International Research Center for Infectious Diseases

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

Impact of amino acid mutations in PB2, PB1-F2, and NS1 on the replication and pathogenicity of pandemic (H1N1) 2009 influenza viruses.

Ozawa M, Basnet S, Burley LM, Neumann G, Hatta M, Kawaoka Y.

Here, we assessed the effects of PB1-F2 and NS1 mutations known to increase the pathogenicity of influenza viruses on the replication and pathogenicity in mice of pandemic (H1N1) 2009 influenza viruses. We also characterized viruses possessing a PB1-F2 mutation that was recently identified in pandemic (H1N1) 2009 influenza virus isolates, with and without simultaneous mutations in PB2 and NS1. Our results suggest that some NS1 mutations and the newly identified PB1-F2 mutation have the potential to increase the replication and/or pathogenicity of pandemic (H1N1) 2009 influenza viruses.

The importance of the NP: VP35 ratio in Ebola virus nucleocapsid formation.

Noda T, Kolesnikova L, Becker S, Kawaoka Y.

Ebola virus VP35 is a cofactor of the viral RNA polymerase complex and, together with NP and VP24, is an essential component for nucleocapsid formation. In the present study, we examined the interactions between VP35 and NP and found that VP35 interacts with helical NP-RNA complexes through the C-terminus of NP. We also found that coexpression of excess VP35 with NP reduced the yields of NP-RNA complexes purified by CsCl gradient ultracentrifugation and inhibited the formation of the NP-induced inclusion bodies that typically form in Ebola virus-infected cells. These findings suggest that the NP to VP35 ratio is important in the Ebola virus replication cycle and advance our knowledge of nucleocapsid morphogenesis.

Determination of a phosphorylation site in Nipah virus nucleoprotein and its involvement in virus transcription.

Huang M, Sato H, Hagiwara K, Watanabe A, Sugai A, Ikeda F, Kozuka-Hata H, Oyama M, Yoneda M, Kai C.

The phosphorylation of viral proteins is

known to play a role in genome transcription and replication in paramyxoviruses. The paramyxovirus nucleocapsid (N) protein, the most abundant protein in infected cells, is a component of the N-RNA complex and supports the transcription and replication of virus mRNA and genomic RNA. In this study, we found a rapid turnover of phosphorylation in the Nipah virus N (NiV-N). The phosphorylated NiV-N was hardly detectable in steady-state cells, but was detected after inhibition of cellular protein phosphatases. We identified a phosphorylated serine residue at Ser451 of NiV-N by peptide mass fingerprinting by electrospray ionization-quadrupole time-of-flight mass spectrometry. In the NiV minigenome assay, using luciferase as a reporter gene, the substitution of Ser451 for alanine in NiV-N resulted in a reduction in luciferase activity of approximately 45% compared with the wild-type protein. Furthermore, the substitution of Ser451 for glutamic acid, which mimics a phosphoserine, led to a more significant decrease in luciferase activity - approximately 81%. Northern blot analysis showed that both virus transcription and replication were reduced by these mutations. These results suggest that a rapid turnover of the phosphorylation of NiV-N plays an important role in virus transcription and replication.

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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 *Shigella flexneri* effector OspI deamidates UBC13 to dampen the inflammatory response.

Sanada T, Kim M, Mimuro H, Suzuki M, Ogawa M, Oyama A, Ashida H, Kobyashi T, Koyama T[1], Nagai S[1], Shibata Y[2], Gohda J[2], Inoue J[2], Mizushima T[3], and Sasakawa C.: [1]Nippon Institute for Biological Science, Shinmachi, Ome, Tokyo, Japan, [2]Division of Cellular and Molecular Biology; Department of Cancer Biology; Institute of Medical Science, University of Tokyo, Tokyo, Japan, [3]Picobiology Institute; Department of Life Science; Graduate School of Life Science; University of Hyogo, Hyogo, Japan.

