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.

Gene end-like sequences within the 3' non-coding region of the Nipah virus genome attenuate viral gene transcription.

Sugai, A., Sato, H., Yoneda, M. and Kai, C.

The regulation of transcription during Nipah virus (NiV) replication is poorly understood. Using a bicistronic minigenome system, we investigated the involvement of non-coding regions (NCRs) in the transcriptional re-initiation efficiency of NiV RNA polymerase. Reporter assays revealed that attenuation of NiV gene expression was not constant at each gene junction, and that the attenuating property was controlled by the 3' NCR. However, this regulation was independent of the gene-end, gene-start and intergenic regions. Northern blot analysis indicated that regulation of viral gene expression by the phosphoprotein (P) and large protein (L) 3' NCRs occurred at the transcription level. We identified uridine-rich tracts within the L 3' NCR that are similar to gene-end signals. These gene-end-like sequences were recognized as weak transcription termination signals by the viral RNA polymerase, thereby reducing downstream gene transcription. Thus, we suggest that NiV has a unique mechanism of transcriptional regulation.

Emergence of oseltamivir-resistant H7N9 influenza viruses in immunosuppressed cynomolgus macaques

Kiso M, Iwatsuki-Horimoto K, Yamayoshi S, Uraki R, Ito M, Nakajima N[1], Yamada S, Imai M, Kawakami E[2], Tomita Y, Fukuyama S[3], Itoh Y[4], Ogasawara K[4], Lopes TJS[5], Watanabe T[3], Moncla LH[5,6], Hasegawa H[1], Friedrich TC[5,6], Neumann G[5], Kawaoka Y.: [1]Department of Pathology, National Institute of Infectious Diseases, Tokyo, [2]Laboratory for Disease Systems Modeling, RIKEN Center for Integrative Medical Sciences, Kanagawa, [3]ERATO Infection-Induced Host Responses Project, Japan Science and Technology Agency, Saitama, [4]Department of Pathology, Shiga University of Medical Science, Japan, [5]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, [6]Wisconsin National Primate Research Center, Madison

Antiviral compounds (eg, the neuraminidase inhibitor oseltamivir) are invaluable for the treatment of individuals infected with influenza A viruses of the H7N9 subtype (A[H7N9]), which have infected and killed hundreds of persons. However, oseltamivir treatment often leads to the emergence of resistant viruses in immunocompromised indi-

viduals. To better understand the emergence and properties of oseltamivir-resistant A(H7N9) viruses in immunosuppressed individuals, we infected immunosuppressed cynomolgus macaques with an A(H7N9) virus and treated them with oseltamivir. Disease severity and mortality were higher in immunosuppressed than in immunocompetent animals. Oseltamivir treatment at 2 different doses reduced A(H7N9) viral titers in infected animals, but even high-dose oseltamivir did not block viral replication sufficiently to suppress the emergence of resistant variants. Some resistant variants were not appreciably attenuated in cultured cells, but an oseltamivir-resistant A(H7N9) virus did not transmit among ferrets. These findings are useful for the control of A(H7N9) virus infections in clinical settings.

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Our 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 Molecular Virology, Department of Microbiology and Immunology.

1. Herpes Simplex Virus 1 UL34 Protein Regulates the Global Architecture of the Endoplasmic Reticulum in Infected Cells

Fumio Maeda, Jun Arii, Yoshitaka Hirohata, Yuhei Maruzuru, Naoto Koyanagi, Akihisa Kato, Yasushi Kawaguchi

