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.

Nipah and Hendra Virus Nucleoproteins Inhibit Nuclear Accumulation of Signal Transducer and Activator of Transcription 1 (STAT1) and STAT2 by Interfering with Their Complex Formation.

Sugai A, Sato H, Takayama I, Yoneda M, Kai C.

Henipaviruses, such as Nipah (NiV) and Hendra (HeV) viruses, are highly pathogenic zoonotic agents within the Paramyxoviridae family. The P gene products of the paramyxoviruses have been well characterized for their interferon (IFN) antagonist activity and their contribution to viral pathogenicity. In this study, we demonstrated that the nucleoprotein (N) of henipaviruses also prevents the host IFN signaling response. Reporter assays demonstrated that the NiV and HeV N proteins (NiV-N and HeV-N, respectively) dose-dependently suppressed both type I and type II IFN responses and that the inhibitory effect was mediated by their core domains. Additionally, NiV-N prevented the nuclear transport of signal transducer and activator of transcription 1 (STAT1) and STAT2. However, NiV-N did not associate with Impα5, Impβ1, or Ran, which are members of the nuclear transport system for STATs. Although P protein is known as a binding partner of N protein and actively retains N protein in the cytoplasm, the IFN antagonist activity of N protein was not abolished by the coexpression of P protein. This suggests that the IFN inhibition by N protein occurs in the cytoplasm. Furthermore, we demonstrated that the complex formation of STATs was hampered in the N protein-expressing cells. As a result, STAT nuclear accumulation was reduced, causing a subsequent downregulation of interferon-stimulated genes (ISGs) due to low promoter occupancy by STAT complexes. This novel route for preventing host IFN responses by henipavirus N proteins provides new insight into the pathogenesis of these viruses.

Cryo-EM structure of the Ebola virus nucleoprotein-RNA complex at 3.6 Å resolution.

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Ebola virus causes haemorrhagic fever with a high fatality rate in humans and non-human primates. It belongs to the family *Filoviridae* in the order *Mononegavirales,* which are viruses that contain

linear, non-segmented, negative-sense, single-stranded genomic RNA. The enveloped, filamentous virion contains the nucleocapsid, consisting of the helical nucleoprotein-RNA complex, VP24, VP 30, VP35 and viral polymerase. The nucleoprotein-RNA complex acts as a scaffold for nucleocapsid formation and as a template for RNA replication and transcription by condensing RNA into the virion. RNA binding and nucleoprotein oligomerization are synergistic and do not readily occur independently. Although recent cryo-electron tomography studies have revealed the overall architecture of the nucleocapsid core, there has been no high-resolution reconstruction of the nucleocapsid. Here we report the structure of a recombinant Ebola virus nucleoprotein-RNA complex expressed in mammalian cells without chemical fixation, at near-atomic resolution using single-particle cryo-electron microscopy. Our structure reveals how the Ebola virus nucleocapsid core encapsidates its viral genome, its sequence-independent coordination with RNA by nucleoprotein, and the dynamic transition between the RNA-free and RNA-bound states. It provides direct structural evidence for the role of the N terminus of nucleoprotein in subunit oligomerization, and for the hydrophobic and electrostatic interactions that lead to the formation of the helical assembly. The structure is validated as representative of the native biological assembly of the nucleocapsid core by consistent dimensions and symmetry with the full virion. The atomic model provides a detailed mechanistic basis for understanding nucleocapsid assembly and highlights key structural features that may serve as targets for anti-viral drug development.

Enhanced Replication of Highly Pathogenic Influenza A(H7N9) Virus in Humans.

Yamayoshi S, Kiso M, Yasuhara A, Ito M, Shu Y[1], Kawaoka Y.: [1]Sun Yat-Sen University, Shenzhen, China

To clarify the threat posed by emergence of highly pathogenic influenza A(H7N9) virus infection among humans, we characterized the viral polymerase complex. Polymerase basic 2-482R, polymerase basic 2-588V, and polymerase acidic-497R individually or additively enhanced virus polymerase activity, indicating that multiple replication-enhancing mutations in 1 isolate may contribute to virulence.