Upon infection of many bacterial pathogens, bacterial invasion is quickly sensed by the innate immune system and triggers acute inflammatory responses. However, it is still unclear how pathogens modulate host inflammatory responses. We found that a *Shigella* OspI effector delivered via the type III secretion system dampens acute inflammatory responses during bacterial invasion by targeting TNF receptor-associated factor 6 (TRAF6). OspI was a glutamine deamidase and selectively deamidated Gln100 to Glu100 in Ubc13. Consequently, the E2 ubiquitin-conjugating activity that is required for TRAF6 activation was inhibited, allowing *Shigella* OspI to modulate the diacylglycerol-CBM complex-TRAF6-NF-κB signaling pathway. We determined the 2.0 Å crystal structure of OspI, which contains a putative Cys-His-Asp catalytic triad. A mutational analysis showed that this catalytic triad was essential for deamidation activity. Our results suggest that *Shigella* inhibits acute inflammatory responses at the initial stage of infection by targeting the Ubc13-TRAF6 complex.

2. BabA-mediated adherence is a potentiator of the *Helicobacter pylori* type IV secretion system activity.

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Chronic infection of Helicobacter pylori in the stomach mucosa with translocation of the bacterial cytotoxin-associated gene A (CagA) effector protein via the cag-Type IV secretion system (TFSS) into host epithelial cells are major risk factors for gastritis, gastric ulcers, and cancer. The blood group antigen-binding adhesin BabA mediates the adherence of *H. pylori* to ABO/Lewis b (Le^b) blood group antigens in the gastric pit region of the human stomach mucosa. We identified both *in vitro* and *in vivo* that BabA-mediated binding of *H. pylori* to Le^b on the epithelial surface augments TFSS-dependent *H. pylori* pathogenicity by triggering the production of proinflammatory cytokines and precancer-related factors. We successfully generated Le^b-positive cell lineages by transfecting Le^b-negative cells with several glycosyltransferase genes. Using these established cell lines, we found increased mRNA levels of proinflammatory cytokines (*CCL5* and *IL-8*) as well as precancer-related factors (CDX2 and MUC2) after the infection of Le^b-positive cells with WT *H. pylori* but not with *babA* or TFSS deletion mutants. This increased mRNA expression was abrogated when Le^b-negative cells were infected with WT *H. pylori*. Thus, *H. pylori* can exploit BabA-Le^b binding to trigger TFSS-dependent host cell signaling to induce the transcription of genes that enhance inflammation, development of intestinal metaplasia, and associated precancerous transformations.

3. HIV-1 envelope phenotypic tropism test using Dual Split Protein (DSP)-mediated membrane fusion detection system

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Human Immunodeficiency virus type I (HIV-1) requires two cell surface receptors to initiate the infectious process. One is CD4 and the other is one of the chemokine receptors, CCR5 or CXCR4. Therefore, HIV-1 can be classified according to the uasage of the chemokine receptor. Viruses using CCR5 are grouped as CCR5-tropic (R5), while those using CXCR4 as CXCR4-tropic (X4) viruses. Viruses which can use both CCR5 and CXCR4 are called as dual-tropic (R5X4) viruses. Although the precise mechanism has not been elucidated, R5 viruses dominate the early phase of the infection. During the course of infection, X4 viruses emerge in about half of the patients.

The major viral determinant for cellular tropism is resided in the highly variable loop 3 (V 3) region of the env gene. Therefore, HIV-1 tropism may be determined by viral sequences or by the phenotype of infectious viral particles using indicator cells. A CCR5 specific inhibitor has been approved for clinical use. Therefore, determination of HIV-1 tropism is crucial for the treatment or studies on prevention.

We developed a novel HIV-1 phenotypic tropism assay based on the cell fusion. The procedure does not include the infectious viruses in the procedure and very rapid to get the results. We employed dual split protein (DSP) composed of split green fluorescent protein (GFP) and split renilla luciferase (RL) as a marker for cell fusion. DSP_{1-7} and DSP_{8-11} are fusion proteins of GFP and RL. Although expression of either of them does not express the activities, both activities can be recovered by after cell fusion events.

