International Research Center for Infectious Diseases

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

Professor	Chieko Kai, D.V.M., Ph.D.	教授（兼）	農学博士	甲斐 知恵子
Professor	Yoshihiro Kawaoka, D.V.M., Ph.D.	教授（兼）	獣医学博士	河岡 義裕
Project Research Associate	Takeshi Kuraishi, Ph. D.	特任助教	農学博士	倉石 武
Project Research Associate	Takeshi Noda, D.V.M., Ph.D.	特任助教	獣医学博士	野田 岳志
Project Research Associate	Makoto Ozawa, D.V.M., Ph.D.	特任助教	医学博士	小澤 真

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. Ebolavirus is internalized into host cells via macropinocytosis in a viral glycoprotein-dependent manner.

Nanbo A, Imai M, Watanabe S, Noda T, Takahashi K, Neumann G, Halfmann P, Kawaoka Y.

Ebolavirus (EBOV) is an enveloped, single-stranded, negative-sense RNA virus that causes severe hemorrhagic fever with mortality rates of up to 90% in humans and nonhuman primates. Previous studies suggest roles for clathrin- or caveolae-mediated endocytosis in EBOV entry; however, ebolavirus virions are long, filamentous particles that are larger than the plasma membrane invaginations that characterize clathrin- or caveolae-mediated endocytosis. The mechanism of EBOV entry remains, therefore, poorly understood. To better understand Ebolavirus entry, we carried out internalization studies with fluorescently labeled, biologically contained Ebolavirus and Ebolavirus-like particles (Ebola VLPs), both of which resemble authentic Ebolavirus in their morphology. We examined the mechanism of Ebolavirus internalization by real-time analysis of these fluorescently labeled Ebolavirus particles and found that their internalization was independent of clathrin- or caveolae-mediated endocytosis, but that they co-localized with sorting nexin (SNX) 5, a marker of macropinocytosis-specific endosomes (macropinosomes). Moreover, the internalization of Ebolavirus virions accelerated the uptake of a macropinocytosis-specific cargo, was associated with plasma membrane ruffling, and was dependent on cellular GTPases and kinases involved in macropinocytosis. A pseudotyped vesicular stomatitis virus possessing the Ebolavirus glycoprotein (GP) also co-localized with SNX5 and its internalization and infectivity were affected by macropinocytosis inhibitors. Taken together, our data suggest that Ebolavirus is internalized into cells by stimulating macropinocytosis in a GP-dependent manner. These findings provide new insights into the lifecycle of Ebolavirus and may aid in the development of therapeutics for Ebolavirus infection.

2. Characterization of the Ebola virus nucleoprotein-RNA complex.

Noda T, Hagiwara K, Sagara H, Kawaoka Y.

When Ebola virus nucleoprotein (NP) is expressed in mammalian cells, it assembles into helical structures. Here, the recombinant NP helix purified from cells expressing NP was characterized biochemically and morphologically. We found that the recombinant NP helix is associated with non-viral RNA, which is not protected from RNase digestion and that the morphology of the helix changes depending on the environmental salt concentration. The N-terminal 450 aa residues of NP are sufficient for these properties. However, digestion of the NP-associated RNA eliminates the plasticity of the helix, suggesting that this RNA is an essential structural component of the helix, binding to individual NP molecules via the N-terminal 450 aa. These findings enhance our knowledge of Ebola virus assembly and understanding of the Ebola virus life cycle.

