KEYSTONE SYMPOSIA™
on Molecular and Cellular Biology
Accelerating Life Science Discovery

KEYSTONE SYMPOSIA 40th MEETING SEASON

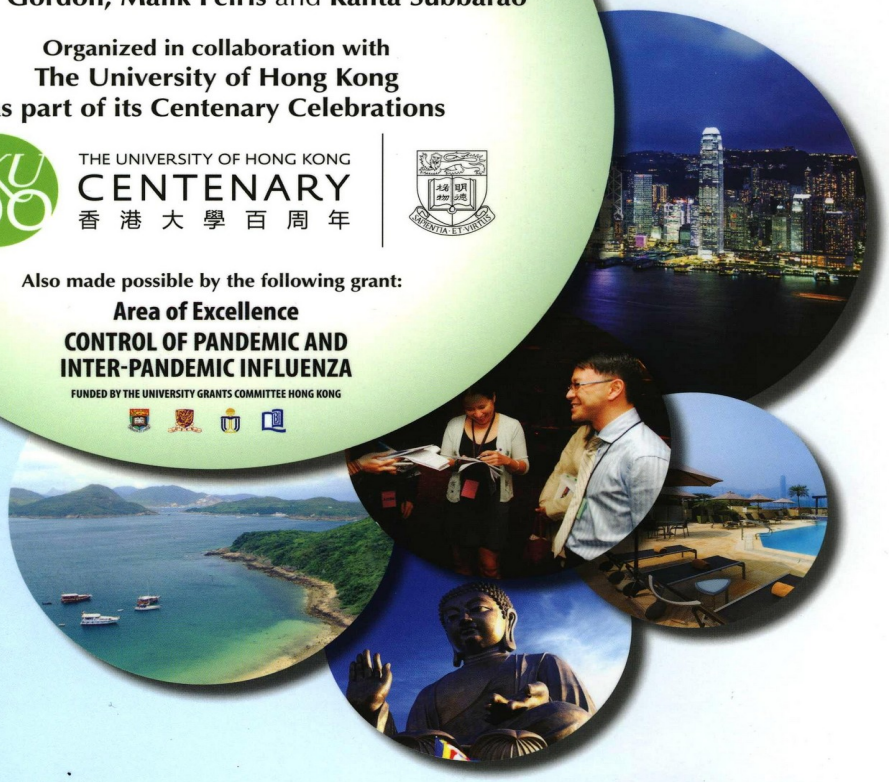
Pathogenesis of Influenza: Virus-Host Interactions

Scientific Organizers:
Siamon Gordon, Malik Peiris and **Kanta Subbarao**

Organized in collaboration with
The University of Hong Kong
as part of its Centenary Celebrations

HKU 100 THE UNIVERSITY OF HONG KONG CENTENARY 香港大學百周年

Also made possible by the following grant:
Area of Excellence
CONTROL OF PANDEMIC AND INTER-PANDEMIC INFLUENZA
FUNDED BY THE UNIVERSITY GRANTS COMMITTEE HONG KONG



May 23–28, 2011
Sheraton Hong Kong Hotel & Towers
Kowloon, Hong Kong SAR, China

129 The Role of Innate Sensing Receptors in Highly Pathogenic Avian Influenza (HPAI) H5N1 Associated Hyper-induction of Cytokines

Kenrie Pui-yan Hui[1], Suki Man-yan Lee[1], Chung-yan Cheung[1] and Malik Peiris[1,2]
[1]Department of Microbiology, The University of Hong Kong, HKSAR, China; [2]HKU-Pasteur Research Centre, HKSAR, China

Cytokine dysregulation contributes to the unusual severity of HPAI H5N1 disease. Previously, we demonstrated that interferon regulatory factor 3 (IRF3) and p38 MAP kinase are separate signaling pathways which contribute to the induction of pro-inflammatory cytokines and chemokines in H5N1-infected cells. However, mechanisms leading to the increased induction of pro-inflammatory cytokines by H5N1 viruses are poorly understood. Here we investigate the role of RIG-I and TLR3 in the H5N1 virus induced inflammatory response.