We chose NP-2 human glioma cell lines modified to express CD4/CCR5 (N4R5) and CD4/CXCR4 (N4X4) as indicator cells. We established NP-2 derived cell lines stably expressing DSP_{1-7}, N4R5-DSP_{1-7} and N4X4-DSP_{1-7}, respectively. We constructed a expression plasmid vector pRE-11 for GFP_{8-11} and HIV-1 envelope genes derived from laboratory strains or patients' plasma (pRE-11-env). pRE-11-env was transfected into 293FT cells. Two days later, transfected 293FT cells were overlaid to N4X4-DSP_{1-7} or N4R5-DSP_{1-7} cells. After 6h of co-cultivation, the tropism could be determined by detection of either GFP signal or RL activity. Reference stains of X4-tropic (HXB2, LAI, NL4-3), R5-tropic (BaL), and dual-tropic (SF2) env were used for assay validation, the tropisms were precisely deter-

mined. Assay sensitivity evaluation revealed at least 0.5% and 10% for detection of X4 and R5 minor population, respectively. Clinical samples were examined. We are comparing the results of DSP cell fusion assay and in-house pseudoviral tropism assay.

4. Structural basis of T cell receptor and viral antigens presented with the major histocompatibility on the cell surface.

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Viral antigens are processed in the infected cells and presented on the cell surface with the major histocompatibility complex class I molecules (HLA class I molecules in humans). Cytotoxic T cells recognize the peptide/MHC (pMHC) by the T cell receptor (TCR). We have been analyzing structural basis between the interaction of TCR and pMHC. The human CD8 functions as a co-receptor for specific T cell recognition. Only one complex structure of human CD8αα binding to HLA-A*0201 has been solved, revealing the molecular basis of CD8 interacting with its ligand pHLA. We revealed the complex structures of human CD8αα bound to HLA-A*2402, which demonstrate two opposite α3 domain CD loop shifts (either pull or push) in the HLA heavy chain upon CD8 engagement. Taking the previously reported mouse CD8-pMHC complex structures into account, from the structural view, all of the data indicate the plasticity of CD8 binding to pMHC/HLA, which facilitates its co-receptor function for T cells. The plasticity of CD8 binding appears not to affect the specificity of TCR recognition, as no peptide conformation change extends to the pMHC interface for TCR contacting.

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International Research Center for Infectious Diseases

Department of Infectious Disease Control Division of Viral Infection

感染制御系・ウイルス学分野

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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. Role of the Herpes Simplex Virus 1 Us3 Kinase Phosphorylation Site and Endocytosis Motifs in Envelope Glycoprotein B in Its Intracellular Transport and Neurovirulence

Takahiko Imai, Jun Arii, Atsuko Minowa, Aya Kakimoto, Naoto Koyanagi, Akihisa Kato and Yasushi Kawaguchi

Herpes simplex virus 1 Us3 protein kinase phosphorylates threonine at position 887 (Thr-887) in the cytoplasmic tail of envelope glycoprotein B (gB) in infected cells. This phosphorylation down-regulates cell surface expression of gB and plays a role in viral pathogenesis in the mouse herpes stromal keratitis model. In the present study, we demonstrated that Us3 phosphorylation of gB Thr-887 up-regulated accumulation of endocytosed gB from the cell surface of infected cells. We also showed that two motifs in the cytoplasmic tail of gB, tyrosine at position 889 (Tyr-889) and di-leucines at positions 871 and 872, were required for efficient down-regulation of gB cell surface expression and up-regulation of accumulation of endocytosed gB in infected cells. A systematic analysis of mutations in these three sequences in gB suggested that cell surface expression of gB in infected cells was down-regulated in part by the increase in accumulation of endocytosed gB, which was coordinately and tightly regulated by the three gB trafficking signals. Tyr-889 appeared to be of predominant importance in regulating gB intracellular transport, and Tyr-889 was linked to HSV-1 neurovirulence in mice following intracerebral infection. These observations support the hypothesis that HSV-1 evolved the three gB sequences for proper regulation of gB intracellular transport and this regulation has a critical role in diverse aspects of HSV-1 pathogenesis.

