL2 locus in CD4(+) T cells is coupled to immunosenescence and plays a critical role in the broad dysfunction that occurs in polyclonal T cells during HIV-1 infection.

2. Development and Customization of a Color-Coded Microbeads-Based Assay for Drug Resistance in HIV-1 Reverse Transcriptase

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Drug resistance (DR) of HIV-1 can be examined genotypically or phenotypically. Although sequencing is the gold standard of the genotypic resistance testing (GRT), high-throughput GRT targeted to the codons responsible for DR may be more appropriate for epidemiological studies and public health research. We used a Japanese database to design and synthesize sequence-specific oligonucleotide probes (SSOP) for the detection of wild-type sequences and 6 DR mutations in the clade B HIV-1 reverse transcriptase region. We coupled SSOP to microbeads of the Luminex 100 xMAP system and developed a GRT based on the polymerase chain reaction (PCR)-SSOP-Luminex method. Sixteen oligoprobes for discriminating DR mutations from wild-type sequences at 6 loci were designed and synthesized, and their sensitivity and specificity were confirmed using isogenic plasmids. The PCR-SSOP-Luminex DR assay was then compared to direct sequencing using 74 plasma specimens from treatment-naïve patients or those on failing treatment. In the majority of specimens, the results of the PCR-SSOP-Luminex DR assay were concordant with sequencing results: 62/74 (83.8%) for M41, 43/74 (58.1%) for K65, 70/74 (94.6%) for K70, 55/73 (75.3%) for K103, 63/73 (86.3%) for M184 and 68/73 (93.2%) for T215. There were a number of specimens without any positive signals, especially for K65. The nucleotide position of A2723G, A2747G and C2750T were frequent polymorphisms for the wild-type amino acids K65, K66 and D67, respectively, and 14 specimens had the D67N mutation encoded by G2748A. We synthesized 14 additional oligoprobes for K65, and the sensitivity for K65 loci improved from 43/74 (58.1%) to 68/74 (91.9%). We developed a rapid high-throughput assay for clade B HIV-1 DR mutations, which could be customized by synthesizing oligoprobes suitable for the circulating viruses. The assay could be a useful tool especially for public health research in both resource-rich and resource-limited settings.

3. Phosphorylation of Herpes Simplex Virus 1 dUTPase Up-regulated Viral dUTPase Activity to Compensate for Low Cellular dUTPase Activity for Efficient Viral Replication

Akihisa Kato, Yoshitaka Hirohata, Jun Arii and Yasushi Kawaguchi:

We recently reported that herpes simplex virus 1 (HSV-1) protein kinase Us3 phosphorylated viral dUTPase (vdUTPase) at serine 187 (Ser-187) to up-regulate its enzymatic activity, which promoted HSV-1 replication in human neuroblastoma SK-N-SH cells but not in human carcinoma HEp-2 cells (J. Virol. 88: 655-666, 2014). In the present study, we showed that endogenous cellular dUTPase activity in SK-N-SH cells was significantly lower than in HEp-2 cells, and that overexpression of cellular dUTPase in SK-N-SH cells increased replication of an HSV-1 mutant with an alanine substitution for Ser-187 (S187A) in vdUTPase to the wild-type level. In addition, we showed that knock-down of cellular dUTPase in HEp-2 cells significantly reduced replication of the mutant vdUTPase (S187A) virus, but not of wild-type HSV-1. Furthermore, the replacement of Ser-187 in vdUTPase with aspartic acid, which is mimics constitutive phosphorylation, and overexpression of cellular dUTPase restored viral replication to the wild-type level in cellular dUTPase knock-down HEp-2 cells. These results indicated that sufficient dUTPase activity was required for efficient HSV-1 replication and supported the hypothesis that Us3 phosphorylation of vdUTPase Ser-187 up-regulated vdUTPase activity in host cells with low cellular dUTPase activity to produce efficient viral replication.

