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ウイルス感染分野

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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.

1. Baloxavir marboxil treatment of nude mice infected with influenza A virus.

Kiso M, Yamayoshi S, Murakami J, Kawaoka Y:

Immunocompromised patients infected with influenza virus require prolonged treatment with neuraminidase inhibitors, because these patients are not able to eradicate the virus from the respiratory tract, leading to the emergence of drug-resistant mutant viruses. Here, we examined the efficacy of baloxavir marboxil in nude mice, which are immunologically deficient. Daily treatment with a suboptimal dose of baloxavir marboxil increased the survival time of the virus-infected nude mice but did not clear the virus from their respiratory organs, resulting in gradual body weight loss after termination of treatment. Despite the prolonged baloxavir marboxil treatment, few resistant mutants were detected.

2. Influenza A variants with reduced susceptibility to baloxavir isolated from Japanese patients are fit and transmit through respiratory droplets.

Imai M, Yamashita M, Sakai-Tagawa Y, Iwatsuki-Horimoto K, Kiso M, Murakami J, Yasuhara A, Takada K, Ito M, Nakajima N[1], Takahashi K[1], Lopes TJS[2], Dutta J[3], Khan Z[3], Kriti D[3], van Bakel H[3], Tokita A[5], Hagiwara H[5], Izumida N[5], Kuroki H[6], Nishino T[5], Wada N[5], Koga M[7], Adachi E[8], Jubishi D, Hasegawa H[9], Kawaoka Y:[1]Department of Pathology, National Institute of Infectious Diseases, Japan. [2]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, USA. [3]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, USA. [5]Members of the Tokyo Pediatric Association Public Health Committee, Japan. [6]Sotobo Children's Clinic, Japan. [7]Division of Infectious Diseases, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Japan.

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Here we report the isolation of the influenza A/H1N1 2009 pandemic (A/H1N1pdm) and A/H3N2 viruses carrying an I38T mutation in the polymerase acidic protein-a mutation that confers reduced susceptibility to baloxavir marboxil-from patients before and after treatment with baloxavir marboxil in Japan. These variants showed replicative abilities and pathogenicity that is similar to those of wild-type isolates in hamsters; they also transmitted efficiently between ferrets by respiratory droplets.

3. Influenza Virus Polymerase Mutation Stabilizes a Foreign Gene Inserted into the Virus Genome by Enhancing the Transcription/Replication Efficiency of the Modified Segment.

Furusawa Y, Yamada S, Lopes TJS[2], Dutta J[3], Khan Z[3], Kriti D[3], van Bakel H[3], Kawaoka Y:

We previously attempted to establish a reporter influenza virus by inserting the gene for the Venus fluorescent protein into the NS segment of influenza A/Puerto Rico/8/34 (PR8, H1N1) virus to yield WT-Venus-PR8. Although the inserted Venus gene was deleted during serial passages of WT-Venus-PR8, we discovered that the PB2-E712D mutation stabilizes the Venus gene. Here, we explored the mechanisms by which Venus gene deletion occurs and how the polymerase mutation stabilizes the Venus gene. Deep sequencing analysis revealed that PB2-E712D does not cause an appreciable change in the mutation rate, suggesting that the stability of the Venus gene is not affected by polymerase fidelity. We found by using quantitative real-time PCR that WT-Venus-PR8 induces high-level interferon beta (IFN-β) expression. The induction of IFN-β expression seemed to result from the reduced transcription/replication efficiency of the modified NS segment in WT-Venus-PR8. In contrast, the transcription/replication efficiency of the modified NS segment was enhanced by the PB2-E712D mutation. Loss of the Venus gene in WT-Venus-PR8 appeared to be caused by internal deletions in the NS segment. Moreover, to further our understanding of the Venus stabilization mechanisms, we identified additional amino acid mutations in the virus polymerase complex that stabilize the Venus gene. We found that some of these amino acids are located near the template exit or the product exit of the viral polymerase, suggesting that these amino acids contribute to the stability of the Venus gene by affecting the binding affinity between the polymerase complex and the RNA template and product.

4. A humanized MDCK cell line for the efficient isolation and propagation of human influenza viruses.

Takada K, Kawakami C[10], Fan S[2], Chiba S[2], Zhong G[2], Gu C[2], Shimizu K[10], Takasaki S, Sakai-Tagawa Y, Lopes TJS[2], Dutta J[3], Khan Z[3], Kriti D[3], van Bakel H[3], Yamada S, Watanabe T, Imai M, Kawaoka Y: [10]Yokohama City Institute of Public Health, Japan

Here, we developed hCK, a Madin-Darby canine kidney (MDCK) cell line that expresses high levels of human influenza virus receptors and low levels of avian virus receptors. hCK cells supported human A/H3N2 influenza virus isolation and growth much more effectively than conventional MDCK or human virus receptor-overexpressing (AX4) cells. A/H3N2 viruses propagated in hCK cells also maintained higher genetic stability than those propagated in MDCK and AX4 cells.

5. Antigenic drift originating from changes to the lateral surface of the neuraminidase head of influenza A virus.

Yasuhara A, Yamayoshi S, Kiso M, Sakai-Tagawa Y, Koga M[7], Adachi E[8], Kikuchi T[11], Wang IH, Yamada S, Kawaoka Y: [11]AIDS Research Center, National Institute of Infectious Diseases, Japan.

Influenza viruses possess two surface glycoproteins, haemagglutinin and neuraminidase (NA). Although haemagglutinin plays a major role as a protective antigen, immunity to NA also contributes to protection. The NA protein consists of a stalk and a head portion, the latter of which possesses enzymatic NA (or sialidase) activity. Like haemagglutinin, NA is under immune pressure, which leads to amino acid alterations and antigenic drift. Amino acid changes accumulate around the enzymatic active site, which is located at the top of the NA head. However, amino acid alterations also accumulate at the lateral surface of the NA head. The reason for this accumulation remains unknown. Here, we isolated seven anti-NA monoclonal antibodies (mAbs) from individuals infected with A(H1N1)pdm09 virus. We found that amino acid mutations on the lateral surface of the NA head abolished the binding of all of these mAbs. All seven mAbs activated Fcγ receptor (FcγR)-mediated signalling pathways in effector cells and five mAbs possessed NA inhibition activity, but the other two did not; however, all seven protected mice from lethal challenge infection through their NA inhibition activity and/or FcγR-mediated antiviral activity. Serological analysis of individuals infected with A(H1N1)pdm09 virus revealed that some possessed or acquired the anti-NA-lateral-surface antibodies following infection. We also found antigenic drift on the lateral surface of the NA head of isolates from 2009 and

2015. Our results demonstrate that anti-lateral-surface mAbs without NA inhibition activity can provide protection by activating FcγR-mediated antiviral activity and can drive antigenic drift at the lateral surface of the NA head. These findings have implications for NA antigenic characterization in that they demonstrate that traditional NA inhibition assays are inadequate to fully characterize NA antigenicity.

Publications

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