RESEARCH ACTIVITIES
1. Evaluation of seasonal influenza vaccines for H1N1pdm09 and type B viruses based on a replication-incompetent PB2-KO virus.

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Vaccination is the first line of protection against influenza virus infection in humans. Although inactivated and live-attenuated vaccines are available, each vaccine has drawbacks in terms of immunogenicity and safety. To overcome these issues, our group has developed a replication-incompetent PB2-knockout (PB2-KO) influenza virus that replicates only in PB2-expressing cells. Here we generated PB 2-KO viruses possessing the hemagglutinin (HA) and neuraminidase (NA) segments from H1N1pdm09 or type B viruses and tested their vaccine potential. The two PB2-KO viruses propagated efficiently in PB2-expressing cells, and expressed chimeric HA as expected. Virus-specific IgG and IgA antibodies were detected in mice immunized with the viruses, and the immunized mice showed milder clinical signs and/or lower virus replication levels in the respiratory tract upon virus challenge. Our results indicate that these PB2-KO viruses have potential as vaccine candidates.

2. Broadly Reactive Human Anti-hemagglutinin Stem Monoclonal Antibody That Inhibits Influenza A Virus


Viruses can cause devastating diseases. The long-term goal of our research is to understand the molecular pathogenesis of viral diseases by using influenza and Ebola virus infections as models. Interactions between viral and host gene products during viral replication cycles determine the consequences of infection (i.e., the characteristics of disease manifestation, whether limited or widespread); hence, our research has centered on such interactions in these viral infections.
Many broadly reactive human monoclonal antibodies against the hemagglutinin (HA) stem of influenza A virus have been developed for therapeutic applications. These antibodies typically inhibit viral entry steps, especially the HA conformational change that is required for membrane fusion. To better understand the mechanisms by which such antibodies inhibit viral replication, we established broadly reactive human anti-HA stem antibodies and determined the properties of these antibodies by examining their reactivity with 18 subtypes of HA, evaluating their in vivo protective efficacy, identifying their epitopes, and characterizing their inhibitory mechanisms. Among the eight human monoclonal antibodies we generated, which recognized at least 3 subtypes of the soluble HA antigens tested, clone 59-1-10/5-1 reacted with 18 subtypes of HA and protected mice from lethal infection with H1N1pdm09, H3N2, H5N1, and H7N9 viruses. This antibody recognized the HA2 helix A in the HA stem, and inhibited virus particle release from infected cells but did not block viral entry completely. These results show that broadly reactive human anti-HA stem antibodies can exhibit protective efficacy by inhibiting virus particle release. These findings expand our knowledge of the mechanisms by which broadly reactive stem-targeting antibodies inhibit viral replication and provide valuable information for universal vaccine development.

3. A highly pathogenic avian H7N9 influenza virus isolated from a human is lethal in some ferrets infected via respiratory droplets


Low pathogenic H7N9 influenza viruses have recently evolved to become highly pathogenic, raising concerns of a pandemic, particularly if these viruses acquire efficient human-to-human transmissibility. We compared a low pathogenic H7N9 virus with a highly pathogenic isolate, and two of its variants that represent neuraminidase inhibitor-sensitive and -resistant subpopulations detected within the isolate. The highly pathogenic H7N9 viruses replicated efficiently in mice, ferrets, and/or nonhuman primates, and were more pathogenic in mice and ferrets than the low pathogenic H7N9 virus, with the exception of the neuraminidase inhibitor-resistant virus, which showed mild-to-moderate attenuation. All viruses transmitted among ferrets via respiratory droplets, and the neuraminidase-sensitive variant killed several of the infected and exposed animals. Neuraminidase inhibitors showed limited effectiveness against these viruses in vivo, but the viruses were susceptible to a polymerase inhibitor. These results suggest that the highly pathogenic H7N9 virus has pandemic potential and should be closely monitored.

4. Multi-platform 'Omics Analysis of Human Ebola Virus Disease Pathogenesis

Eisfeld AJ, Halfmann PJ, Wendler JP, Kyle JE, Burnum-Johnson KE, Peralta Z, Maemura T, Walters KB, Watanabe T, Fukuyama S, Yamashita M, Jacobs JM, Kim Y-M, Casey CP, Stratton KG, Webb-Robertson BM, Gritsenko MA, Monroe ME, Weitz KK, Shukla AK, Tian M, Neumann G, Reed JL, van Bakel H, Metz TO, Smith RD, Waters KM, N’jai A, Sahr F, Kawaoa Y: 'Biological Sciences Division, Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory (PNNL), USA,'Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai (ISMMS), USA, 'Computing and Analytics Division, National Security Directorate, PNNL, USA,'Department of Chemical and Biological Engineering, UW-Madison, USA, 'Department of Biological Sciences, Fourah Bay College, College of Medicine & Allied Health Sciences, University of Sierra Leone, Sierra Leone, '34(th) Regimental Military Hospital at Wilberforce, Sierra Leone."

