

28 April 2009

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## **Chem-Bio News – Pandemic Influenza Edition #58**

- 1. 'TOO LATE' TO CONTAIN SWINE FLU:** *"The swine flu virus first detected in Mexico can no longer be contained and countries should focus on mitigating its effects, a top UN official said."*
- 2. HK STEPS UP SARS-LIKE EMERGENCY PRECAUTIONS AGAINST SWINE FLU:** *"Hong Kong has activated SARS-like emergency preparations for the deadly swine flu, raising its alert level to "serious".*
- 3. MITOGEN-ACTIVATED PROTEIN KINASE-ACTIVATED KINASE RSK2 PLAYS A ROLE IN INNATE IMMUNE RESPONSES TO INFLUENZA VIRUS INFECTION:** *"These findings establish a role for RSK2 in the cellular antiviral response."*
- 4. MONOCLONAL ANTIBODIES AGAINST THE FUSION PEPTIDE OF HEMAGGLUTININ PROTECT MICE FROM LETHAL INFLUENZA A VIRUS H5N1 INFECTION:** *"The study shows that MAb 1C9, which is specific to the antigenically conserved fusion peptide of HA2, can contribute to the cross-clade protection of mice infected with H5N1 virus and mediate more effective recovery from infection."*
- 5. INFLUENZA VIRUS AND POLY(I:C) INHIBIT MHC CLASS I-RESTRICTED PRESENTATION OF CELL-ASSOCIATED ANTIGENS DERIVED FROM INFECTED DEAD CELLS CAPTURED BY HUMAN DENDRITIC CELLS:** *"Thus, DCs [dendritic cells] have a mechanism that prevents MHC [major histocompatibility complex] class I-restricted cross-presentation of cell-associated Ag when they have captured dead infected cells."*
- 6. DEVELOPMENTAL REGULATION OF MHC II EXPRESSION AND TRANSPORT IN HUMAN PLASMACYTOID-DERIVED DENDRITIC CELLS:** *"Our data demonstrate that the regulation of MHC II expression and transport is drastically different in pDCs compared with conventional DCs, indicating distinct and potentially complementary immunoregulatory functions."*
- 7. ANTIGENIC FINGERPRINTING OF H5N1 AVIAN INFLUENZA USING CONVALESCENT SERA AND MONOCLONAL ANTIBODIES REVEALS POTENTIAL VACCINE AND DIAGNOSTIC TARGETS:** *"This is the first study, to our knowledge, describing the complete antibody repertoire following H5N1 infection."*
- 8. INFERRING STABILIZING MUTATIONS FROM PROTEIN PHYLOGENIES: APPLICATION TO INFLUENZA HEMAGGLUTININ:** *"We present a conceptual framework that explains how this requirement causes the probability that a particular amino acid mutation is fixed during evolution to depend on its effect on protein stability."*
- 9. WORLD COUNTING DOWN TO PANDEMIC, SAYS TOP VIROLOGIST:** *"I think the spread of this virus in humans cannot possibly be contained within a short time ... there are already cases in almost every region. The picture is changing every moment."*
- 10. NEW RESPIRATORY ENTEROVIRUS AND RECOMBINANT RHINOVIRUSES AMONG CIRCULATING PICORNAVIRUSES:** *"Finally, we identified recombinants among circulating rhinoviruses and mapped their recombination sites, thereby demonstrating that rhinoviruses can recombine in their natural host."*

**CB Daily Report**

### **'TOO LATE' TO CONTAIN SWINE FLU**

BBC

April 28, 2009

"The swine flu virus first detected in Mexico can no longer be contained and countries should focus on mitigating its effects, a top UN official said.

World Health Organization deputy chief Keiji Fukuda was speaking as the WHO raised its alert level to four, or two steps short of a full pandemic."

"Mr Fukuda said this was a "significant step towards pandemic influenza" but a pandemic should not be considered inevitable."

Experts did not recommend closing borders or restricting travel, he stressed.

"With the virus being widespread... closing borders or restricting travel really has very little effects in stopping the movement of this virus," he said."

#### **"WHO PANDEMIC ALERT PHASES**

Phase 1: No viruses circulating among animals causing infections in humans

Phase 2: Animal influenza virus causes infection in humans, and is considered potential pandemic threat

Phase 3: Influenza causes sporadic cases in people, but no significant human-to-human transmission

Phase 4: Verified human-to-human transmission able to cause community-level outbreaks. Significant increase in risk of a pandemic

Phase 5: Human-to-human transmission in at least two countries. Strong signal pandemic imminent

Phase 6: Virus spreads to another country in a different region. Global pandemic under way

The full article can be found at: <http://news.bbc.co.uk/1/hi/world/americas/8021827.stm>

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### **HK STEPS UP SARS-LIKE EMERGENCY PRECAUTIONS AGAINST SWINE FLU**

By Leslie Tang

Asia Pacific News

April 28, 2009

"Hong Kong has activated SARS-like emergency preparations for the deadly swine flu, raising its alert level to "serious".