Primary human macrophages derived from peripheral blood monocytes were infected with HPAI H5N1 (483/97) or seasonal H1N1 (54/98) viruses. The role of innate sensing receptors in cytokine induction by the viruses was investigated using siRNAs to knock-down selected innate immune sensing receptors. Non targeting controls were included and controls to detect off-target effects of siRNA were included. Paracrine activation (by virus free supernatants of infected cells) of innate sensing receptors on adjacent cells was monitored by real-time PCR and Western Blotting. The involvement of IFN receptors and JAK signaling pathways in these autocrine / paracrine cascades was investigated using siRNAs and a JAK inhibitor.

Here we demonstrate that RIG-I, to a lesser extent TLR3 (but not MDA5) plays the major role in the induction of TNF-alpha and IFN-beta in response to H5N1 infection in macrophages via the regulation of IRF3 and NF-kB nuclear translocation. In addition to the direct effects on virus infected cells, paracrine interactions between macrophages and alveolar epithelial cells contributed to cytokine cascades via modulation of IFNAR1/JAK signaling and by the up-regulation of RIG-I in adjacent uninfected cells. Compared with mediators induced by H1N1 infection, those associated with H5N1 infection differentially enhanced the expression of RIG-I and the cytokine responses to PolyIC or infection with seasonal or H5N1 influenza virus. Thus, sensitizing neighbouring cells by up-regulation of RIG-I contributes to the amplified cytokine cascades during H5N1 infection. This may lead to broadened and amplified cytokine signals within the microenvironment of the infected lung.

This work was supported by a RFCID grant (Project No 10091062) from Food and Health Bureau of Hong Kong SAR, an Area of Excellence grant from the University Grants Committee from the Hong Kong SAR (Project No AoE/M-12/06).

130 Validation of Multiplex Real-Time Reverse Transcription Polymerase Chain Reaction Assay with an Internal Control for the Detection of Influenza A/H1N1 2009

Kyung-Ah Hwang[1,2], Kyung-Hee Lee[2], Jae-Hee Park[2], Hee-Suk Chung[2], Young-Sook Cho[2], Jae-Hwan Nam[1]
[1]Department of Biotechnology, The Catholic University of Korea
[2]Division of Research and Development, Samkwang Medical Laboratories, Korea,

Molecular tests for rapid detection of influenza A/H1N1(2009) are necessary. In this regard, real-time polymerase chain reaction (PCR) based on TaqMan technology enables the accurate detection of viral genome over a broad range without the necessity for post-PCR handling. In this study, we developed a multiplex real-time reverse transcription-PCR (rRT-PCR) assay capable of simultaneously detecting influenza A, which includes novel influenza A/H1N1. This assay detected influenza A/H1N1 RNA in a linear range from 500 to 10^{10} copies/mL. The detection limit ranged between $1X10^2$ and $5X10^2$ copies/mL. The percent coefficient of variation (%CV) value in the intra- and inter analysis was found to be almost under 5%. There was no interference with the internal control on RNA extraction. The prevalence of influenza A/H1N1 2009 in the respiratory samples was 16.5% in the case of the multiplex rRT-PCR, 16.0% where the conventional RT-PCR was concerned, and 16.1% in the case of the commercial kit. The mutiplex rRT-PCR assay designed to detect influenza A/H1N1 2009 and the influenza A group showed good analytical sensitivity, specificity, high reliability, and a broad reportable range.

131 Molecular characteristic of new pandemic influenza A virus circulating on the territory of Kazakhstan during the 2009-2010 epidemic season

Ikranbegiin R.[1] Kusnetsova T.V.,[1] Grudinin M.P.,[2] Kiselev O.I.,[2] Glebova T.I.,[1] Baymahanova B.,[1] Shamenova M.G.,[1] Asekenov A.K.,[3] Achmetjhanova K.B.,[4] Kiltbaeva U.D.,[5] Umarkulova N.K.,[6] Elpaeva E.A.,[2] Pisareva M.M.,[2] and Komissarov A.B.[2]
[1] Institute of Microbiology and Virology, Almaty, Kazakhstan, 050010;
[2] Research Institute of Influenza, Saint Petersburg, Russia
[3] Military Hospital of Kazakhstan Ministry of Defense, Almaty, Kazakhstan;
[4] Municipal Children's Polyclinic #7, Almaty, Kazakhstan;
[5] Almaty Multidisciplinary Hospital, Kazakhstan;
[6] Municipal Adult's Polyclinic #7, Almaty, Kazakhstan.