The pathogenesis of human Ebola virus disease (EVD) is complex. EVD is characterized by high levels of virus replication and dissemination, dysregulated immune responses, extensive virus- and host-mediated tissue damage, and disordered coagulation. To clarify how host responses contribute to EVD pathophysiology, we performed multi-platform 'omics analysis of peripheral blood mononuclear cells and plasma from EVD patients. Our results indicate that EVD molecular signatures over-
lap with those of sepsis, imply that pancreatic enzymes contribute to tissue damage in fatal EVD, and suggest that Ebola virus infection may induce aberrant neutrophils whose activity could explain hallmarks of fatal EVD. Moreover, integrated biomarker prediction identified putative biomarkers from different data platforms that differentiated survivors and fatalities early after infection. This work reveals insight into EVD pathogenesis, suggests an effective approach for biomarker identification, and provides an important community resource for further analysis of human EVD severity.

5. Syrian hamster as an animal model for the study of human influenza virus infection.

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Ferrets and mice are frequently used as animal models for influenza research. However, ferrets are demanding in terms of housing space and handling, whereas mice are not naturally susceptible to infection with human influenza A or B viruses. Therefore, prior adaptation of human viruses is required for their use in mice. In addition, there are no mouse-adapted variants of the recent H3N2 viruses, because these viruses do not replicate well in mice. In this study, we investigated the susceptibility of Syrian hamsters to influenza viruses with a view to using them as an alternative animal model to mice. We found that hamsters are sensitive to influenza viruses, including the recent H3N2 viruses, without adaptation. Although the hamsters did not show weight loss or clinical signs of H3N2 virus infection, we observed pathogenic effects in the respiratory tracts of the infected animals. All of the H3N2 viruses tested replicated in the respiratory organs of the hamsters, and some of them were detected in the nasal washes of infected animals. Moreover, a pdm09 and a seasonal H1N1 virus, as well as one of the two H3N2 viruses, but not a type B virus, were airborne transmissible in these hamsters. Hamsters thus have potential as a small animal model for the study of influenza virus infection, including studies of the pathogenicity of H3N2 viruses and other strains, as well as H1N1 virus transmission studies.

Publications


20. Watanabe T, Imai M, Kawaoka Y. NSI is the fluid for "flu-transmission". Proc Natl Acad Sci U S A. 14: 11012-11014, 2017


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Immune cells express multiple Toll-like receptors (TLRs) that are concomitantly activated by a variety of pathogen products derived from microbes and viruses. TLRs also sense host derived products such as RNAs and DNAs. Recent reports have indicated that losing the balance of TLRs responses result in autoimmune diseases. Hence, there must exist regulatory mechanisms coordinating the expression, the localization and the function of TLRs to avoid excessive immune responses for endogenous ligands. We have found recently a candidate for endogenous ligand for TLRs. Our research focuses on regulatory mechanisms controlling pathogenic ligand recognition by TLRs.

1. The protective effect of the anti-Toll-like receptor 9 antibody against acute cytokine storm caused by immunostimulatory DNA

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Toll-like Receptor 9 (TLR9) is an innate immune receptor recognizing microbial DNA. TLR9 is also activated by self-derived DNA, such as mitochondrial DNA, in a variety of inflammatory diseases. We show that TLR9 activation in vivo is controlled by an anti-TLR9 monoclonal Ab (mAb). A newly established mAb, named NaR9, clearly detects endogenous TLR9 expressed in primary immune cells. NaR9 inhibited TLR9-dependent cytokine production in vitro by bone marrow-derived macrophages and conventional dendritic cells, but not plasmacytoid dendritic cells. The difference of inhibitory effect among cell types depends on the uptake activity of antibody. Furthermore, NaR9 treatment rescued mice from fulminant hepatitis caused by administering the TLR9 ligand CpGB and D-(+)-galactosamine. The production of proinflammatory cytokines induced by CpGB and D-(+)-galactosamine was significantly impaired by the mAb. These results suggest that a mAb is a promising tool for therapeutic intervention in TLR9-dependent inflammatory diseases.