Officials said chances are likely the flu will spread to the city. But the two women and a child, who were suspected to have caught the virus after travelling to the United States and Mexico, have been cleared by health authorities.

Hong Kong was badly hit in 2003 by the SARS outbreak and now, with another possible pandemic at large, the government is not taking any chances."

"The Hong Kong International Airport has stepped up temperature screening at all checkpoints. Those who are down with a fever and who have travelled to affected areas in the past seven days, will be sent to hospital for tests immediately."

The full article can be found at:

<http://www.channelnewsasia.com/stories/eastasia/view/425512/1/.html>

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## **MITOGEN-ACTIVATED PROTEIN KINASE-ACTIVATED KINASE RSK2 PLAYS A ROLE IN INNATE IMMUNE RESPONSES TO INFLUENZA VIRUS INFECTION**

Drug Week

May 1, 2009

"Viral infections induce signaling pathways in mammalian cells that stimulate innate immune responses and affect cellular processes, such as apoptosis, mitosis, and differentiation. Here, we report that the ribosomal protein S6 kinase alpha 3 (RSK2), which is activated through the "classical" mitogen-activated protein kinase pathway, plays a role in innate immune responses to influenza virus infection."

"RSK2 functions in the regulation of cell growth and differentiation but was not known to play a role in the cellular antiviral response. We have found that knockdown of RSK2 enhanced viral polymerase activity and growth of influenza viruses. Influenza virus infection stimulates NK-kappa B- and beta interferon-dependent promoters. This stimulation was reduced in RSK2 knockdown cells, suggesting that RSK2 executes its effect through innate immune response pathways. Furthermore, RSK2 knockdown suppressed influenza virus-induced phosphorylation of the double-stranded RNA-activated protein kinase PKR, a known antiviral protein."

"These findings establish a role for RSK2 in the cellular antiviral response."

The full article can be found at: (Mitogen-Activated Protein Kinase-Activated Kinase RSK2 Plays a Role in Innate Immune Responses to Influenza Virus Infection. Journal of Virology, 2009;83(6):2510-2517). Link not available.

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## **MONOCLONAL ANTIBODIES AGAINST THE FUSION PEPTIDE OF HEMAGGLUTININ PROTECT MICE FROM LETHAL INFLUENZA A VIRUS H5N1 INFECTION**

Biotech Week

April 29, 2009

"The HA2 glycopolyptide (gp) is highly conserved in all influenza A virus strains, and it is known to play a major role in the fusion of the virus with the endosomal membrane in host cells during the course of viral infection. Vaccines and therapeutics targeting this HA2 gp could induce efficient broad-spectrum immunity against influenza A virus infections."

"So far, there have been no studies on the possible therapeutic effects of monoclonal antibodies (MAbs), specifically against the fusion peptide of hemagglutinin (HA), upon lethal infections with highly pathogenic avian influenza (HPAI) H5N1 virus. We have identified MAb 1C9, which binds to GLFGAIAGF, a part of the fusion peptide of the HA2 gp. We evaluated the efficacy of MAb 1C9 as a therapy for influenza A virus infections. This MAb, which inhibited cell fusion in vitro when administered passively, protected 100% of mice from challenge with five 50% mouse lethal doses of HPAI H5N1 influenza A viruses from two different clades. Furthermore, it caused earlier clearance of the virus from the lung. The influenza virus load was assessed in lung samples from mice challenged after pretreatment with MAb 1C9 (24 h prior to challenge) and from mice receiving early treatment (24 h after challenge)."

"The study shows that MAb 1C9, which is specific to the antigenically conserved fusion peptide of HA2, can contribute to the cross-clade protection of mice infected with H5N1 virus and mediate more

effective recovery from infection."

The full article can be found at: (N. Pabhu, et. al., "Monoclonal Antibodies against the Fusion Peptide of Hemagglutinin Protect Mice from Lethal Influenza A Virus H5N1 Infection". Journal of Virology, 2009;83(6):2553-2562). Link not available.