It has long been assumed that dUTPase activity is important for replication of viruses encoding a dUTPase and that the viral dUTPase activity was needed if host cell dUTPase activity was not sufficient for efficient viral replication. In the present study, we showed that the S187A mutation in HSV-1 vdUTPase, which impaired its enzymatic activity, reduced viral replication in SK-N-SH cells, which have low endogenous cellular dUTPase activity, and overexpression of cellular dUTPase restored viral replication to its wild-type level. We also showed that knock-down of cellular dUTPase in HEp-2 cells, which have higher dUTPase activity than SK-N-SH cells, reduced replication of HSV-1 with the vdUTPase mutation but had no effect on wild-type virus replication. This is the first report,

to our knowledge, directly showing that dUTPase activity was critical for efficient viral replication and that vdUTPase compensated for low host cell dUTPase activity to produce efficient viral replication.

4. The UL12 Protein of Herpes Simplex Virus 1 Is Regulated by Tyrosine Phosphorylation

Hikaru Fujii, Akihisa Kato, Michio Mugitani, Yukie Kashima, Masaaki Oyama[1], Hiroko Kozuka-Hata[1], Jun Arii and Yasushi Kawaguchi: [1]Medical Proteomics Laboratory, The Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo 108-8639, Japan

The herpes simplex virus 1 UL12 protein (pUL12) is a nuclease that is critical for viral replication in vitro and neurovirulence in vivo. In this study, mass spectrometric analysis of pUL12 and phosphate-affinity SDS-polyacrylamide gel electrophoresis analysis identified tyrosine at pUL12 residue 371 (Tyr-371) as a pUL12 phosphorylation site: Tyr-371 is conserved in pUL12 homologs in herpesviruses in all *Herpesviridae* subfamilies. Replacement of Tyr-371 with phenylalanine (Y371F) in pUL12 (i) abolished its exonuclease activity in HSV-1-infected Vero, HEL and A549 cells; (ii) reduced viral replication and cell-cell spread, and expression of pUL12 in infected cells in a cell type-dependent manner; (iii) led to aberrant subcellular localization of pUL12 in infected cells in a cell type-dependent manner; and (iv) reduced HSV-1 neurovirulence in mice. The effects of the pUL12 Y371F mutation in cell cultures and mice were similar to those of a nuclease-dead double mutation in pUL12, although the Y371F mutation reduced viral replication several-fold more than the nuclease-dead double mutation in a cell type- and multiplicity of infection-dependent manner. Replacement of Tyr-371 with glutamic acid, which mimics constitutive phosphorylation, restored the wild-type phenotype in cell cultures and mice. These results suggested that phosphorylation of pUL12 Tyr-371 was essential for pUL12 to express its nuclease activity in HSV-1-infected cells, and that this phosphorylation promoted viral replication and cell-cell spread in cell cultures and nerurovirulence in mice mainly by up-regulating pUL12 nuclease activity and, in part, by regulating subcellular localization and expression of pUL12 in HSV-1-infected cells.

Herpesviruses encode a considerable number of enzymes for their replication. Like cellular enzymes, the viral enzymes need to be properly regulated in infected cells. Although the functional aspects of herpesvirus enzymes have gradually been clarified, there is a lack of information on how most of these enzymes are regulated in infected cells. In the present study, we have reported that the enzymatic activity of herpes simplex virus 1 alkaline nuclease pUL12 was regulated by phosphorylation of pUL12 Tyr-371 in infected cells, and that this phosphorylation promoted viral replication and cell-cell spread in cell cultures and nerurovirulence in mice, mainly by up-regulating pUL12 nuclease activity. Interestingly, pUL12 and tyrosine at pUL12 residue 371 appeared to be conserved in all herpesviruses in the family *Herpesviridae*, raising the possibility that the herpesvirus pUL12 homologs may also be regulated by phosphorylation of the conserved tyrosine residue.