Upon herpes simplex virus 1 (HSV-1) infection, the CD98 heavy chain (CD98hc) is redistributed around the nuclear membrane (NM), where it promotes vi-ral de-envelopment during the nuclear egress of nucleocapsids. In this study, we attempted to identify the factor(s) involved in CD98hc accumulation and demon-strated the following: (i) the null mutation of HSV-1 UL34 caused specific dispersion throughout the cytoplasm of CD98hc and the HSV-1 de-envelopment regulators, gly-coproteins B and H (gB and gH); (ii) as observed with CD98hc, gB, and gH, wild-type HSV-1 infection caused redistribution of the endoplasmic reticulum (ER) markers cal-nexin and ERp57 around the NM, whereas the UL34-null mutation caused cytoplas-mic dispersion of these markers; (iii) the ER markers colocalized efficiently with CD98hc, gB, and gH in the presence and absence of UL34 in HSV-1-infected cells; (iv) at the ultrastructural level, wild-type HSV-1 infection caused ER compression around the NM, whereas the UL34-null mutation caused cytoplasmic dispersion of the ER; and (v) the UL34-null mutation significantly decreased the colocalization effi-ciency of lamin protein markers of the NM with CD98hc and gB. Collectively, these results indicate that HSV-1 infection causes redistribution of the ER around the NM, with resulting accumulation of ER-associated CD98hc, gB, and gH around the NM and that UL34 is required for ER redistribution, as well as for efficient recruitment to the NM of the ER-associated de-envelopment factors. Our study suggests that HSV-1 induces remodeling of the global ER architecture for recruitment of regulators medi-ating viral nuclear egress to the NM.

2. Herpes Simplex Virus 1 Small Capsomere-Interacting Protein VP26 Regulates Nucleocapsid Maturation.

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VP26 is a herpes simplex virus 1 (HSV-1) small capsomere-interacting protein. In this study, we investigated the function of VP26 in HSV-1-infected cells with the following results. (i) The VP26 null mutation significantly impaired incorpo-ration of minor capsid protein UL25 into nucleocapsids (type C capsids) in the nu-cleus. (ii) The VP26 mutation caused improper localization of UL25 in discrete punc-tate domains containing multiple capsid proteins (e.g., the VP5 major capsid protein) in the nucleus; these domains corresponded to capsid aggregates. (iii) The VP26 mu-tation significantly impaired packaging of replicated viral DNA genomes into capsids but had no effect on viral DNA concatemer cleavage. (iv) The VP26 mutation re-duced the frequency of type C capsids, which contain viral DNA but not scaffolding proteins, and produced an accumulation of type A capsids, which lack both viral DNA and scaffold proteins, and had no effect on accumulation of type B capsids, which lack viral DNA but retain cleaved scaffold proteins. Collectively, these results indicated that VP26 was required for efficient viral DNA packaging and proper local-ization of nuclear capsids. The phenotype of the VP26 null mutation was similar to that reported previously of the UL25 null mutation and of UL25 mutations that pre-clude UL25 binding to capsids. Thus, VP26 appeared to regulate nucleocapsid matu-ration by promoting incorporation of UL25 into capsids, which is likely to be re-quired for proper capsid nuclear localization.

3. Herpes simplex virus-1 evasion of CD8$^+$ T cell accumulation contributes to viral encephalitis

Naoto Koyanagi, Takahiko Imai, Keiko Shindo, Ayuko Sato[1], Wataru Fujii[2], Takeshi Ichinohe[3], Naoki Takemura[4,5], Shigeru Kakuta[6], Satoshi Uematsu[4,5], Hiroshi Kiyono[1,4,7], Yuhei Maruzuru, Jun Arii, Akihisa Kato, and Yasushi Kawaguchi: [1]Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan [2]Department of Animal Resource Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan [3]Department of Infectious Disease Control, International Research Center for Infectious Diseases, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan [4]International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan [5]Department of Mucosal Immunology, School of Medicine, Chiba University, Chiba, Japan [6]Department of Biomedical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan [7]Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Tokyo, Japan

Herpes simplex virus-1 (HSV-1) is the most common cause of sporadic viral encephalitis, which can be lethal or result in severe neurological defects even with antiviral therapy. While HSV-1 causes encephalitis in spite of HSV-1-specific humoral and cellular immunity, the mechanism by which HSV-1 evades the immune system in the central nervous system (CNS) remains unknown. Here we describe a strategy by which HSV-1 avoids immune targeting in the CNS. The HSV-1 UL13 kinase promotes evasion of HSV-1-specific CD8$^+$ T cell accumulation in infection sites by downregulating expression of the CD8$^+$ T cell attractant chemokine CXCL9 in the CNS of infected mice, leading to increased HSV-1 mortality due to encephalitis. Direct injection of CXCL9 into the CNS infection site enhanced HSV-1-specific CD8$^+$ T cell accumulation, leading to marked improvements in the survival of infected mice. This previously uncharacterized strategy for HSV-1 evasion of CD8$^+$ T cell accumulation in the CNS has important implications for understanding the pathogenesis and clinical treatment of HSV-1 encephalitis.