3. Novel Phosphoprotein-interacting Region in Nipah Virus Nucleocapsid Protein

Mio Omi-Furutani, Misako Yoneda, Kentaro Fujita, Fusako Ikeda, and Chieko Kai

The interaction of Nipah virus (NiV) nucleocapsid (N) protein with phosphoprotein (P) during nucleocapsid assembly is the essential process in the viral life cycle, since only the encapsidated RNA genome can be used for replication. In our study, we utilized fluorescent protein tags to visualize NiV N and P proteins in live cells and analyzed their cellular localization in order to identify the region responsible for N-P interaction. N protein fused to monomeric enhanced cyan fluorescence protein (N-ECFP) exhibited a dotted pattern in transfected cells, while P protein fused to monomeric red fluorescent protein (P-mRFP) showed diffuse distribution. When the two proteins were coexpressed, P-mRFP colocalized with N-ECFP dots. N-ECFP mutants with serial amino-acid deletions were generated to search for the region(s) responsible for this N-P colocalization, and we have found that aa 135-146 were responsible for the N-P colocalization. The residues crucial for N-P interaction were further investigated by introducing Alanine substitutions to the untagged N protein. Alanine scanning combined with coimmunoprecipitation and minigenome assay has revealed that there are two distinct regions within aa 135-146 region, each of which is essential for the interaction of N-P and the function of N.

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International Research Center for Infectious Diseases

Department of Infectious Disease Control
感染制御分野

Professor	Chihiro Sasakawa, Ph.D.	教授(兼)	医学博士	笹	川 千 尋
Professor	Aikichi Iwamoto, M.D., D.M.Sc.	教授(兼)	医学博士	岩	本 愛 吉
Project Assistant Professor	Noriaki Hosoya, Ph.D.	特任助教	医学博士	細	谷 紀 彰
Research Associate	Takahito Sanada, Ph.D.	特任研究員	医学博士	真	田 貴 人
Research Associate	Hitomi Nakamura, M.D., Ph.D.	特任研究員	医学博士	中	村 仁 美
Research Associate	Michiko Koga, M.D., Ph.D.	特任研究員	医学博士	古	賀 道 子

Our special interest is focused upon searching for effective methods to protect or regulate bacterial infection by using accumulated knowledge based on molecular pathogenicity, and developing animal models for studying the bacterial pathogens and attenuated strains for novel vaccines. Our other research targets are HIV and other pathogens responsible for emerging infectious diseases. Cellular immunity is important in controlling the pathogenic viruses. In order to understand the viral pathogenesis, we analyzed the relationship between antigen presentation and recognition by T cells. The works have been conducted by close collaboration with Division of Bacterial Infection, Division of Infectious Diseases and Department of Infectious Diseases and Applied Immunology.

1. The Attenuated Pathogenicity of Helicobacter pylori CagA from Native Americans in Peruvian Amazon.

Masato Suzuki, Kotaro Kiga, Hitomi Mimuro, Takahito Sanada, Shiho Suzuki and Chihiro Sasakawa

The clinical outcome of host-microbe interactions must involve in geographical genotypes of microbial virulence-associated factors. *Helicobacter pylori*, a cause of gastric diseases, is known as one of the most successful bacterial pathogens, which chronically infects human stomachs of at least half the world's population. Recent epidemiological studies revealed that this bacterium and human have been evolving intimately ever since, and at present the genetic geography of *H. pylori* affects the risk of gastric diseases including gastric adenocarcinoma. We showed that *H. pylori* isolates from Native Americans in Peruvian Amazonian villages, where all residents were infected with *H. pylori*, but had few gastric diseases, had a novel *cagA* genotype that closely associates with attenuated bacterial pathogenesis. CagA is a major virulence factor of *H. pylori* and injected from bacteria into the gastric epithelium in which CagA elicits pleiotropic host responses, such as cell proliferation and inflammation, leading to the development of *H. pylori*-related gastric diseases, suggesting that these attenuated strains are promising novel vaccines.

2. The bacterial effector Cif interferes with SCF ubiquitin ligase function by inhibiting deneddylation of Cullin1.

Hanako Morikawa[1], Minsoo Kim, Hitomi Mimuro[1], Claire Punginelli[1], Tomohiro Koyama[2], Shinya Nagai[2], Atsushi Miyawaki[3], Kazuhiro Iwai[4], Chihiro Sasakawa: [1]Depart-

ment of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo, Japan, [2]Nippon Institute for Biological Science, Tokyo, Japan, [3]Laboratory for Cell Function and Dynamics, Advanced Technology Development Group, Brain Science Institute, RIKEN, Saitama, Japan, [4]Department of Biophysics and Biochemistry, Graduate School of Frontier Biosciences/Graduate School of Medicine Osaka University, Osaka, Japan

