Annual Report 2014

HKU-Pasteur Research Pole

7/F Jockey Club Building for Interdisciplinary Research

5, Sassoon Road, Hong Kong SAR

Malik Peiris, Director

Roberto Bruzzone, Co-Director

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1. Summary

The University of Hong Kong (HKU) and Institut Pasteur (IP) have established the HKU-Pasteur Research Pole (HKU-PRP), a laboratory integrated into the School of Public Health of HKU, with a signing ceremony on December 17, 2013. HKU-PRP will further develop the successful partnership between HKU and IP by focusing on the cell biology of infections. HKU-PRP is expanding its current strength on respiratory diseases by implementing research programs that tackle neglected tropical diseases, such as arboviroses, as well as basic cell biology that is relevant to the advancement of our understanding of pathogenetic mechanism. HKU-PRP is strengthning its position as a regional reference for the Institut Pasteur International Network, with particular emphasis on postgraduate training, and is firmly engaged also in undergraduate teaching with the School of Public Health and the Li Ka Shing Faculty of Medicine at HKU. We have recruited a new Group Leader at the end of 2013 and are launching a new search in 2015 to expand our critical mass.

Research. Our vision is to generate biological knowledge to better understand and treat infectious diseases by bringing together scientists with an interdisciplinary mind. We have re-organized the structure of the lab, which now consists of three Group Leaders – one of which has been recruited in 2013, and have secured several independent extramural grants to continue our research focus on respiratory infections and the mosquito borne disease of dengue. With respect to influenza research, we have extended our exploration of virus-host interaction and host response to viral infection by combining clinical studies and basic research investigations to gain insight into the mechanistic aspects of disease pathogenesis. We have been the driving force to set up a new research platform between HKU and the First Affiliated Hospital of Guangzhou Medical University. This initiative will cover a wide range of investigations related to new emerging viruses identified in Guangzhou and laboratory space will be made available to HKU-PRP to develop collaborative research projects. In addition, we are still actively engaged in research on the newly identified Middle East respiratory syndrome coronavirus (MERS-CoV) and have validated a neutralization assay for seroepidemiological studies on MERS-CoV. The newly developed assay does not require Biosafety Level 3 containment and is thus a relatively high-throughput assay, well suited for large-scale seroepidemiology studies, which we are planning in collaboration with Pasteur Institutes in Northern Africa to better understand the ecology and epidemiology of MERS-CoV. With respect to dengue research, we have identified a crucial cellular factor that is necessary for the virus to exit from the infected cell. This is the first characterization of a host intracellular receptor that is necessary for trafficking and secretion of progeny virus. We are now extending our investigations on the characterization of host factors that are exploited in extensive remodeling of the endoplasmic reticulum to facilitate virus replication, trafficking, assembly and egress. The scientific output of HKU-PRP has been of the highest quality, with 27 papers published since January 2014.

Teaching. Our program of courses for postgraduate students and young scientists has become a reference beyond the Asia region, drawing an increasing number of highly qualified applications from around the world. This educational program has established a worldwide network of trainees, who seek our mentorship well after the course. In addition, we have started in 2012 collaboration with the Pasteur Institute of Ho Chi Minh City (Vietnam) to establish an annual international course on epidemiology and public health. The 2014 edition focused on influenza-like illness and was organized in collaboration with the School of Public Health.

Perspectives. We have developed a strong identity that is contributing to promote the presence and image of HKU, as well as of IP and its international network, in the region through research, teaching and public health activities. We have now the opportunity to expand our critical mass and plan to recruit a new Group Leader towards the end of 2015. In recognition of our achievements, we have been designated as the Asian hub of the Pasteur Network and will be playing a key role in the Center for Global Health Research and Education that Institut Pasteur has established to develop throughout the network the capacity to confront major current and upcoming global health challenges through an innovative model for research collaboration. In summary, the results obtained in 2014 are clearly in line with our strategic objectives and represent a solid foundation to position HKU-PRP as a cluster of excellence within the School of Public Health.