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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 VP22 Inhibits AIM2-Dependent Inflammasome Activation to Enable Efficient Viral Replication

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The AIM2 inflammasome is activated by DNA, leading to caspase-1 activation and release of pro-inflammatory cytokines interleukin 1β (IL-1β) and IL-18, which are critical mediators in host innate immune responses against various pathogens. Some viruses employ strategies to counteract inflammasome-mediated induction of pro-inflammatory cytokines, but their *in vivo* relevance is less well understood. Here we show that the herpes simplex virus 1 (HSV-1) tegument protein VP22 inhibits AIM 2-dependent inflammasome activation. VP22 interacts with AIM2 and prevents its oligomerization, an initial step in AIM2 inflammasome activation. A mutant virus lacking VP22 (HSV-1ΔVP22) activates AIM2 and induces IL-1β and IL-18 secretion, but these responses are lost in the absence of AIM2. Additionally, HSV-1ΔVP22 infection results in diminished viral yields *in vivo*, but HSV-1ΔVP22 replication is largely restored in AIM2-deficient mice. Collectively, these findings reveal a mechanism of HSV-1 evasion of the host immune response that enables efficient viral replication *in vivo*.

2. Roles of the Phosphorylation of Herpes Simplex Virus 1 UL51 at a Specific Site in Viral Replication and Pathogenicity

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Herpes simplex virus 1 (HSV-1) UL51 is a phosphoprotein that functions in the final envelopment in the cytoplasm and viral cell-cell spread, leading to efficient viral replication in cell cultures. To clarify the mechanism by which UL51 is regulated in HSV-1-infected cells, we focused on the phosphorylation of UL51. Mass spectrometry analysis of purified UL51 identified five phosphorylation sites in UL51. Alanine replacement of one of the identified phosphorylation sites in UL51, serine 184 (Ser-184), but not the other identified phosphorylation sites, significantly reduced viral replication and cell-cell spread in HaCaT cells. This mutation induced membranous invaginations adjacent to the nuclear membrane, the accumulation of primary enveloped virions in the invaginations and perinuclear space, and mislocalized UL34 and UL31 in punctate structures at the nuclear membrane; however, it had no effect on final envelopment in the cytoplasm of HaCaT cells. Of note, the alanine mutation in UL51 Ser-184 significantly reduced the mortality of mice following ocular infection. Phosphomimetic mutation in UL51 Ser-184 partly restored the wild-type phenotype in cell cultures and in mice. Based on these results, we concluded that some UL51 functions are specifically regulated by phosphorylation at Ser-184 and that this regulation is critical for HSV-1 replication in cell cultures and pathogenicity *in vivo*.

3. ESCRT-III mediates budding across the inner nuclear membrane and regulates its integrity

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Vesicle-mediated nucleocytoplasmic transport is a nuclear pore-independent mechanism for the nuclear export of macromolecular complexes, but the molecular basis for this transport remains largely unknown. Here we show that endosomal sorting complex required for transport-III (ESCRT-III) is recruited to the inner nuclear membrane (INM) during the nuclear export of herpes simplex virus 1 (HSV-1). Scission during HSV-1 budding through the INM is prevented by depletion of ESCRT-III proteins. Interestingly, in uninfected human cells, the depletion of ESCRT-III proteins induces aberrant INM proliferation. Our results show that HSV-1 expropriates the ESCRT-III machinery in infected cells for scission of the INM to produce vesicles containing progeny virus nucleocapsids. In uninfected cells, ESCRT-III regulates INM integrity by downregulating excess INM.

4. Regulation of Herpes Simplex Virus 2 Protein Kinase UL13 by Phosphorylation and Its Role in Viral Pathogenesis

Naoto Koyanagi, Akihisa Kato, Kosuke Takeshima, Yuhei Maruzuru, Hiroko Kozuka-Hata, Masaaki Oyama, Jun Arii, Yasushi Kawaguchi