2. Herpes Simplex Virus 1 Protein Kinase Us3 and Major Tegument Protein UL47 Reciprocally Regulate Their Subcellular Localization in Infected Cells

Akihisa Kato, Zhuoming Liu, Atsuko Minowa, Takahiko Imai, Michiko Tanaka[1], Ken Sugimoto, Yukihiro Nishiyama[2], Jun Arii, and Yasushi Kawaguchi: [1]Department of Pathology, National Institute of Infectious Disease; [2]De-

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Us3 is a serine-threonine protein kinase encoded by herpes simplex virus 1 (HSV-1). We have identified UL47, a major virion protein, as a novel physiological substrate of Us3. In vitro kinase assays and systematic analysis of mutations at putative Us3 phosphorylation sites near the nuclear localization signal of UL47 showed that serine at residue 77 (Ser-77) was required for Us3 phosphorylation of UL47. Substitution of UL47 Ser-77 by alanine produced aberrant accumulation of UL47 at the nuclear rim and impaired nuclear localization of UL47 in a significant fraction of infected cells. The same defect in UL47 localization was produced by an amino acid substitution in Us3 that inactivated its protein kinase activity. In contrast, a phosphomimetic mutation at UL47 Ser-77 restored wild-type nuclear localization. The UL47 S77A mutation also reduced viral replication in the mouse cornea and development of herpes stroma keratitis in mice. In addition, UL47 formed a stable complex with Us3 in infected cells and nuclear localization of Us3 was significantly impaired in the absence of UL47. These results suggested that Us3 phosphorylation of UL47 Ser -77 promoted nuclear localization of UL47 in cell cultures and had a critical role in viral replication and pathogenesis in vivo. Furthermore, UL47 appeared to be required for efficient nuclear localization of Us3 in infected cells. Therefore, Us3 protein kinase and its UL47 substrate demonstrated a unique regulatory feature in that they reciprocally regulated the subcellular localization of each other in infected cells.

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International Research Center for Infectious Diseases

Pathogenic Microbes Repository Unit
病原微生物資源室

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This unit is collecting standardized bacterial strains and distributing to research organizations, hospital laboratories, and medical educational institutions throughout the country. In addition, under cooperation with the Japanese Society for Bacteriology, we are distributing authorized bacterial strains for microbiology course for medical school.

Our society is always threatened by emerging and reemerging infectious diseases with various kinds of altitude pathogenic microbes owing to increased foreign tourism, import increase including food, food poisoning such as the O-157 epidemic, and bioterrorism. In addition, by advanced medical developments, the aging society, and increased HIV infection, the quick identification of and therapy for opportunistic infection causative agents and multiple drug resistance bacteria have become important in the medical field.

The need for researchers and clinical practitioners specialized in bacteriology and infectious diseases have risen remarkably, and the substantial study and education required is an emergent problem. For thorough study and education, knowledge of bacteriology, a system of collecting pathogenic microorganism strains of reliable origin, to maintain and save them appropriately, and to provide them to cutting-edge researchers or educational establishments is indispensable. However, in Japan, research into pathogenic microorganisms and infectious diseases is performed mainly in universities, where there is no system for conservation and supply. Therefore, valuable bacterial strains have faced disappearance. Furthermore, under the CARTAGENA PROTOCOL ON BIOSAFETY for conventions of biological diversity, the provision and purchase of pathogenic microorganisms from foreign countries has become difficult.

In such circumstances, we are collecting, saving, and analyzing the pathogenicity of microorganisms and distributing pathogenic bacteria to 1) offer type cultures as a positive control in research, education and examinations, 2) prepare pathogenic bacterial strains that have socially high importance, and 3) offer microbes to universities or public research organizations for training or research. We possess about 1,500 strains that almost cover the main pathogenic microbes, including strains valuable internationally such as pathogenic *E. coli* of Orskov's collection, which is stored only in our laboratory in Japan. Furthermore, it is important to secure their utility as type cultures by preparing genomic and genetic information about the pathogenicity of our bacterial collection based on the researches of the Division of Bacterial Infection. Thus, our laboratory is expected to contribute to countermeasures against infectious disease, and to the education and research of medical microbiology in our country.