Publications

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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. Consecutive inoculations of influenza virus vaccine and poly (I:C) protects mice against homologous and heterologous virus challenge

Moriyama M, Chino S and Ichinohe T

Mucosal immunity induced through natural infection by influenza virus has potent cross-protective activity, compared to subcutaneous vaccination-induced systemic immunity. Compared to natural infection with influenza virus, however, a single intranasal vaccination with an inactivated influenza virus vaccine and poly (I:C) is not sufficient to induce primary immune response in naïve animals. The reasons for this moderate effect are not fully understood. Here, we demonstrated that intranasal vaccination with formalin-inactivated influenza virus vaccine and poly (I:C) for five consecutive days elicits high levels of virus-specific nasal IgA and serum IgG responses, while vaccination without poly (I:C) induced little response. Mice immunized with influenza virus vaccine and poly (I:C) for five consecutive days sustained high levels of virus-specific IgA in nasal wash and IgG in serum until at least 6months after vaccination. Furthermore, intranasal vaccination with influenza virus vaccine and poly (I:C) protected mice against homologous and heterologous influenza virus challenge. These results suggest that consecutive inoculations of influenza virus vaccine and poly (I:C) is an alternative method to induce primary immune responses in naïve subjects.

2. Induction of lung $CD8^+$ T cell responses by consecutive inoculations of a poly (I:C) influenza vaccine.

Moriyama M, Takeyama H, Hasegawa H and Ichinohe T

The cytotoxic T lymphocyte (CTL) response plays a key role in host recovery from influenza virus infection and in subsequent immunity. Compared to natural infection with influenza virus, however, intranasal vaccination with adjuvant-combined inactivated vaccine elicits only moderate CTL responses. Here we demonstrate that 5 days of consecutive, intranasal vaccination with a combination of inactivated influenza vaccine and poly (I:C) elicits a strong CTL response in the lung. Antigen-captured respiratory DCs did efficiently migrate from the

lung to the mediastinal lymph node (mLN) after the 5 day series of inoculations with vaccine and poly (I:C). Importantly, formalin-inactivated whole virus vaccine and poly (I:C) adjuvant have synergic effects on consecutive vaccinations to elicit a strong CTL response in the lung. Although the CTL response was less effective against heterologous influenza virus, we show for the first time that intranasal administration of inactivated influenza virus vaccine and poly (I:C) for 5 consecutive days can elicit high levels of influenza virus-specific $CD8^+$ T cells in the lung.

3. Evasion of antiviral immunity by SFTSV.

Moriyama M, Igarashi M, Koshiba T, Takada A and Ichinohe T

Recognition of viruses by host innate immune systems plays a critical role not only in providing resistance to viral infection, but also in initiation of antigen-specific adaptive immune responses against viruses. Severe fever with thrombocytopenia syndrome (SFTS) is a newly emerging infectious disease caused by the SFTS phlebovirus (SFTSV), a highly pathogenic tick-borne phlebovirus. The 294 amino acid nonstructural protein (NSs) of SFTSV associates with TANK-binding kinase 1 (TBK1), a key regulator of host innate antiviral immunity, to inhibit interferon beta (IFN-β) production and enhance viral replication. Herein, we demonstrate that two conserved amino acids in the NSs of SFTSV and heartland virus, another tick-borne phlebovirus, are essential for association with TBK1 and suppression of IFN-β production. Our results provide important insight into the molecular mechanisms by which SFTSV NSs helps to counteract host antiviral strategies.

Publications

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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. Characterization of morphological conversions of *Helicobacter pylori* under anaerobic conditions