Cycle inhibiting factor (Cif) is one of the effectors delivered into epithelial cells by enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *Escherichia coli* (EHEC) via the type III secretion system (TTSS). Cif family proteins, which inhibit host cell-cycle progression via mechanisms not yet precisely understood, are highly conserved among EPEC, EHEC, *Yersinia pseudotuberculosis, Photorhabdus luminescens* and *Burkholderia pseudomallei*. Levels of several proteins relevant to cell-cycle progression are modulated by Cullin-RING ligases (CRLs), which in turn are activated by conjugation and deconjugation of NEDD8 to Cullins. Here we show that Cif interacts with NEDD8 and interferes with SCF (Skp1-Cullin1-F-box protein) complex ubiquitin ligase function. We found that neddylated Cullin family proteins accumulated and ubiquitination of p27 decreased in cells infected with EPEC. Consequently, Cif stabilized SCF substrates such as CyclinD1, Cdt1, and p27, and caused G1 cell-cycle arrest. Using time-lapse-imaging of fluorescent ubiquitination-based cell-cycle indicator (Fucci)-expressing cells, we were able to monitor cell-cycle progression during EPEC infection and confirmed the arrest of infected cells at G1. Our in vitro and in vivo data show that Cif-NEDD8 interaction inhibits deneddylation of Cullins, suppresses CRL activity and induces G1 arrest. We thus conclude that the bacterial effector Cif interferes with neddylation-mediated cell-cycle control.

3. Bacterial Interactions with the Host Epithelium.

Minsoo Kim, Hiroshi Ashida[1], Michinaga Ogawa[1], Yuko Yoshikawa, Hitomi Mimuro, and Chihiro Sasakawa: [1]Department of Microbiology and Immunology, Institute of Medical Science University of Tokyo, Tokyo, Japan

The gastrointestinal epithelium deploys multiple innate defense mechanisms to fight microbial intruders, including epithelial integrity, rapid epithelial cell turnover, quick expulsion of infected cells, autophagy, and innate immune responses. Nevertheless, many bacterial pathogens are equipped with highly evolved infectious stratagems that circumvent these defense systems and use the epithelium as a replicative foot- hold. During replication on and within the gastrointestinal epithelium, gastrointestinal bacterial pathogens secrete various components, toxins, and effectors that can subvert, usurp, and exploit host cellular functions to benefit bacterial survival. In addition, bacterial pathogens use a variety of mechanisms that balance breaching the epithelial barrier with maintaining the epithelium in order to promote bacterial colonization. These complex strategies represent a new paradigm of bacterial pathogenesis.

4. *Shigella* deploy multiple countermeasures against host innate immune responses.

Hiroshi Ashida, Michinaga Ogawa, Minsoo Kim, Shiho Suzuki[1], Takahito Sanada, Claire Punginelli, Hitomi Mimuro and Chihiro Sasakawa: [1]Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo, Japan

Although the intestinal epithelium is equipped with multiple defense systems that sense bacterial components, transmit alarms to the immune system, clear the bacteria, and renew the injured epithelial lining, mucosal bacterial pathogens are capable of efficiently colonizing the intestinal epithelium, because they have evolved systems that modulate the inflammatory and immune responses of the host and exploit the harmful environments as replicative niches. In this review we highlight current topics concerning Shigella's tactics that interfere with the innate immune systems.

5. Changes in impact of HLA class I allele expression on HIV-1 plasma virus loads at a population level over time.

Michiko Koga, Ai Kawana-Tachikawa[1], David Heckerman[2], Takashi Odawara[1], Hitomi Nakamura, Tomohiko Koibuchi[3], Takeshi Fujii[3], Toshiyuki Miura[1] and Aikichi Iwamoto: [1]Division of Infectious Disease, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan, [2]Microsoft Research, Redmond, Washington, USA, [3]Department of Infectious Diseases and Applied Immunology, Research Hospital, Institute of Medical Science, University of Tokyo, Tokyo, Japan