2. Licensing Toll-like receptor 7 to induce type I interferon by CD11a/CD18 Integrin

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Plasmacytoid dendritic cells (pDCs) sense viral RNA through Toll-like receptor (TLR) 7 and produce type I interferons (IFN-1) to initiate pDC responses against viral infection. pDCs form clusters upon virus infection and cell adhesion enhances IFN-1 responses. Little is known, however, about the molecular mechanism linking cell adhesion with IFN-1 expression. Here we show that cell adhesion licenses TLR7 to traffic for IFN-1 induction. Liganded TLR7 trafficked to mTORC1 and induced association of TRAF6 with mTORC1. Liganded TLR7 trafficked to mTORC1 and induced association of TRAF6 with TRAF3, constitutively associated factor 3 (TRAF3), and interferon regulatory factor 7 (IRF7). IFN-1 was produced predominantly in pDCs in cell cluster rather than isolated pDCs. These results suggest that IFN-1 induction by TLR7 is limited to clustered pDCs through licensing by cell adhesion molecules.

3. ADP-ribosylation factor-like 8b is required for development of Systemic Lupus Erythematosus in BXSB.Yaa mice

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Toll-like receptor 7 (TLR7) in the endolysosome is a sensor for single-stranded RNA (ssRNA) from viruses and it induces antiviral immune response. In addition, this receptor also responds to synthetic endogenous ligands for TLR7.

4. Guanosine and its modified derivatives are endogenous ligands for TLR7

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Toll-like receptor 7 (TLR7) in the endolysosome is a sensor for single-stranded RNA (ssRNA) from viruses and it induces antiviral immune response. In addition, this receptor also responds to synthetic endogenous ligands for TLR7.

Plasmacytoid dendritic cell (pDC) senses viral RNA through Toll-like receptor (TLR7) and expresses type I interferons (IFN-1) to induce defense responses against viruses. pDC also responds to self RNA and expresses IFN-1, which plays pathogenic roles in systemic lupus erythematosus (SLE). We have reported the requirement of ADP-ribosylation factor-like 8b (Arl8b) for TLR7 dependent IFN-1 production in pDC. We here studied the role of Arl8b in a well-studied SLE model, BXSB.Yaa mice. Arl8b<sup>Gt/Gt</sup> gene trap mice were back-crossed more than 13 times with BXSB. Yaa mice. BXSB.Yaa mice began to die from 13 weeks old, and 9 out of 10 mice died by 33 weeks old. Our data suggest the key role of Arl8b in the SLE model, probably by enabling TLR7 dependent IFN-1 production in pDC. Our data suggest that Arl8b is an attractive new target for therapeutic intervention in SLE.
Publications


Hansbro PM, Haw TJ, Starkey MR, Miyake K. Toll-like receptors in COPD. Eur Respir J. 49. 2017


1. Development of nanogel-based nasal vaccination system for various infectious diseases

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Nasal vaccination is one of the most effective immunization methods because it can induce effective antigen-specific immune responses not only at the mucosal site of administration but also at distant mucosal surfaces, as well as in the systemic compartment. Based on this advantage, we have been promoting the development of novel nasal vaccination system using cholesteryl group-bearing pullulan (CHP) nanogels. CHP nanogels have been developed as novel drug delivery system, and a cationic CHP nanogels have been demonstrated to induce effective immunity as a nasal vaccine antigen carrier. Since vaccine antigens incorporated into CHP nanogels have exhibited no brain deposition after nasal administration in mice and nonhuman primates, the vaccine seems safe, and could be a promising new delivery system. Recently we have established the CHP nanogel-based vaccines against various infectious pathogens such as S. pneumoniae to combine specific recombinant protein antigens respectively. In both cases, antigen specific antibody responses or cell mediated immunity was effectively induced after nasal vaccine administration. Moreover, we demonstrated the efficacy of the vaccination in the murine bacteria airway infection model. Thus, CHP nanogel-based nasal vaccination system provide effective approach for various infectious diseases.

2. The development of nasal anti-hypertension vaccine

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Mucosal surfaces are the first line of host defense against foreign substances such as pathogenic microorganisms and allergens. In addition, the mucosal immune system not only senses harmful foreign antigens, but also establishes a tolerance that does not react excessively to antigens such as food-derived proteins and commensal bacteria. Our mission is the understanding molecular and cellular aspects of the mucosal immune system, providing mucosal vaccines to prevent infectious diseases, and establishing mucosal immune therapy to control food allergy and autoimmune diseases such as inflammatory bowel diseases.
3. Investigation on the crosstalk between nasal- and protect from lethal pneumococcal infection.