Collection, preservation and data management of bacterial strains

It is necessary for us to collect representative type strains and the derivatives of pathogenic microbes corresponding to the following six items.

a) Comprehensive collection of genome sequencing strains.
b) The causative agents of hospital-acquired (nosocomial) infection, such as opportunistic infectious bacteria and antibiotic-resistant bacteria.
c) Pathogenic *Escherichia coli* associated with the intestinal and urinary tract or meningeal infections, including *Shigella*, EPEC and EHEC O-157.
d) Intracellular bacterial pathogens such as *Mycobacterium avium* and obligate intracellular bacteria.
e) Zoonotic agents causing brucellosis (*Brucella*), leptospirosis (*Leptospira*), and so on.
f) Pathogens causing newly emerging infections and outbreaks, such as *Helicobacter pylori, Salmonella* spp. and *Clostridium* perfringens.

We dissect the biochemical properties of bacterial strains collected by deposition, and maintain them appropriately. We are also opening the database of our collection to the public.

Distribution of bacterial strains

We are distributing standardized bacterial strains to research organizations, hospital laboratories, and medical educational institutions throughout the country. In addition, under cooperation with the Japanese Society for Bacteriology, we are distributing authorized bacterial strains for microbiology course for medical school.

Value-added creation of a bacterial strain collection by pathogenic analysis

We are analyzing the pathogenicity of pathogenic microorganisms, especially pathogenic *E. coli*, the pathogenicity of new bacterial infection causative agents in cooperation with the Division of Bacterial Infection. Our collection has original added value by offering this information to users.

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.

准教授　医学博士　　三　室　仁　美

Bacteria-gut epithelium 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 gastrointestinal bacteria, such as Helicobacter pylori, enteropathogenic E. coli, and Shigella, 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 vaccines, animal models, and drugs.

1. BabA-mediated adherence is a potentiator of the *Helicobacter pylori* type IV secretion system activity.

Ishijima N, Suzuki M, Ashida H, Ichikawa Y, Kanegae Y[1], Saito I[1], Borén T[2], Haas R[3], Sasakawa C, and Mimuro H.: [1]Laboratory of Molecular Genetics, Institute of Medical Science, University of Tokyo, Tokyo, Japan, [2]Department of Medical Biochemistry and Biophysics, Umeå University, SE-901 87 Umeå, Sweden, [3]Max von Pettenkofer-Institut für Hygiene und Medizinische Mikrobiologie, Department Bakteriologie, Ludwig-Maximilians-Universität, Pettenkoferstrasse 9a, D-80336 München, Germany.

Chronic infection of Helicobacter pylori in the stomach mucosa with translocation of the bacterial cytotoxin-associated gene A (CagA) effector protein via the *cag*-Type IV secretion system (TFSS) into host epithelial cells are major risk factors for gastritis, gastric ulcers, and cancer. The blood group antigen-binding adhesin BabA mediates the adherence of *H. pylori* to ABO/Lewis b (Le^b) blood group antigens in the gastric pit region of the human stomach mucosa. We identified both *in vitro* and *in vivo* that BabA-mediated binding of *H. pylori* to Le^b on the epithelial surface augments TFSS-dependent *H. pylori* pathogenicity by triggering the production of proinflammatory cytokines and precancer-related factors. We successfully generated Le^b-positive cell lineages by transfecting Le^b-negative cells with several glycosyltransferase genes. Using these established cell lines, we found increased mRNA levels of proinflammatory cytokines (*CCL5* and *IL-8*) as well as precancer-related factors (*CDX2* and *MUC2*) after the infection of Le^b-positive cells with WT *H. pylori* but not with *babA* or TFSS deletion mutants. This increased mRNA expression was abrogated when Le^b-negative cells were infected with WT *H. pylori*. Thus, *H. pylori* can exploit BabA-Le^b binding to trigger TFSS-dependent host cell signaling to induce the transcription of genes that enhance inflammation, development of intestinal metaplasia, and associated precan-

cerous transformations.