HLA class I allele types have differential impacts on the level of the pVL and outcome of HIV-1 infection. While accumulations of CTL es-

cape mutations at population levels have been reported, their actual impact on the level of the pVL remains unknown. In this study HLA class I types from 141 untreated, chronically HIV-1 infected Japanese patients diagnosed from 1995? 2007 were determined, and the associations between expression of individual HLA alleles and level of pVL analyzed. It was found that the Japanese population has an extremely narrow HLA distribution compared to other ethnic groups, which may facilitate accumulation of CTL escape mutations at the population level. Moreover while they uniquely lack the most protective HLA-B27/B57, they commonly express the alleles that are protective in Caucasians (A11:10.4%, A26:11.55%, B51:8.6% and Cw14:12.7%). Cross-sectional analyses revealed no significant associations between expression of individual alleles and the level of the pVL. The patients were then stratified by the date of HIV diagnosis and the analyses repeated. It was found that, before 2001, B51+ individuals displayed significantly lower pVL than the other patients (median: 5150 vs. 18 000 RNA copies/ml, P=0.048); however thereafter this protective effect waned and disappeared, whereas no changes were observed for any other alleles over time. These results indicate that, at a population level, some HLA alleles have been losing their beneficial effects against HIV disease progression over time, thereby possibly posing a significant challenge for HIV vaccine development. However such detrimental effects may be limited to particular HLA class I alleles.

6. Long-term successful control of super-multi-drug resistant Human Immunodeficiency Virus type I infection by a novel combination therapy of Raltegravir, Etravirine and boosted-Darunavir.

Hitomi Nakamura, Naoko Miyazaki[1], Noriaki Hosoya, Michiko Koga, Takashi Odawara, Tadashi Kikuchi[2], Tomohiko Koibuchi, Ai Kawana-Tachikawa, Takeshi Fujii, Toshiyuki Miura, Aikichi Iwamoto: [1]Department of Infectious Diseases and Applied Immunology, Research Hospital, Institute of Medical Science, University of Tokyo, Tokyo, Japan, [2]Division of Infectious Disease, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan

Drug-resistant virus infection has been a major hurdle in the management of human immunodeficiency virus type 1 (HIV-1) infection. Recently, three novel antiretrovirals [raltegravir (RAL), etravirine (ETR), and darunavir (DRV)] were introduced into the market almost simultaneously, and salvage regimens containing these three antiretrovirals have been reported to exhibit strong potency against drug-resistant HIV-1 infection. However, the sustainability of such regimens remains unclear, particularly for patients infected with multidrug-resistant viruses. Here we report a case of super-multidrug-resistant HIV-1 infection which has been successfully controlled by novel combination therapy including RAL, ETR, and DRV for over 2 years, indicating that the novel combination could become an ultimate weapon against drug-resistant HIV infection and could alter the landscape of HIV salvage therapy.

7. Identification and structural definition of H 5-specific CTL epitopes restricted by HLA-A*0201 derived from the H5N1 subtype of influenza A viruses.

Yeping Sun[1], Jun Liu[1], Meng Yang[1], Feng Gao[1], Jianfang Zhou[2], Yoshihiro Kitamura[1,3], Bin Gao[1,3], Po Tien[1], Yuelong Shu[2], Aikichi Iwamoto, Zhu Chen[4] and George F. Gao[1,3]: [1]CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences (CAS), Beijing, China, [2]Chinese National Influenza Center, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China, [3]China-Japan Joint Laboratory of Molecular Immunology and Molecular Microbiology, Institute of Microbiology, CAS, Beijing, China, [4]Institute of Hematology, Rui Jin Hospital affiliated to Shanghai Second Medical University, Shanghai, China