Increase in the incidence of influenza-like illness has been registered in Kazakhstan from October, 2009. The severe form of the disease was observed from the first days with the temperature rise to 39.5-C.

The comparative genetic analysis of HA1 amino acid sequences of isolated viruses A/Almaty/347/2009(H1N1), A/Almaty/330/2009(H1N1), A/Almaty/352/2009(H1N1), A/Almaty/373/2009(H1N1) (corresponding GenBank accession numbers: CY067263, HQ008269, CY067267, CY067269) with sequences from GenBank database showed that viruses isolated in July were almost identical to the reference strain A/California/04/2009 (H1N1v), whereas the viruses isolated in November during the morbidity rise had a number of group and strain specific amino acid substitutions in HA.

The appearance of mutations S120S in E2 antigenic site of viruses isolated in November 2009 as well as D221E in viruses isolated in July 2009 on the territory of South-East Kazakhstan and Kirgizia indicates that the pandemic influenza virus A/H1N1v underwent additional changes from the beginning of its release in human population of Middle Asian Republics. Evolutionary significance of the HA substitution A138V in all influenza viruses isolated on the territory of South-East Kazakhstan and Kirgizia in November 2009 remains unclear. However, according to the published data this mutation could cause changes in interaction of the receptor with sialic acids.

Thus the pandemic influenza A/H1N1 virus circulating during the 2009-2010 epidemic season on the territory of Middle Asian Republics has undergone significant changes. Besides the co-circulation of the new virus with seasonal human H3N2 influenza virus in these regions could be the impetus for mutations leading to the appearance of a new drift variant containing either H1 or H3 hemagglutinin subtype.

132 Influenza A/H1N1 pdm virus in Western Siberia in 2009–2011

A. Ilyicheva[a, b], O. Kurskaya[a], A. Durymanov[b], I. Susloparov[b], I. Sobolev[b], A. Shestopalov[b]
[a] Novosibirsk State University, Pirogov St., 2, Novosibirsk, 630090 Russia;
[b] State Research Center of Virology and Biotechnology VECTOR, Koltsovo, Novosibirsk region, 630559 Russia

The first case of human infection with influenza A/H1N1 pdm in Russia was identified in a tourist who returned from the USA on May 18, 2009. The virus spread over the Russian Federation with lightning speed; and in July, confirmed human cases were reported in population aggregates over the country.

Fifty-six influenza A/H1N1pdm viruses were isolated during 2009-2010. Full genome nucleotide sequences for 23 strains were completed and deposited into GenBank. The strains had sporadic nucleotide substitutions in genome and biologically and antigenically were equal to the vaccinal strain A/California/07/09 (H1N1 2009).

Thirty-eight strains were isolated during 2011 in Western Siberia. The isolates were biologically unique compared to the strains, isolated in this region during 2009-2010. Thus, isolates 2011 had the same infectivity in chick embryos (10^7-10^8), but more low HA titer (2^3-2^5 lower) with cock, goose and guinea pig red blood cells compared to isolates 2009-2010. All studied isolates had amino-acid substitutions in antigenic sites. Antigenic characterization of isolates performed by HI test using reference sera to A/California/04/2009 (H1N1) pdm, A/England/195/2009 (H1N1) pdm, A/South Carolina/2/2010 (H1N1) pdm, A/Texas/77/2009 (H1N1) pdm. The titers for isolates 2011 were 4-8-fold lower compared to 2009-2010.

Documenting the measures taken and lessons learnt provides a learning opportunity for both doctors and policy makers, and can help fortify Russia's ability to respond to future major disease outbreaks.

This work was supported by grants of the Russian Federation program, project no. RNP 14.740.11.0247.