2. Overview of the Programs

2.1 Research

Our research projects address key biological questions to gain insight into how viruses function and interact with their hosts in the cellular environment that constitutes their battleground. There is a major emphasis on influenza and other viruses that are both global and regional threats with a high burden of disease. Three questions are of particular interest to us:

1. *How do viruses invade, replicate and escape infected cells?* This question encompasses both the cellular view of the infectious process – by studying molecules and machinery of the host cells that are utilized during the viral life cycle, as well as the virus point of view – to dissect novel functions of viral genes.
2. *What makes a microbe pathogenic?* This question addresses the genetic determinants of virulence and the acquisition of traits that favor crossing of species barriers by zoonotic viruses.
3. *How do pathogens withstand the host immune response?* This question zooms in on the first lines of defense of the host and the complex strategies devised by viruses to foil them.

The scientific activity of HKU-PRP has been significantly re-organized to become an important component of the Division of Public Health Laboratory Sciences of the School of Public Health in the LKS Faculty of Medicine. The lab now consists of three teams headed by Junior Group Leaders with demonstrated ability to obtain independent grants and a track record of productivity. All groups are actively engaged in the Teaching and Training program.

A search for a new Group Leader is being launched in 2015 to complement our research portfolio and augment the intellectual creativity, skills, and innovation potential of HKU-Pasteur Research Pole and the Center of Influenza Research. The successful candidate will develop a competitive research program that will address fundamental questions in the cell biology and/or immune response of viral infections. The primary selection criterion will be scientific excellence and demonstrated potential for leadership, but we are particularly interested in candidates with international experience, applying innovative approaches to understand the cell biology of virus-host interactions and the immune response at the cellular and/or organismal level.

Research in the Suki Lee lab focuses on virus-host interaction and host response to viral infection. The most significant achievement has been the discovery that TLR10, an orphan receptor, functions as a new innate immune sensor, by demonstrating that influenza virus infection increased TLR10 expression and led to cytokine and interferon induction. Suki Lee was the recipient of the Most Promising Young Researcher Award 2014 from the Research Office of the Food and Health Bureau of Hong Kong SAR.

The lab of Chris Mok uses a combination of in vitro and in vivo models to understand the behavior and pathogenicity of emerging viruses. In this context, he has been the driving force behind the establishment of a new research platform, the "Guangdong-Hong Kong Joint Research Centre for Clinical and Preventive Medicine against Emerging Infectious Diseases", between HKU and the First Affiliated Hospital of Guangzhou Medical University. This initiative will cover a wide range of research interests related to new emerging viruses identified in Guangzhou and laboratory space will be made available to HKU-PRP to develop collaborative research projects.

The main objectives of the Sumana Sanyal lab are to combine methods of cell biology and immunology to address aspects of host-pathogen interactions. Using influenza and dengue as model systems, the lab aims to determine the identity and function of specific host factors that are exploited by these viruses to complete their intracellular life cycle. A related research area is the investigation of counterstrategies employed by the host – either through upregulation of immune signaling pathways or expression of virus restriction factors – in order to prevent virus infection at various steps, including replication, assembly and release.

The research activity of HKU-PRP includes work by Jimmy Lai (joint appointment in the Department of Pathology), who combines chemical, biochemical and cell biological methods to unravel the mechanisms of influenza virus-cell receptor interaction at the atomic level. Research projects that had been initiated by staff that have since left the lab are now close to completion under supervision by Roberto Bruzzone. Work on MERS coronavirus is coordinated by Malik Peiris.

2.2 Teaching and Education

The main objective of our educational pillar is to further develop an advanced teaching program in life sciences that will train a highly selected group of students who will be at the forefront of biomedical research in their countries. This program is extremely competitive and comparable in quality to that of established benchmarks, such as EMBO and Cold Spring Harbor and, therefore, is solidifying the reputation of HKU-PRP and Hong Kong as the premier regional hub for education. Some figures collected during a 4-year span (2010-2013), during which the complete series of three courses has been operational, help to illustrate the overall impact of the program in Hong Kong. We have received more than 750 applications from over 25 countries; 303 students with the same global geographic representation were selected for participation. We have also expanded the number of subscriptions to the HKU-Pasteur Courses Series Newsletter, which now totals 494, and our groups in social media have grown, reaching 265 and 262 members for LinkedIn and Facebook, respectively.