Objective: To combat global increases in the prevalence of lifestyle-related diseases and concomitant infectious diseases, we aimed to develop an innovative intranasal vaccine that simultaneously targets both hypertension and pneumonia, is not given by invasive injection, and offers prolonged therapeutic effect and reduced frequency of administration. Methods: AT1R-PspA vaccine, consisting of a cationic nanogel incorporating angiotensin II type 1 receptor (AT1R) partial peptide conjugated with pneumococcal surface protein A (PspA) and cyclic di-GMP adjuvant, was created and given intranasally to spontaneously hypertensive rats (SHRs). Antigen-specific antibodies and blood pressure were examined to evaluate immune responses and the anti-hypertensive effect of the vaccine. To examine the protective effect of antibodies induced by vaccination on pneumococcal infection, sera obtained from immunized SHRs were incubated with a lethal dose of Streptococcus pneumoniae and then administered to mice. Results: Five doses of AT1R-PspA nasal vaccine induced AT1R-specific serum IgG antibody production and attenuated the development of hypertension in SHRs in the long term. Both in vitro and in vivo studies revealed that responses to angiotensin II were suppressed in vaccinated rats. Anti-AT1R IgG antibody incubated with angiotensin II in rat aortic vascular smooth muscles directly inhibited downstream AT1R signaling pathways. Mice passively immunized with sera obtained from AT1R-PspA-vaccinated SHRs were protected from lethal pneumococcal infection. Conclusions: Intranasal immunization with AT1R-PspA vaccine has the potential to simultaneously attenuate the development of hypertension and protect from lethal pneumococcal infection.

4. Analysis of miRNA candidates as biomarkers for prediction and evaluation of mucosal vaccination

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We have been developing a rice-based oral vaccine system, MucoRice system, as a next generation of mucosal vaccine. MucoRice-CTB is a vaccine that...
incorporates a cholera toxin B subunit (CTB) which does not have toxicity into rice by genetic engineering technologies. When the MucoRice-CTB is administrated orally to mice, pigs and macaques, cholera toxin-induced diarrhea is inhibited by antigen-specific secretory IgA with its neutralizing activity. Recently we reported the first production according to current Good Manufacturing Practices of the MucoRice-CTB at an academic institution. Then, we conducted a doctor-led Phase I clinical trial using MucoRice-CTB at the Hospital of Institute of Medical Science, the University of Tokyo since 2015 to 2016. In the Phase I clinical trial, the dose of MucoRice-CTB was performed by 3 cohorts of 1 g (CTB 3 mg), 3 g (CTB 9 mg), 6 g (CTB 18 mg), with a double blind test on 20-40 years old healthy adult male who have no allergic reaction to rice. MucoRice-CTB was administered every two weeks, four times, and CTB-specific antibody titer in serum and feces in each subject was confirmed by ELISA assay. In parallel, we proceeded with the search of serum miRNA biomarkers using microarray analysis. In recent years, several miRNAs have been identified as biomarkers for disease discrimination. In this study, we aimed to investigate the miRNA biomarkers for evaluating the effect of mucosal vaccine by identifying serum miRNA that are specifically induced by administration of MucoRice-CTB. Moreover, we would like to analyze whether the responsiveness to MucoRice-CTB is due to the difference in potentially expressed miRNA leading to the miRNA biomarkers for predicting susceptibility to mucosal vaccine.

5. Innate and adaptive immune cells regulate Paneth cell granule formation and α-defensin secretion

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The gastrointestinal tract is constantly exposed to numerous foreign antigens. Intestinal epithelial cell layer acts as a first line of defense and is divided into villi and crypt regions. In the crypts, epithelial stem cells and Paneth cells are preferentially located. Paneth cells release granules containing a variety of antimicrobial peptides as a major part of the host innate immune system. α-defensin is most abundant and highly bactericidal peptide specifically produced by Paneth cells.

It has been known that crypts are surrounded by immune cells. Type3 innate lymphoid cells located beneath of crypts preferentially produce Interleukin 22 (IL-22). We found that IL-22 induces the expression of the glutathione peroxidase gene family and promotes the differentiation of Paneth cells with matured granules containing α-defensin. The lack of IL-22 thus resulted in the decreased amount of fecal α-defensin. We further found that Rag1-deficient mice which lack both T and B cells had also reduced amount of fecal α-defensin. In addition, Rag1-deficient mice had lower expression of Rab-family gene which are known to regulate granule secretion. These results indicated that granule release of Paneth cells is regulated by acquired immune cells via Rab-family gene expression.