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International Research Center for Infectious Diseases

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感染制御系　ウイルス学分野

Associate Professor　Takeshi Ichinohe, Ph.D.

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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. Inflammasomes in antiviral immunity: clues for influenza vaccine development.

Yamazaki T and Ichinohe T.

Inflammasomes are cytosolic multiprotein complexes that sense microbial motifs or cellular stress and stimulate caspase-1-dependent cytokine secretion and cell death. Recently, it has become increasingly evident that both DNA and RNA viruses activate inflammasomes, which control innate and adaptive immune responses against viral infections. In addition, recent studies suggest that certain microbiota induce inflammasomes-dependent adaptive immunity against influenza virus infections. Here, we review recent advances in research into the role of inflammasomes in antiviral immunity.

2. Influenza A virus protein PB1-F2 translocates into mitochondria via Tom40 channels and impairs innate immunity.

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Mitochondria contribute to cellular innate immunity against RNA viruses. Mitochondrial-mediated innate immunity is regulated by signalling molecules that are recruited to the mitochondrial membrane, and depends on the mitochondrial inner membrane potential ($\Delta\psi m$). Here we examine the physiological relevance of $\Delta\psi m$ and the mitochondrial-associating influenza A viral protein PB1-F2 in innate immunity. When expressed in host cells, PB 1-F2 completely translocates into the mitochondrial inner membrane space via Tom40 channels, and its accumulation accelerates mitochondrial fragmentation due to reduced $\Delta\psi m$. By contrast, PB1-F2 variants lacking a C-terminal polypeptide, which is frequently found in low pathogenic subtypes, do not affect mitochondrial function. PB1-F2-mediated attenuation of $\Delta\psi m$ suppresses the RIG-I signalling pathway and activation of NLRP3 inflammasomes. PB1-F2 translocation into mitochondria strongly correlates with impaired cellular innate immunity, making this translocation event a potential therapeutic target.

3. 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.

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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. Epigenetic silencing of miR-210 increases the proliferation of gastric epithelium during chronic *Helicobacter pylori* infection.

Kiga K, Mimuro H, Suzuki M[1], Shinozaki-Ushiku A[2], Kobayashi T, Sanada T, Kim M[3], Ogawa M[3], Iwasaki YW[4], Kayo H[4], Fukuda-Yuzawa Y[4], Yashiro M[5], Fukayama M[2], Fukao T[4], Sasakawa C[1,3,5,6]: [1]Department of Microbiology and Immunology, Institute of Medical Science, The University of Tokyo, [2]Department of Pathology, Graduate School of Medicine, The University of Tokyo, [3]Division of Bacterial Infection Biology, Institute of Medical Science, The University of Tokyo, [4]Max-Planck-Institute of Immunobiology and Epigenetics, [5]Department of Surgical Oncology, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan. [5]Nippon Institute for Biological Science, [6]Medical Mycology Research Center, Chiba University.

Persistent colonization of the gastric mucosa by *Helicobacter pylori* (Hp) elicits chronic inflammation and aberrant epithelial cell proliferation, which increases the risk of gastric cancer. Here we examine the ability of microRNAs to modulate gastric cell proliferation in response to persistent Hp infection and find that epigenetic silencing of miR-210 plays a key role in gastric disease progression. Importantly, DNA methylation of the miR-210 gene is increased in Hp-positive human gastric biopsies as compared with Hp-negative controls. Moreover, silencing of miR-210 in gastric epithelial cells promotes proliferation. We identify *STMN1* and *DIMT1* as miR-210 target genes and demonstrate that inhibition of miR-210 expression augments cell proliferation by activating *STMN1* and *DIMT1*. Together, our results highlight inflammation-induced epigenetic silencing of miR-210 as a mechanism of induction of chronic gastric diseases, including cancer, during Hp infection.

2. *Shigella* IpaH7.8 E3 ubiquitin ligase targets glomulin and activates inflammasomes to demolish macrophages.