The haemagglutinin (HA) glycoprotein of influenza A virus is a major antigen that initiates humoral immunity against infection; however, the cellular immune response against HA is poorly understood. Furthermore, HA-derived cytotoxic T-lymphocyte (CTL) epitopes are relatively rare in comparison to other internal gene products. Here, CTL epitopes of the HA serotype H5 protein were screened. By using in silico prediction, in vitro refolding and a T2 cell-binding assay, followed by immunization of HLA-A2.1/K(b) transgenic mice, an HLA-A*0201-restricted decameric epitope, RI-10 (H5 HA205-214, RLYQNPTTYI), was shown to elicit a robust CTL epitope-specific response. In addition, RI-10 and its variant, KI-10 (KLYQNPTTYI), were also demonstrated to be able to induce a higher CTL epitope-specific response than the influenza A virus dominant CTL epitope GL-9 (GILGFVFTL) in peripheral blood mononuclear cells of HLA-A*0201-positive patients who had

recovered from H5N1 virus infection. Furthermore, the crystal structures of RI-10-HLA-A*0201 and KI-10-HLA-A*0201 complexes were determined at 2.3 and 2.2 A resolution, respectively, showing typical HLA-A*0201-restricted epitopes. The conformations of RI-10 and KI-10 in the antigen-presenting grooves in crystal structures of the two complexes show significant differences, despite their nearly identical sequences. These results provide implications for the discovery of diagnostic markers and the design of novel influenza vaccines.

8. Novel Immunodominant Peptide Presentation Strategy: a Featured HLA-A*2402-Restricted Cytotoxic T-Lymphocyte Epitope Stabilized by Intrachain Hydrogen Bonds from Severe Acute Respiratory Syndrome Coronavirus Nucleocapsid Protein.

Jun Liu, Peng Wu[1], Feng Gao, Jianxun Qi[2], Ai Kawana-Tachikawa, Jing Xie[1], Christopher J. Vavricka[2], Aikichi Iwamoto, Taisheng Li[1], and George F. Gao: [1]Department of Infectious Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China, [2]CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China

Antigenic peptides recognized by virus-specific cytotoxic T lymphocytes (CTLs) are presented by major histocompatibility complex (MHC; or human leukocyte antigen [HLA] in humans) molecules, and the peptide selection and presentation strategy of the host has been studied to guide our understanding of cellular immunity and vaccine development. Here, a severe acute respiratory syndrome coronavirus (SARS-CoV) nucleocapsid (N) protein-derived CTL epitope, N1 (QFKDNVILL), restricted by HLA-A*2402 was identified by a series of in vitro studies, including a computer-assisted algorithm for prediction, stabilization of the peptide by co-refolding with HLA-A*2402 heavy chain and β_2-microglobulin (β_2m), and T2-A24 cell binding. Conse- quently, the antigenicity of the peptide was confirmed by enzyme-linked immunospot (ELISPOT), proliferation assays, and HLA-peptide complex tetramer staining using peripheral blood mononuclear cells (PBMCs) from donors who had recovered from SARS donors. Furthermore, the crystal structure of HLA-A*2402 complexed with peptide N1 was determined, and the featured peptide was characterized with two unexpected intrachain hydrogen bonds which augment the central residues to bulge out of the binding groove. This may contribute to the T-cell receptor (TCR) interaction, showing a host immunodominant peptide presentation strategy. Meanwhile, a rapid and efficient strategy is presented for the determination of naturally presented CTL epitopes in the context of given HLA alleles of interest from long immunogenic overlapping peptides.

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International Research Center for Infectious Diseases

Department of Infectious Disease Control Division of Microbial Infection

感染制御系　微生物学分野

Professor	Tetsuro Matano, M.D., D.M.Sc.		教　授	医学博士	俣　野　哲　朗
Project Assistant Professor	Hiroaki Takeuchi, D.M.Sc.		特任助教	医学博士	武　内　寛　明

We are working on Microbiology and Immunology to elucidate the molecular mechanism of viral replication in vivo. We focus on HIV, a representative virus inducing chronic persistent infection. Our current projects are elucidation of AIDS pathogenesis and development of an AIDS vaccine. For clarifying the mechanism of persistent HIV replication and developing an effective AIDS vaccine interfering with its establishment, we are studying acquired immune responses in non-human primate AIDS models.