We have established in 2013, with the support of L'Oreal Hong Kong Ltd, an exchange program of short-term scholarships at Institut Pasteur for Hong Kong students. The Scholarship is offered to postgraduate students who are permanent residents of Hong Kong or Macau and demonstrate a keen and devoted capability and intention to pursue research studies in France at the doctoral or postdoctoral level. This initiative is showing the potential to create a very solid bridge between Hong Kong and Institut Pasteur.

In line with the regional training needs in the Asian-Pacific Region, we have launched with the Pasteur Institute of Ho Chi Minh City and the support of the Institut Pasteur International Network and the French Regional Scientific Cooperation a series of international workshops for epidemiologists and public health personnel involved in surveillance activities. The 2014 workshop has been devoted to analyze and discuss methods and impact of surveillance programs of influenza-like illness (ILI).

We are actively engaged in postgraduate training in our lab with two PhD students having defended their thesis in 2014.

2.3 International Activity

We have leadership roles in a number of global projects with a major focus on viral respiratory infections. Roberto Bruzzone is a member of the Executive Committee of the **International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC)**, a global initiative aiming to ensure that clinical researchers have the open access protocols and data-sharing processes needed to facilitate a rapid response to emerging diseases that may turn into epidemics or pandemics. Moreover, we are part of an EU-funded consortium (**Funding scheme: Large-scale integrating project**) supported through the 7th Framework Program with Malik Peiris as coordinator of our work package.

Institut Pasteur and the Institut Pasteur International Network are developing a Center for Global Health Research and Education (CGH) that aims to improve the health of populations worldwide. HKU-PRP has been designated as the Asian the hub of the CGH and Roberto Bruzzone has been appointed Direcotr of the International Teaching and Training Progam of Institut Pasteur.

Malik Peiris is the Coordinator of the Theme-based Research Scheme (TRS): "**Viral, host and environmental determinants of influenza virus transmission and pathogenesis**", which has been awarded a HK$75 million grant. This large-scal multidisciplinary project builds on the success of the Area of Excellence scheme on "**Control of Pandemic and Inter-pandemic Influenza**", which was also initiated and coordinated by Malik Peiris. The newly funded TRS aims at enhancing global public health by identifying the viral and host determinants of influenza virus transmission and pathogenesis leading to evidence-based interventions.

Malik Peiris continues to serve on a number of WHO working groups in relation to both avian and swine origin influenza virus. Malik Peiris received special mention in the Nature Index 2014 – China Supplement, as one of the most significant contributors in this area (microbiology/virology), with three articles in the index on the infectivity and transmission of avian and swine influenza viruses.

3. Progress Report

3.1 SUKI LEE LAB

Main Objectives and Strategy

Influenza virus infections remain one of the major causes of mortality in the developing world and of morbidity worldwide. Animal influenza A viruses that have zoonotically transmitted to humans include H5N1, H9N2 and the lately H7N9 virus, whereas those that have adapted to sustained human transmission include the 2009 pandemic H1N1 virus. The lab focuses on virus-host interaction and host response to viral infection, with a major objective to investigate the underlining mechanisms of influenza virus pathogenesis and to explore the potential of novel therapeutic targets for the treatment of diseases caused by these viruses. More specifically, we are focusing on two major projects in 2014.

Molecular and functional characterization of TLR10

Toll-like receptors (TLRs) play key roles in innate immune recognition of pathogen-associated molecular patterns (PAMPs) of invading microbes. Among the ten TLR family members identified in humans, TLR10 remains an orphan receptor without known agonist or function. Recent studies have reported genetic polymorphisms of TLR10 in humans in association with diverse diseases, including inflammatory and respiratory diseases, but the mechanisms remain obscure. Recently, we have proposed a role for TLR10 as a new innate immune sensor by demonstrating that influenza virus infection increased TLR10 expression and led to cytokine and interferon (IFN) induction. However, two key questions regarding this orphan receptor remain unanswered: What is the ligand for TLR10 sensing during viral infection, and what is the function of TLR10 in host response following viral infection? By addressing these knowledge gaps our studies will open a new area in innate immunity and provide fundamentally important information in understanding virus-host interactions.