Suzuki S[1,2,3], Mimuro H, Kim M[2], Ogawa M[1], Ashida H[2], Toyotome T[1], Franchi L[3], Suzuki M[1], Sanada T, Suzuki T[4], Tsutsui H[5], Núñez G[3], Sasakawa C[1,2,6,7]: [1]Department of Microbiology and Immunology, Institute of Medical Science, The University of Tokyo, [2]Division of Bacterial Infection Biology, Institute of Medical Science, The University of Tokyo, [3]Department of Pathology and Comprehensive Cancer Center, University of Michigan Medical School, [4]Department of Molecular Bacteriology and Immunology, Graduate School of Medicine, University of the Ryukyus, [5]Department of Microbiology, Hyogo College of Medicine, [6]Nippon Institute for Biological Science, [7]Medical Mycology Research Center, Chiba University.

When nucleotide-binding oligomerization domain-like receptors (NLRs) sense cytosolic-invading bacteria, they induce the formation of inflammasomes and initiate an innate immune response. In quiescent cells, inflammasome activity is tightly regulated to prevent excess inflammation and cell death. Many bacterial pathogens provoke inflammasome activity and induce inflammatory responses, including cell death, by delivering type III secreted effectors, the rod component flagellin, and toxins. Recent studies indicated that *Shigella* deploy multiple mechanisms to stimulate NLR inflammasomes through type III secretion during infection. Here, we show that *Shigella* induces rapid macrophage cell death by delivering the invasion plasmid antigen H7.8 (IpaH7.8) enzyme 3 (E3) ubiquitin ligase effector via the type III secretion system, thereby activating the NLR family pyrin domain-containing 3 (NLRP3) and NLR family CARD domain-containing 4 (NLRC4) inflammasomes and caspase-1 and leading to macrophage cell death in an IpaH7.8 E3 ligase-dependent manner. Mice infected with *Shigella* possessing IpaH7.8, but not with *Shigella* possessing an IpaH7.8 E3 ligase-null mutant, exhibited enhanced bacterial multiplication. We defined glomulin/flagellar-associated protein 68 (GLMN) as an IpaH7.8 target involved in IpaH7.8 E3 ligase-dependent inflammasome activation. This protein originally was identified through its association with glomuvenous malformations and more recently was described as a member of a Cullin ring ligase inhibitor. Modifying GLMN levels through overexpression or knockdown led to reduced or augmented inflammasome activation, respectively. Macrophages stimulated with lipopolysaccharide/ATP induced GLMN puncta that localized with the active form of caspase-1. Macrophages from GLMN(+/−) mice were more responsive to inflammasome activation than those from GLMN(+/+) mice. Together, these results highlight a unique bacterial adaptation that hijacks inflammasome activation via interactions between IpaH7.8 and GLMN.

3. The immune receptor NOD1 and kinase RIP2 interact with bacterial peptidoglycan on early endosomes to promote autophagy and inflammatory signaling.

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The intracellular innate immune receptor NOD1 detects Gram-negative bacterial peptidoglycan (PG) to induce autophagy and inflammatory responses in host cells. To date, the intracellular compartment in which PG is detected by NOD1 and whether NOD1 directly interacts with PG are two questions that remain to be resolved. To address this, we used outer membrane vesicles (OMVs) from pathogenic bacteria as a physiological mechanism to deliver PG into the host cell cytosol. We report that OMVs induced autophagosome formation and inflammatory IL-8 responses in epithelial cells in a NOD1- and RIP2-dependent manner. PG contained within OMVs colocalized with both NOD1 and RIP 2 in EEA1-positive early endosomes. Further, we provide evidence for direct interactions between NOD1 and PG. Collectively, these findings demonstrate that NOD1 detects PG within early endosomes, thereby promoting RIP2-dependent autophagy and inflammatory signaling in response to bacterial infection.

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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 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.