UL13 proteins are serine/threonine protein kinases encoded by herpes simplex virus 1 (HSV-1) and HSV-2. Although the downstream effects of the HSV protein kinases, mostly those of HSV-1 UL13, have been reported, there is a lack of information on how these viral protein kinases are regulated in HSV-infected cells. In this study, we used a large-scale phosphoproteomic analysis of HSV-2-infected cells to identify a physiological phosphorylation site in HSV-2 UL13 (i.e., Ser-18) and investigated the significance of phosphorylation of this site in HSV-2-infected cell cultures and mice. Our results were as follows. (i) An alanine substitution at UL13 Ser-18 (S18A) significantly reduced HSV-2 replication and cell-to-cell spread in U2OS cells to a level similar to those of the UL13-null and kinase-dead mutations. (ii) The UL13 S18A mutation significantly impaired phosphorylation of a cellular substrate of this viral protein kinase in HSV-2-infected U2OS cells. (iii) Following vaginal infection of mice, the UL13 S18A mutation significantly reduced mortality, HSV-2 replication in the vagina, and development of vaginal disease to levels similar to those of the UL13-null and the kinase-dead mutations. (iv) A phosphomimetic substitution at UL13 Ser-18 significantly restored the phenotype observed with the UL13 S18A mutation in U2OS cells and mice. Collectively, our results suggested that phosphorylation of UL13 Ser-18 regulated UL13 function in HSV-2-infected cells and that this regulation was critical for the functional activity of HSV-2 UL13 *in vitro* and *in vivo* and also for HSV-2 replication and pathogenesis.

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. Two conserved amino acids within the NSs of severe fever with thrombocytopenia syndrome phlebovirus are essential for anti-interferon activity.

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

The nonstructural protein (NSs) of severe fever with thrombocytopenia syndrome phlebovirus (SFTSV) sequesters TANK-binding kinase 1 (TBK1) into NSs-induced cytoplasmic structures to inhibit the phosphorylation and nuclear translocation of interferon (IFN) regulatory factor 3 (IRF3) and subsequent interferon beta (IFN-β) production. Although the C-terminal region of SFTSV NSs (NSs_{66-249}) has been linked to the formation of NSs-induced cytoplasmic structures and inhibition of host IFN-β responses, the role of the N-terminal region in antagonizing host antiviral responses remains to be defined. Here, we demonstrate that two conserved amino acids at positions 21 and 23 in the SFTSV and heartland virus (HRTV) NSs are essential for suppression of IRF3 phosphorylation and IFN-β mRNA expression following infection with SFTSV or recombinant influenza virus lacking the NS1 gene. Surprisingly, formation of SFTSV/HRTV NSs-induced cytoplasmic structures is not essential for inhibition of host antiviral responses. Rather, an association between SFTSV/HRTV NSs and TBK1 is required for suppression of mitochondrial antiviral signaling protein (MAVS)-mediated activation of IFN-β promoter activity. Although SFTSV NSs did not prevent the ubiquitination of TBK1, it associates with TBK1 through its N-terminal kinase domain (residues 1 to 307) to block the autophosphorylation of TBK1. Furthermore, we found that both wild-type NSs and the 21/23A mutant (NSs in which residues at positions 21 and 23 were replaced with alanine) of SFTSV suppressed NLRP3 inflammasome-dependent interleukin-1β (IL-1β) secretion, suggesting that the importance of these residues is restricted to TBK1-dependent IFN signaling. Together, our findings strongly implicate the two conserved amino acids at positions 21 and 23 of SFTSV/HRTV NSs in the inhibition of host interferon responses.

2. High ambient temperature dampens adaptive immune responses to influenza A virus infection.

Moriyama M, and Ichinohe T.

Although climate change may expand the geographical distribution of several vector-borne diseases, the effects of environmental temperature in host defense to viral infection in vivo are unknown. Here, we demonstrate that exposure of mice at high ambient temperature of 36°C impaired adaptive immune responses against infection with viral pathogens, influenza, Zika, and sever fever with thrombocytopenia syndrome phlebovirus. Following influenza virus infection, the high heat-exposed mice failed to stimulate inflammasome-dependent cytokine secretion and respiratory DC migration to lymph nodes. Although commensal microbiota composition remained intact, the high heat-exposed mice decreased their food intake and increased autophagy in the lung tissue. Induction of autophagy in room temperature-exposed mice severely impaired virus-specific CD8 T cells and antibody responses following respiratory influenza virus infection. In addition, we found that administration of glucose or dietary short-chain fatty acids (SCFAs) restored influenza virus-specific adaptive immune responses in high heat-exposed mice. These findings uncover an unexpected mechanism by which the ambient temperature and nutritional status control the virus-specific adaptive immune responses.