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### **INFLUENZA VIRUS AND POLY(I:C) INHIBIT MHC CLASS I-RESTRICTED PRESENTATION OF CELL-ASSOCIATED ANTIGENS DERIVED FROM INFECTED DEAD CELLS CAPTURED BY HUMAN DENDRITIC CELLS**

Preventive Medicine Week

May 3, 2009

"During viral infection, dendritic cells (DCs) capture infected cells and present viral Ags to CD8(+) T cells. However, activated DCs might potentially present cell-associated Ags derived from captured dead cells."

"In this study, we find that human DCs that captured dead cells containing the TLR3 agonist poly(I:C) produced cytokines and underwent maturation, but failed to elicit autologous CD8(+) T cell responses against Ags of dead cells. Accordingly, DCs that captured dead cells containing poly(I:C), or influenza virus, are unable to activate CD8(+) T cell clones specific to cell-associated Ags of captured dead cells. CD4(+) T cells are expanded with DCs that have captured poly (I:C)-containing dead cells, indicating the inhibition is specific for MHC class I-restricted cross-presentation. Furthermore, these DCs can expand naive allogeneic CD8(+) T cells. Finally, soluble or targeted Ag is presented when coloaded onto DCs that have captured poly(I:C)-containing dead cells, indicating the inhibition is specific for dead cell cargo that is accompanied by viral or poly(I:C) stimulus. Thus, DCs have a mechanism that prevents MHC class I-restricted cross-presentation of cell-associated Ag when they have captured dead infected cells."

The full article can be found at: (D. Frleta, et. al., "Influenza Virus and Poly(I:C) Inhibit MHC Class I-Restricted Presentation of Cell-Associated Antigens Derived from Infected Dead Cells Captured by Human Dendritic Cells". Journal of Immunology, 2009;182(5):2766-2776). Link not available.

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### **DEVELOPMENTAL REGULATION OF MHC II EXPRESSION AND TRANSPORT IN HUMAN PLASMACYTOID-DERIVED DENDRITIC CELLS**

Drug Week

April 24, 2009

"Plasmacytoid predendritic cells (pDCs) play a key role in antiviral immunity through their capacity to produce large amounts of type I interferons in response to Toll-like receptor triggering, and to differentiate into dendritic cells (DCs). However, their antigen processing and presentation pathways remain poorly characterized."

"In this study, we analyzed major histocompatibility complex class II (MHC II) synthesis and transport in primary human pDCs. We show that stimulation of pDCs with influenza virus leads to a sustained neosynthesis of MHC II molecules, which rapidly accumulate in antigen loading compartments organized around the microtubule organization center. MHC II endocytosis as well as antigen internalization remain active during the entire process of pDC differentiation into DCs, suggesting a capacity to constantly renew surface peptide-MHC II complexes. Formation of the intracellular pool of MHC II in activated pDCs is nuclear factor-kappa B-dependent and associated with acquisition of a dendritic phenotype, but independent of the IRF7-type I interferon-dependent pathway, suggesting that innate and adaptive functions of pDCs are differentially regulated."

"Our data demonstrate that the regulation of MHC II expression and transport is drastically different in

pDCs compared with conventional DCs, indicating distinct and potentially complementary immunoregulatory functions.”

The full article can be found at: (C. Sadaka, et. al., “Developmental regulation of MHC II expression and transport in human plasmacytoid-derived dendritic cells”. *Blood*, 2009;113(10):2127-2135). Link not available.

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## **ANTIGENIC FINGERPRINTING OF H5N1 AVIAN INFLUENZA USING CONVALESCENT SERA AND MONOCLONAL ANTIBODIES REVEALS POTENTIAL VACCINE AND DIAGNOSTIC TARGETS**

By Surender Khurana, Amorsolo L. Suguitan, Jr., Yonaira Rivera, Cameron P. Simmons, Antonio Lanzavecchia, Federica Sallusto, Jody Manischewitz, Lisa R. King, Kanta Subbarao, Hana Golding  
PLoS Medicine  
April 21, 2009

### “Methods and Findings

To address this need, we generated whole-genome-fragment phage display libraries (GFPDL) expressing fragments of 15–350 amino acids covering all the proteins of A/Vietnam/1203/2004 (H5N1). These GFPDL were used to analyze neutralizing human monoclonal antibodies and sera of five individuals who had recovered from H5N1 infection. This approach led to the mapping of two broadly neutralizing human monoclonal antibodies with conformation-dependent epitopes. In H5N1 convalescent sera, we have identified several potentially protective H5N1-specific human antibody epitopes in H5 HA[(-10)-223], neuraminidase catalytic site, and M2 ectodomain. In addition, for the first time to our knowledge in humans, we identified strong reactivity against PB1-F2, a putative virulence factor, following H5N1 infection. Importantly, novel epitopes were identified, which were recognized by H5N1-convalescent sera but did not react with sera from control individuals (H5N1 naïve, H1N1 or H3N2 seropositive).