Our results indicate that the cell fate and function of Paneth cells are dually regulated by innate and adaptive immune cells for the production and secretion of α-defensin in gastrointestinal tract. α-defensin plays a crucial role for the creation and maintenance of intestinal homeostasis, thus we concluded that the mutual interaction of Paneth cells and immune cells provide healthy intestinal environment.

6. Functional analysis of the tissue-specific molecule expressed by skin mast cells

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Mast cells (MCs) are located at the tissues associated with body surface such as skin and mucosa. These tissues are continuously exposed to physical and chemical stimuli leading to the unexpected activation of MCs. Once MC activation occurs locally, various inflammatory mediators are released and excessive immune reactions such as allergic and inflammatory responses are subsequently induced. To avoid unnecessary activation of MCs and maintain appropriate immunological homeostasis, there exists a unique suppressive pathway in MCs, mediated by fibroblasts. In this study, a novel regulatory pathway mediated by skin fibroblasts via the usage of a novel molecule X in skin MCs was newly identified. Molecule X was specifically and highly expressed by skin MCs (unpublished data). In addition, in vitro co-culture of bone marrow-derived MCs and skin fibroblasts revealed that the expression of molecule X on MCs was induced by skin fibroblasts. Molecule X deficient and WT mice were subjected to hapten-induced contact dermatitis and it was demonstrated that deficiency of Molecule X enhanced ear swelling response and vascular permeability in comparison to WT mice. These results revealed that the skin MCs and fibroblasts form anti-inflammatory pathway by cell to cell interaction using molecule X.

Publications

Journals (Refereed)

Reviews (Refereed)

Japanese Journals and Reviews

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

Naoto Koyanagi, Takahiko Imai, Keiko Shindo, Ayuko Sato, Wataru Fujii, Takeshi Ichinohe, Naoki Takemura, Shigeru Kakuta, Satoshi Uematsu, Hiroshi Kiyono, Yuhei Maruzuru, Jun Arii, Akihisa Kato, and Yasushi Kawaguchi: Department of Mucosal Immunology, Division of Molecular Virology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

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

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

To date, approximately 250 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 and manifest diseases in their hosts. Our goal is to apply our fundamental findings for the development of anti-herpetic drugs and vaccines for the control of these viral infections.
Upon herpes simplex virus 1 (HSV-1) infection, the CD98 heavy chain (CD98hc) is redistributed around the nuclear membrane (NM), where it promotes viral de-envelopment during the nuclear egress of nucleocapsids. In this study, we attempted to identify the factor(s) involved in CD98hc accumulation and demonstrated the following: (i) the null mutation of HSV-1 UL34 caused specific dispersion throughout the cytoplasm of CD98hc and the HSV-1 de-envelopment regulators, glycoproteins B and H (gB and gH); (ii) as observed with CD98hc, gB, and gH, wild-type HSV-1 infection caused redistribution of the endoplasmic reticulum (ER) markers calnexin and ERP57 around the NM, whereas the UL34-null mutation caused cytoplasmic dispersion of these markers; (iii) the ER markers colocalized efficiently with CD98hc, gB, and gH in the presence and absence of UL34 in HSV-1-infected cells; (iv) at the ultrastructural level, wild-type HSV-1 infection caused ER compression around the NM, whereas the UL34-null mutation caused cytoplasmic dispersion of the ER; and (v) the UL34-null mutation significantly decreased the colocalization efficiency of lamin protein markers of the NM with CD98hc and gB. Collectively, these results indicate that HSV-1 infection causes redistribution of the ER around the NM, with resulting accumulation of ER-associated CD98hc, gB, and gH around the NM and that UL34 is required for ER redistribution, as well as for efficient recruitment to the NM of the ER-associated de-envelopment factors. Our study suggests that HSV-1 induces remodeling of the global ER architecture for recruitment of regulators mediating viral nuclear egress to the NM.

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

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

Publications


Inoue, Y., Saga, T., Aikawa, T., Kumagai, M., Shimada, A., Kawaguchi, Y., Naruse, K., Morishita, S., Koga, A. and Takeda, H. OPEN Complete fusion of a transposon and herpesvirus created the
Teratorn mobile element in medaka fish. Nat. Commun. 8: 551, 2017