1. Broadening of $CD8^+$ cell responses in vaccine-based simian immunodeficiency virus controllers

Nami Iwamoto, Tetsuo Tsukamoto, Miki Kawada, Akiko Takeda, Hiroyuki Yamamoto, Hiroaki Takeuchi, and Tetsuro Matano

In our prior study on a prophylactic T cell-based vaccine, some vaccinated macaques controlled a simian immunodeficiency virus (SIV) challenge. These animals allowed viremia in the acute phase but showed persistent viral control after the setpoint. Here, we examined the breadth of postchallenge virus specific cellular immune responses in these SIV controllers. We previously reported that in a group of Burmese rhesus macaques possessing the MHC haplotype 90-120-Ia, immunization with a Gag-expressing vaccine results in nonsterile control of a challenge with SIVmac239 but not a mutant SIV carrying multiple cytotoxic T lymphocyte (CTL) escape gag mutations. In the present study, we investigated whether broader cellular immune responses effective against the mutant SIV replication are induced after challenge in those vaccinees that maintained wild-type SIVmac239 control. We analyzed cellular immune responses in these SIV controllers (n＝8). These controllers elicited CTL responses directed against SIV non-Gag antigens as well as Gag in the chronic phase. Postvaccinated, prechallenge $CD8^+$ cells obtained from these animals suppressed wild-type SIV replication in vitro, but mostly had no suppressive effect on the mutant SIV replication, whereas $CD8^+$ cells in the chronic phase after challenge showed efficient anti-mutant SIV efficacy. The levels of in vitro anti-mutant SIV efficacy of $CD8^+$ cells correlated with Vif-specific $CD8^+$ T cell frequencies. Plasma viremia was kept undetectable even after the mutant SIV superchallenge in the chronic phase. These results suggest that vaccine-based wild-type SIV controllers can acquire $CD8^+$ cells with the potential to suppress replication of SIV variants carrying CTL escape mutations.

2. A structural constraint for functional interaction between N-terminal and C-terminal domains in simian immunodeficiency virus capsid proteins

Natsuko Inagaki, Hiroaki Takeuchi, Masaru Yokoyama[1], Hironori Sato[1], Akihide Ryo[2], Hiroyuki Yamamoto, Miki Kawada, and Tetsuro Matano: [1]Pathogen Genomic Center, National Institute of Infectious Diseases; [2]Department of Microbiology, Yokohama City University School of Medicine

The Gag capsid (CA) is one of the most conserved proteins in highly-diversified HIV and SIV. Understanding the limitations imposed on amino acid sequences in CA could provide valuable information for vaccine immunogen design or anti-HIV drug development. Here, by comparing two pathogenic SIV strains, SIVmac239 and SIVsmE543-3, we found critical amino acid residues for functional interaction between the N-terminal and the C-terminal domains in CA. We first examined the impact of Gag residue 205, aspartate (Gag205D) in SIVmac239 and glutamate (Gag205E) in SIVsmE543-3, on viral replication; due to this difference, Gag206-216 (IINEEAADWDL) epitope specific CTLs were previously shown to respond to SIVmac239 but not SIVsmE543-3 infection. A mutant SIVmac 239, SIVmac239Gag205E, whose Gag205D is replaced with Gag205E showed lower replicative ability. Interestingly, however, SIVmac239Gag 205E passaged in macaque T cell culture often resulted in selection of an additional mutation at Gag residue 340, a change from SIVmac239 valine (Gag340V) to SIVsmE543-3 methionine (Gag340M), with recovery of viral fitness. Structural modeling analysis suggested possible intermolecular interaction between the Gag205 residue in the N-terminal domain and Gag340 in the C-terminal in CA hexamers. The Gag205D to Gag205E substitution in SIVmac239 resulted in loss of in vitro core stability, which was recovered by additional Gag340V to Gag340M substitution. Finally, selection of Gag205E plus Gag 340M mutations, but not Gag205E alone was observed in a chronically SIVmac239 infected rhesus macaque eliciting Gag206-216 specific CTL responses. These results present in vitro and in vivo evidence implicating the interaction between Gag residues 205 in CA NTD and 340 in CA CTD in SIV replication. Thus, this study indicates a structural constraint for functional interaction between SIV CA NTD and CTD, providing insight into immunogen design to limit viral escape options.