3. Severe acute respiratory syndrome coronavirus viroporin 3a activates the NLRP3 inflammasome.

Chen IY, Moriyama M, Chang MF, and Ichinohe T.

Nod-like receptor family, pyrin domain-containing 3 (NLRP3) regulates the secretion of proinflammatory cytokines interleukin 1 beta (IL-1β) and IL-18. We previously showed that influenza virus M2 or encephalomyocarditis virus (EMCV) 2B proteins stimulate IL-1β secretion following activation of the NLRP3 inflammasome. However, the mechanism by which severe acute respiratory syndrome coronavirus (SARS-CoV) activates the NLRP3 inflammasome remains unknown. Here, we provide direct evidence that SARS-CoV 3a protein activates the NLRP3 inflammasome in lipopolysaccharide-primed macrophages. SARS-CoV 3a was sufficient to cause the NLRP3 inflammasome activation. The ion channel activity of the 3a protein was essential for 3a-mediated IL-1β secretion. While cells uninfected or infected with a lentivirus expressing a 3a protein defective in ion channel activity expressed NLRP3 uniformly throughout the cytoplasm, NLRP 3 was redistributed to the perinuclear space in cells infected with a lentivirus expressing the 3a protein. K^+ efflux and mitochondrial reactive oxygen species were important for SARS-CoV 3a-induced NLRP3 inflammasome activation. These results highlight the importance of viroporins, transmembrane pore-forming viral proteins, in virus-induced NLRP3 inflammasome activation.

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International Research Center for Infectious Diseases

Department of Infectious Disease Control Division of Bacteriology

感染制御系・細菌学分野

Associate Professor Hitomi Mimuro, Ph.D.

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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. Small RNA regulates pathogenicity of *Helicobacter pylori* during persistent infection.

Kinoshita DR[1], Kiga K[1], Otsubo R[2], Sanada T[1,2], Okano T[3], Subsomwong P[2], Iida T[1], Zhu B[1], Ogura Y[4], Yokomori R[5], Hayashi T[4], Suzuki T[3], Nakai K[5], Mimuro H[1,2].: [1]Division of Bacteriology, Department of Infectious Diseases Control, International Research Center for infectious Diseases, Institute of Medical Science, The University of Tokyo, Tokyo, Japan. [2]Department of infection Microbiology, Research Institute for Microbial Diseases, Osaka University, Suita City, Osaka, Japan. [3]Department of Bacterial pathogenesis, Infection and Host Response, Graduate of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan. [4]Department of Bacteriology, Faculty of Medicine Sciences, Kyushu University, Fukuoka, Japan. [5]Human Genome Center, the Institute of Medical Science, the University of Tokyo, Tokyo, Japan

Helicobacter pylori (*H. pylori*) establish persistent infection in the human stomach and induce stomach diseases such as gastritis and gastric cancer. To find out the mechanisms of sustained *H. pylori* infection, we analyzed whole genome sequences of strains isolated from stomachs of Mongolian gerbils infected with *H. pylori*, and extract all point mutations acquired during infection. We found extended poly(T) sequences located in the upstream region of non-coding small RNA HPncX through the infection. Using genetically-modified *H. pylori*, we confirmed that the expression levels of HPncX altered with increasing lengths of poly(T) stretches. A comparative transcriptomic (RNA-seq) and proteomic (iTRAQ, isobaric tags for relative and absolute quantitation) analysis revealed that both expression levels of mRNA and protein of major pathogenic factor CagA were increased in HPncX deletion mutant compared with wild type. Furthermore, electrophoretic mobility shift assay (EMSA) revealed that HPncX bound to *cagA* mRNA to degrade *cagA* mRNA by RNase III. Actually, coculture of HPncX deletion mutant of *H. pylori* with AGS cells, gastric epithelial cell line, showed increased levels of intra-

cellular CagA protein compared with coculture of wild type *H. pylori*, which resulted in increased cell motility and IL-8 secretion. The increased effects of CagA in HPncX deletion mutant were cancelled in the HPncX-complemented strain. These results are the first to demonstrate that HPncX is the newly identified virulence-controlling small RNA to regulate expression of CagA.