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Helicobacter pylori (*H. pylori*) is a Gram-negative microaerophilic bacterial pathogen which colonizes the stomach of more than half the human population, and is linked to chronic gastritis, peptic ulcers, and gastric cancer. Spiral-shaped *H. pylori* undergo morphologic conversion to the viable but not culturable (VBNC) coccoid form as they transit from the microaerobic stomach into the anaerobic intestinal tract. However, little is known about the morphological and pathogenic characteristics of *H. pylori* under prolonged anaerobic conditions. In this study, we used scanning electron microscopy to document the anaerobiosis-induced morphological change of *H. pylori*, from helical to coccoid to the newly defined fragmented form. Western blot analysis indicated that all three forms expressed certain pathogenic proteins, including the bacterial cytotoxin-associated gene A (CagA), components of the *cag*-Type IV secretion system (TFSS), the blood group antigen-binding adhesin BabA, and UreA (apoenzyme of urease) at nearly equivalent levels. Similar urease activities were also detected in each form of *H. pylori*. However, in contrast to the helical form, the anaerobiosis-induced coccoid and fragmented forms of *H. pylori* were abrogated for bacterial motility and TFSS activity. Notably, we demonstrated that some of the anaerobiosis-induced fragmented state cells could be converted to proliferation-competent helical bacteria *in vitro*. These results indicate that prolonged exposure to the anaerobic intestine might not eliminate the potential for *H. pylori* to recover into the helical pa-

thogenic state.

2. Novel *H. pylori* prophylactic vaccine using anaerobiosis-induced *H. pylori*

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Helicobacter pylori (*H. pylori*) is a Gram-negative, microaerophilic bacterium adapted to survive in the stomach of humans where it can cause peptic ulcers and gastric cancer. Even though effective antibiotics treatment exists, there is a consensus that vaccines are necessary to limit the severity of this infection. While several studies were reported that recombinant antigens of bacterial factors conferred protection against infectious challenge with *H. pylori* in experimental animal models, the protective effects were limited because of antigen variation of *H. pylori*. We previously reported that anaerobiosis-induced *H. pylori* entered Peyer's patches in mice intestine and induced *H. pylori* antigen-specific CD4 +T cells, which is indispensable for *H. pylori*-induced gastritis. Notably, bacteria in Peyer's patches were phagocytosed by dendritic cells. These phenomena suggested an idea that the *H. pylori* antigen-specific immune response induced by anaerobiosis-induced *H. pylori* via intestine might increase protective immune response against *H. pylori* infection. In this study, we used Mongolian gerbil as human pathology model of *H. pylori*, which is more similar than mouse model, and aimed to evaluate protective effects of oral administration of anaerobiosis-induced *H. pylori* on *H. pylori* infection. We found that administration of inactivated anaerobiosis-induced *H. pylori*, but not microaerobiosis-induced *H. pylori*, with cholera toxin, prevented *H. pylori* colonization in the stomach and reduced the levels of gastric inflammation. Although anaerobiosis-induced *H. pylori* entered Peyer's patches in Mongolian gerbil at almost the same levels of microaerobiosis-induced *H. pylori*, anaerobiosis-induced bacteria were phagocytosed by dendritic cells more efficiently than microaerobiosis-induced bacteria. These data indicated that effective phagocytosis of anaerobiosis-induced *H. pylori* by dendritic cells is indispensable for prophylactic vaccine effect of anaerobiosis-induced *H. pylori*.

3. Analysis of persistent infection mechanism of *Helicobacter pylori* using hypermutator strain.

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Helicobacter pylori (*H. pylori*) establish persistent infection in the human stomach and induce stomach diseases such as gastritis and gastric cancer. In most case, the patients infect with *H. pylori* in childhood and develop stomach diseases in late middle age. During long-term infection in the stomach, *H. pylori* can adapt to environmental change through introducing beneficial own genome mutations. However, there is no report on comprehensive genetic mutations of *H. pylori* acquired during long-term infection, since it is difficult to reproduce the events during long-term infection in a short period experimentally. In this study, we established hypermutator of *H. pylori*, to analyze mutation in a shorter period. We have selected 7 factors concerning gene restoration and made *H. pylori* mutants deleted with the genes. By comparing mutation rate in *rpoB* gene by analyzing acquired tolerance against Rifampicin, we selected hypermutator of *H. pylori* having the highest mutation acquisition capability. We isolated output strains yielded by the challenge of Mongolian gerbils with the hypermutator for 8 weeks, analyzed the whole genome sequences by next-generation sequencing, and identified several genetic mutations differed from those in wild-type. The effects of the newly-identified genes on the long-term infection of *H. pylori* are under investigation.

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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. Besides, in 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. Also, 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-resistant 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 research 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 have 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 bacteria, 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 essential 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) A comprehensive collection of genome sequenc-

ing 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.