These studies were performed with the help of National Institute of Infectious Diseases, Tsukuba Primate Research Center in National Institute of Biomedical Innovation, Institute for Virus Research in Kyoto University, and Medical Research Institute in Tokyo Medical and Dental University. A project for a clinical trial of an AIDS vaccine using Sendai virus vectors is proceeding in collaboration with DNAVEC Corp. and International AIDS Vaccine Initiative (IAVI).

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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

Associate Professor Yasushi Kawaguchi, D.V.M., Ph.D. | 准教授 獣医学博士 川口 寧

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. Non-muscle myosin IIA is a functional entry receptor for herpes simplex virus 1.

Jun Arii, Hideo Goto, Tadahiro Suenaga, Masaaki Oyama, Hiroko Kozuka-Hata, Takahiko Imai, Atsuko Minowa, Hiroomi Akashi, Hisashi Arase, Yoshihiro Kawaoka, and Yasushi Kawaguchi

Herpes simplex virus 1 (HSV-1), the prototype of the alphaherpesviruses family, causes lifelong infections in humans. Although generally associated with various mucocutaneous diseases, HSV-1 is also involved in lethal encephalitis. HSV-1 entry into host cells requires cellular receptors for both envelope glycoprotein B (gB) and gD. However, the gB receptors responsible for its broad host-range in vitro and infection of critical targets in vivo remain unknown. Here, we show that non-muscle myosin heavy chain IIA (NMHC-IIA), a subunit of non-muscle myosin IIA (NM-IIA), functions as an HSV-1 entry receptor by interacting with gB. A cell line that is relatively resistant to HSV-1 infection became highly susceptible to infection by this virus when NMHC-IIA was overexpressed. Antibody to NMHC-IIA blocked HSV-1 infection in naturally permissive target cells. Furthermore, knockdown of NMHC-IIA in the permissive cells inhibited HSV-1 infection as well as cell-cell fusion when gB, gD, gH, and gL were coexpressed. Cell surface expression of NMHC-IIA was markedly and rapidly induced during the initiation of HSV-1 entry. A specific inhibitor of myosin light chain kinase, which regulates NM-IIA by phosphorylation, reduced the redistribution of NMHC-IIA as well as HSV-1 infection in cell culture and in a murine model for herpes stromal keratitis. NMHC-IIA is ubiquitously expressed in various human tissues and cell types and, therefore, is implicated as a functional gB receptor that mediates broad HSV-1 infectivity both in vitro and in vivo. The identification of NMHC-IIA as an HSV-1 entry receptor and the involvement of NM-IIA regulation in HSV-1 infection shed new light on HSV-1 entry and identify new targets for antiviral drug development.

2. A Single Amino Acid Substitution in Herpes simplex virus 1 Envelope Glycoprotein B at a Site Required for Binding to the Paired Immunoglobulin-like Type 2 Recep-

tor α (PILRα) Abrogates PILRα-dependent Viral Entry and Reduces Pathogenesis.

Jun Arii, Jing Wang, Tomomi Morimoto, Tadahiro Suenaga, Hiroomi Akashi, Hisashi Arase, and Yasushi Kawaguchi

Paired immunoglobulin-like type 2 receptor α (PILRα) is a herpes simplex virus 1 (HSV-1) entry receptor that associates with *O*-glycans on HSV-1 envelope glycoprotein B (gB). Two threonine residues (Thr-53 and Thr-480) in gB, that are required for addition of the principal gB *O*-glycans, are essential for binding to soluble PILRα. However, the role of the two threonines in PILRα-dependent viral entry remain to be elucidated. Therefore, we constructed a recombinant HSV-1 carrying an alanine replacement of gB Thr-53 alone (gB-T53A) or of both gB Thr-53 and Thr-480 (gB-T53/480A) and demonstrated that these mutations abrogated viral entry in CHO cells expressing PILRα. In contrast, the mutations had no effect on viral entry in CHO cells expressing known host cell receptors for HSV-1 gD, viral entry in HL60 cells expressing MAG (another HSV-1 gB receptor), viral attachment to heparan sulfate, and viral replication in PILRα-negative cells. These results support the hypothesis that gB Thr-53 and Thr-480 as well as gB *O*-glycosylation, probably at these sites, are critical for PILRα-dependent viral entry. Interestingly, in mice following corneal inoculation, the gB-T53A and gB-T53/480A mutations significantly reduced viral replication in the cornea, development of herpes stroma keratitis, and neuroinvasiveness. The ability of HSV-1 to enter cells in a PILRα-dependent manner and to acquire specific carbohydrates on gB, therefore, are linked to an increase in viral replication and virulence in the experimental murine model.