2. *Shigella* effector IpaH4.5 targets 19S regulatory particle subunit RPN13 in the 26S proteasome to dampen cytotoxic T lymphocyte activation.

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Subversion of antigen-specific immune responses by intracellular pathogens is pivotal for successful colonisation. Bacterial pathogens, including *Shigella*, deliver effectors into host cells via the type III secretion system (T3SS) in order to manipulate host innate and adaptive immune responses, thereby promoting infection. However, the strategy for subverting antigen-specific immunity is not well understood. Here, we show that *Shigella flexneri* invasion plasmid antigen H (IpaH) 4.5, a member of the E3 ubiquitin ligase effector family, targets the proteasome regulatory particle non-ATPase 13 (RPN13) and induces its degradation via the ubiquitin-proteasome system (UPS). IpaH4.5-mediated RPN13 degradation causes dysfunction of the 19S regulatory particle (RP) in the 26S proteasome, inhibiting guidance of ubiquitinated proteins to the proteolytically active 20S core particle (CP) of 26S proteasome and thereby suppressing proteasome-catalysed peptide splicing. This, in turn, reduces antigen cross-presentation to CD8+ T cells via major histocompatibility complex (MHC) class I in vitro. In RPN13 knockout mouse embryonic fibroblasts (MEFs), loss of RPN13 suppressed CD8+ T cell priming during *Shigella* infection. Our results uncover the unique tactics employed by *Shigella* to dampen the antigen-specific cytotoxic T lymphocyte (CTL) response.

3. The *Shigella* effector OspI dampens γδ+ T cell-mediated epithelial cell death.

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Shigella flexneri, a Gram-negative enteroinvasive bacterium, is the causal agent of shigellosis in humans. *Shigella* is a group of gram-negative, facultative intracellular pathogens. *Shigella* multiplies in intestinal epithelial cells after orally entering the host. By invading the colonic mucosa, *Shigella* induces an acute inflammatory response, leading to massive tissue destruction, as reflected by the emission of bloody, mucopurulent stools. *Shigella* use a type III secretion system (T3SS) to deliver virulence effector proteins into host cells during infection that promote colonization and interfere with antimicrobial host responses. Our previous study demonstrated that OspI, which is delivered by the T3SS into host epithelial cells, deamidated the ubiquitin-conjugating enzyme UBC13, an essential factor for the TNF receptor-associated factor 6 (TRAF6) E3 ligase activity. Deamidation of UBC13 leads to reduced TRAF6 activity, resulting in a decreased NF-κB activation, and impaired gene expression of proinflammatory cytokines and chemokines during *Shigella* infection *in vitro*. However, the physiological role of OspI remains unclear. In this study, we performed animal infection models to discover the physiological role of OspI. As expected from our previous study, guinea pigs and mice infected with Δ*ospI* exhibited increased production of proinflammatory cytokines and chemokines, thereby demonstrating the negative regulatory roles of OspI in inflammatory responses *in vivo*. In addition, the analysis of mice infected with Δ*ospI* led us to identify previously unknown function of OspI as a negative regulator of γδ+ T cell-mediated epithelial cell death at the early phase of infection. We found that γδ+ T cells exhibited cytotoxic activity against Δ*ospI*-infected epithelial cells through Fas-FasL pathway, and the cell death could be avoided by NF-κB inactivation in the epithelial cells treated with NF-κB inhibitor. Furthermore, wild-type but not Δ*ospI* infection did not reduce bacterial numbers in the cells when the infected epithelial cells were co-incubated with γδ+ T cells. Together, these results provide the first evidence supporting the negative regulatory role of OspI in host immune re-

sponse, by downregulation of Fas expression in the *Shigella*-infected epithelial cells.

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International Research Center for Infectious Diseases

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感染制御系・システムウイルス学分野

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The aim of our laboratory is to expand the knowledge and methodology on virology, which were unable to shed light on by conventional experimental approach. To investigate the dynamics of virus infections including HIV and other human-specific viruses, we use a hematopoietic stem cell-transplanted humanized mouse model. We also address the complex dynamics of viral replications and immune responses against viruses through bioinformatics and mathematical approaches. We further study the evolutionary episode of virus-host interaction through molecular phylogenetic. The interdisciplinary investigations based on experimental virology and other scientific fields/methods will pioneer a new science for deeply understanding infectious diseases.