### Conclusion

This is the first study, to our knowledge, describing the complete antibody repertoire following H5N1 infection. Collectively, these data will contribute to rational vaccine design and new H5N1-specific serodiagnostic surveillance tools.”

The full article can be found at: <http://www.plosmedicine.org/article/info%3Adoi%2F10.1371%2Fjournal.pmed.1000049>

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## **INFERRING STABILIZING MUTATIONS FROM PROTEIN PHYLOGENIES: APPLICATION TO INFLUENZA HEMAGGLUTININ**

By Jesse D. Bloom, Matthew J. Glassman  
PLoS Computational Biology  
April 17, 2009

“One selection pressure shaping sequence evolution is the requirement that a protein fold with sufficient stability to perform its biological functions. We present a conceptual framework that explains how this requirement causes the probability that a particular amino acid mutation is fixed during evolution to depend on its effect on protein stability. We mathematically formalize this framework to develop a Bayesian approach for inferring the stability effects of individual mutations from homologous protein sequences of known phylogeny. This approach is able to predict published experimentally measured mutational stability effects ( $\Delta\Delta G$  values) with an accuracy that exceeds both a state-of-the-art physicochemical modeling program and the sequence-based consensus approach. As a further test, we use our phylogenetic inference approach to predict stabilizing mutations to influenza hemagglutinin. We introduce these mutations into a temperature-sensitive influenza virus with a defect in its hemagglutinin

gene and experimentally demonstrate that some of the mutations allow the virus to grow at higher temperatures. Our work therefore describes a powerful new approach for predicting stabilizing mutations that can be successfully applied even to large, complex proteins such as hemagglutinin. This approach also makes a mathematical link between phylogenetics and experimentally measurable protein properties, potentially paving the way for more accurate analyses of molecular evolution."

The full article can be found at: <http://www.ploscompbiol.org/article/info%3Adoi%2F10.1371%2Fjournal.pcbi.1000349;jsessionid=33F48BE396F208DB6C9F24DCC408562C>

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## **WORLD COUNTING DOWN TO PANDEMIC, SAYS TOP VIROLOGIST**

By Tan Ee Lyn

Reuters

April 27, 2009

"We are counting down to a pandemic," said Guan Yi, a professor at the University of Hong Kong who helped trace the outbreak of SARS in 2003 to the civet cat.

"I think the spread of this virus in humans cannot possibly be contained within a short time ... there are already cases in almost every region. The picture is changing every moment."

Guan, who has been studying and tracking the spread of the H5N1 bird flu virus ever since it was discovered in people in Hong Kong in 1997, said there would be "many problems" if swine flu reached China and India, "where populations are so dense and health infrastructure is still insufficient."

The full article can be found at: <http://www.reuters.com/article/newsOne/idUSTRE53Q0KQ20090427>

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## **NEW RESPIRATORY ENTEROVIRUS AND RECOMBINANT RHINOVIRUSES AMONG CIRCULATING PICORNAVIRUSES**

By Caroline Tapparel, Thomas Junier, Daniel Gerlach, Sandra Van Belle, Lara Turin, Samuel Cordey, Kathrin Mühlemann, Nicolas Regamey, John-David Aubert, Paola M. Soccal, Philippe Eigenmann, Evgeny Zdobnov, and Laurent Kaiser

Emerging Infectious Diseases, US Centers for Disease Control and Prevention

May 2009

"Rhinoviruses and enteroviruses are leading causes of respiratory infections. To evaluate genotypic diversity and identify forces shaping picornavirus evolution, we screened persons with respiratory illnesses by using rhinovirus-specific or generic real-time PCR assays. We then sequenced the 5' untranslated region, capsid protein VP1, and protease precursor 3CD regions of virus-positive samples. Subsequent phylogenetic analysis identified the large genotypic diversity of rhinoviruses circulating in humans. We identified and completed the genome sequence of a new enterovirus genotype associated with respiratory symptoms and acute otitis media, confirming the close relationship between rhinoviruses and enteroviruses and the need to detect both viruses in respiratory specimens. Finally, we identified recombinants among circulating rhinoviruses and mapped their recombination sites, thereby demonstrating that rhinoviruses can recombine in their natural host. This study clarifies the diversity and explains the reasons for evolution of these viruses."

The full article can be found at: <http://www.cdc.gov/eid/content/15/5/719.htm>

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