3. Nucleolin Is Required for Efficient Nuclear Egress of Herpes Simplex Virus 1 Nucleocapsids.

Ken Sagou, Masashi Uema, and Yasushi Kawaguchi

Herpesvirus nucleocapsids assemble in the nucleus and must cross the nuclear membrane for final assembly and maturation to form infectious progeny virions in the cytoplasm. It has been proposed that nucleocapsids enter the perinuclear space by budding through the inner nuclear membrane and these enveloped nucleocapsids then fuse with the outer nuclear membrane to enter the cytoplasm. Little is known about the mechanism(s) for nuclear egress of herpesvirus nucleocapsids and, in particular, which, if any, cellular proteins are involved in the nuclear egress pathway. UL12 is an alkaline nuclease encoded by herpes simplex virus 1 (HSV-1) and has been suggested to be involved in viral DNA maturation and nuclear egress of nucleocapsids. Using a live-cell imaging system to study cells infected by a recombinant HSV-1 expressing UL12 fused to a fluorescent protein, we observed the previously unreported nucleolar localization of UL12 in live infected cells and, with co-immunoprecipitation analyses, showed that UL12 formed a complex with nucleolin, a nucleolus marker, in infected cells. Knock-down of nucleolin in HSV-1-infected cells reduced capsid accumulation as well as the amount of viral DNA resistant to staphylococcal nuclease in the cytoplasm, which represented encapsidated viral DNA, but had little effect on these viral components in the nucleus. These results indicated that nucleolin is a cellular factor required for efficient nuclear egress of HSV-1 nucleocapsids in infected cells.

4. Effects of Phosphorylation of Herpes Simplex Virus 1 Envelope Glycoprotein B by Us3 Kinase In Vivo and In Vitro

Takahiko Imai, Ken Sagou, Jun Arii and Yasushi Kawaguchi

We recently reported that herpes simplex virus 1 (HSV-1) Us3 protein kinase phosphorylates threonine at position 887 (Thr-887) in the cytoplasmic tail of envelope glycoprotein B (gB) (A. Kato, J. Arii, I. Shiratori, H. Akashi, H. Arase, and Y. Kawaguchi, J. Virol. 83: 250-261, 2009; T. Wisner, C. C. Wright, K. Kato, Y. Kawaguchi, F. Mou, J. D. Baines, R. J. Roller and D. C. Johnson, J. Virol. 83: 3115-3126, 2009). In the studies reported here, we examined the effect(s) of this phosphorylation on viral replication and pathogenesis in vivo and present data showing that substitution of gB Thr-887 by alanine significantly reduced viral replication in the mouse cornea and development of herpes stroma keratitis and periocular skin disease in mice. The same effects have been reported for mice infected with a recombinant HSV-1 carrying a kinase-inactive mutant of Us3. These observations suggested that Us3 phosphorylation of gB Thr-887 played a critical role in viral replication in vivo and in HSV-1 pathogenesis. In addition, we generated a monoclonal antibody that specifically reacted with phosphorylated gB Thr-887 and used this antibody to show that Us3 phosphorylation of gB Thr-887 regulated subcellular localization of gB, particularly on the cell surface of infected cells.

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International Research Center for Infectious Diseases

Pathogenic Microbes Repository Unit
感染症国際研究センター
病原微生物資源室

Professor	Chihiro Sasakawa, Ph.D.		教　授	医学博士	笹　川　千　尋
Project Assistant Professor	Minsoo Kim, Ph.D.		特任助教	医学博士	金　　玟　秀

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.