2. Attenuated CagA oncoprotein in *Helicobacter pylori* from Amerindians in Peruvian Amazon.

Suzuki M, Kiga K, Kersulyte D[1], Cok J[2], Hooper CC[2], Mimuro H, Sanada T, Suzuki S, Oyama M[3], Kozuka-Hata H[3], Kamiya S[4], Zou QM[5], Gilman RH[6], Berg DE[1,7], and Sasakawa C.: [1]Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110, USA, [2]Department of Microbiology, Facultad de Medecina, Universidad Peruana Cayetano Heredia, Lima 31, Peru,: [3]Medical Proteomics Laboratory, Institute of Medical Science, University of Tokyo, Tokyo, Japan, [4]Department of Infectious Diseases, Kyorin University School of Medicine, Tokyo, Japan, [5]Department of Clinical Microbiology and Immunology, College of Medical Laboratory Science, The Third Military Medical University, Chongqing 400038, China, [6]Department of International Health, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland 21205 [7]Departments of Genetics and Medicine, Washington University School of Medicine, St. Louis, Missouri 63110, USA.

Population genetic analyses of bacterial genes whose products interact with host tissues can give new understanding of infection and disease processes. We found that strains of the genetically diverse gastric pathogen *Helicobacter pylori* from Amerindians from the remote Peruvian Amazon contain novel alleles of *cagA*, a major virulence gene, and reveal distinctive properties of their encoded CagA proteins. CagA is injected into the gastric epithelium where it hijacks pleiotropic signaling pathways, helps Hp exploit its special gastric mucosal niche, and affects the risk that infection will result in overt gastroduodenal diseases including gastric cancer. The Amerindian CagA proteins contain unusual but functional tyrosine phosphorylation motifs and attenuated CRPIA motifs, which affect gastric epithelial proliferation, inflammation, and bacterial pathogenesis. Amerindian CagA proteins induced less production of IL-8 and cancer-associated Mucin 2 than did those of prototype Western or East Asian strains and behaved as dominant negative inhibitors of action of prototype CagA during mixed infection of Mongolian gerbils. We suggest that Amerindian *cagA* is of relatively low virulence, that this may have been selected in ancestral strains during infection of the people who migrated from Asia into the Americas many thousands of years ago, and that such attenuated CagA proteins could be useful therapeutically.

3. The *Shigella flexneri* effector OspI deamidates Ubc13 to dampen the inflammatory response.

Sanada T, Kim M, Mimuro H, Suzuki M, Ogawa M, Oyama A, Ashida H, Kobyashi T, Koyama T[1], Nagai S[1], Shibata Y[2], Gohda J[2], Inoue J[2], Mizushima T[3], and Sasakawa C.; [1]Nippon Institute for Biological Science, Shinmachi, Ome, Tokyo, Japan, [2]Division of Cellular and Molecular Biology; Department of Cancer Biology; Institute of Medical Science, University of Tokyo, Tokyo, Japan, [3]Picobiology Institute; Department of Life Science; Graduate School of Life Science; University of Hyogo, Hyogo, Japan.

Upon infection of many bacterial pathogens, bacterial invasion is quickly sensed by the innate immune system and triggers acute inflammatory responses. However, it is still unclear how pathogens modulate host inflammatory responses. We found that a *Shigella* OspI effector delivered via the type III secretion system dampens acute inflammatory responses during bacterial invasion by targeting TNF receptor-associated factor 6 (TRAF6). OspI was a glutamine deamidase and selectively deamidated Gln100 to Glu100 in Ubc13. Consequently, the E 2 ubiquitin-conjugating activity that is required for TRAF6 activation was inhibited, allowing *Shigella* OspI to modulate the diacylglycerol-CBM complex-TRAF6-NF-κB signaling pathway. We determined the 2.0 Å crystal structure of OspI, which contains a putative Cys-His-Asp catalytic triad. A mutational analysis showed that this catalytic triad was essential for deamidation activity. Our results suggest that *Shigella* inhibits acute inflammatory responses at the initial stage of infection by targeting the Ubc13-TRAF6 complex.

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