1. Evolutionary arms race between great apes and primate lentiviruses

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The HIV-1-encoded accessory protein viral protein U (Vpu) exerts several immunomodulatory functions, including counteraction of the host restriction factor tetherin, downmodulation of CD4, and inhibition of NF-kB activity to facilitate HIV-1 infection. However, the relative contribution of individual Vpu functions to HIV-1 infection *in vivo* remained unclear. In this suty, we used a humanized mouse model and HIV-1 strains with selective mutations in *vpu* to demonstrate that the anti-tetherin activity of Vpu is a prerequisite for efficient viral spread during the early phase of infection. Mathematical modeling and gain-of-function mutations in SIVcpz, the simian precursor of pandemic HIV-1, corroborate this finding. Blockage of interferon signaling combined with transcriptome analyses revealed that basal tetherin levels are sufficient to control viral replication. These results establish tetherin as a key effector of the intrinsic immune defense against HIV-1, and they demonstrate that Vpu-mediated tetherin antagonism is critical for efficient viral spread during the initial phase of HIV-1 replication.

2. Experimental adaptive evolution of SIVcpz to HIV-1

Kei Sato and Yoshio Koyanagi[1]: [1]Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

From the mid-20th century, humans have been exposed to the menace of infectious viral diseases, such as severe acute respiratory syndrome coronavirus, Ebola virus, and Zika virus. These outbreaks of emerging/reemerging viruses can be trig-

gered by cross-species viral transmission from wild animals to humans, or zoonoses. HIV-1, the causative agent of AIDS, emerged by the cross-species transmission of SIVcpz, the HIV-1 precursor in chimpanzees, around 100 years ago. However, the process by which SIVcpz evolved to become HIV-1 in humans remains unclear. In this study, by using a hematopoietic stem cell-transplanted humanized-mouse model, we experimentally recapitulated the evolutionary process of SIVcpz to become HIV-1. We provided evidence suggesting that a strain of SIVcpz, MB897, preadapted to infect humans over other SIVcpz strains. We further demonstrated a gain-of-function evolution of SIVcpz in infected humanized mice. Our study revealed that pandemic HIV-1 has emerged through at least two steps: preadaptation and subsequent gain-of-function mutations.

3. Adaptive evolution of feline lentiviruses in the New World

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Apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3 (APOBEC3) gene family appears only in mammalian genomes. Some APOBEC 3 proteins can be incorporated into progeny virions and inhibit lentiviral replication. In turn, the lentiviral viral infectivity factor (Vif) counteracts the APOBEC3-mediated antiviral effect by degrading APOBEC3 proteins. Recent investigations have suggested that lentiviral *vif* genes evolved to combat mammalian APOBEC3 proteins, and have further proposed that the Vif-APOBEC3 interaction may help determine the co-evolutionary history of cross-species lentiviral transmission in mammals. In this study, we addressed the co-evolutionary relationship between two New World felids, the puma (*Puma concolor*) and the bobcat (*Lynx rufus*), and their lentiviruses, which are designated puma lentiviruses (PLVs). We demonstrated that PLV-A Vif counteracts the antiviral action of APOBEC3Z3 (A3 Z3) of both puma and bobcat, whereas PLV-B Vif counteracts only puma A3Z3. The species specificity of PLV-B Vif was irrespective of the phylogenic relationships of feline species in the genera *Puma, Lynx* and *Acinonyx*. We revealed that the amino acid at position 178 in the puma and bobcat A3Z3 is exposed on the protein surface and determined the sensitivity to PLV-B Vif-mediated degradation. Moreover, although both the puma and bobcat A3Z 3 genes are polymorphic, their sensitivity/resistance to PLV Vif-mediated degradation was conserved. To the best of our knowledge, this is the first study suggesting that the host APOBEC3 protein potently controls inter-genus lentiviral transmission. Our findings provide the first evidence suggesting that the co-evolutionary arms race between lentiviruses and mammals has occurred in the New World.

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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. 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.