Neuropathogenicity of avian H7N9 and pdmH1N1

The recent avian H7N9 infection cases caused much concern due to its severity. The case fatality rate of H7N9 infection is around 30% with 16% ICU admission rate. The main symptoms of H7N9 patients include fever, cough and dyspnea. As disease progresses, acute respiratory distress syndrome (ARD) develops. Encephalopathy was reported as a complication in a few patients, but the potential of avian H7N9 to spread to the mammalian brain has been demonstrated in a study in which viral RNA of the avian H7N9 virus was detected in brains of infected ferrets. Similarly, neurological complications, particularly in pediatric patients, have also been associated with pdmH1N1. Acute encephalopathy is the most commonly reported neurological complication and some patients can develop delirious behavior and visual hallucinations, with rapid deterioration of consciousness into coma. Other less common neurological complications such as ischemia stroke, focal neurological deficits, acute disseminated encephalomyelitis (ADEM) and Guillain-Barre syndrome (GBS) and transverse myelitis have also been described. Our previous study on H5N1 suggested that human astrocytes and neuronal cells could be infected by highly pathogenic H5N1 virus, and produce potent cytokine response that trigger CNS injury, and may play an important role in viral pathogenesis (Ng et al., 2010). The objective of this study is to extend our observations by investigating the tropism and innate immune response of avian H7N9 and pdmH1N1 viruses in human brain cells.

Achievements and Ongoing Research

We have recently reported that influenza virus infection increased TLR10 expression and TLR10 contributed to innate immune sensing of viral infection leading to induction of pro-inflammatory cytokines and interferons (IFNs). Signaling via TLR10 was activated by the functional RNA-protein complex of influenza virus leading to robust induction of cytokine expression. Our findings have indicate that TLR10 is an important innate immune sensor of viral infection and we are now extending our investigations to identify its ligand(s) and determine the biological function of this receptor in viral infection.

Biochemical and functional characterization of TLR10 [Funding: Funding: RGC-GRF and AoE/M-12/16]

Subcellular localization of TLR10 in human monocytic cells

In the present study, we first determined the exact localization of TLR10 using confocal microscopy. TLR10 was found to be predominantly expressed intracellularly, mainly co-localized with endosomal markers in human monocytic cells.

We next screened known agonists for TLR1-9 to determine their ability to be recognized by TLR10 and activate downstream IFN expression. IFN expression was found to be induced in cells over-expressing TLR10, compared to wild-type THP-1, upon stimulation with a TLR3 agonist, poly (I:C), whereas an opposite effect on IFN expression was observed in TLR10 knock-down cells. Because of the structural similarity of poly (I:C) these data suggest that double-stranded RNA could be a possible natural ligand of TLR10 and induce its signaling to trigger IFN expression.

We speculated, therefore, that TLR10 may be responsible for sensing viral nucleic acids upon influenza virus infection. We isolated viral RNA from live influenza A viruses and used it to stimulate cells. Similar to the poly (I:C) data, IFN expression was found to be upregulated in TLR10 over-expressed cells compared to wild-type control cells, whereas, IFN expression was suppressed in the TLR10 knock-down cells. These data further confirm our hypothesis that TLR10 is a *bona fide* pattern recognition that senses viral RNA as its natural ligand during influenza virus infection.

As influenza A virus can induce both pro-inflammatory cytokines and IFNs, we next investigated whether this TLR10-mediated effect occurred via activation of NFKB.

We found that NFKB activity was increased after influenza A virus infection in TLR10 over-expressing cells compared to controls, suggesting that activation of TLR10 signaling triggered IFN responses via NFKB. As poly (I:C) was found to be a possible ligand of TLR10, we also challenged poly (I:C) under the same experimental conditions. A slight increase of NFKB activity was also measured in TLR10 over-expressing cells, albeit of a much lesser extent compared to influenza A virus infection. Together, our data support the notion that viral RNA could be the natural ligand for TLR10 signaling and extend our working model of TLR10 as a nucleotide-sensing receptor.

In the context of this project we have obtained a provisional patent entitled: TLR10 is an innate immune sensor of virus infections and provides a therapeutic target for immune-modulatory interventions and have started up a strategic collaboration with an industrial partner (Invivogen SAS).

Characterization of neuropathogenicity of avian H7N9 and pdmH1N1 [Funding: HMRF]

We and the others have used *in vitro* and *in vivo* models to demonstrate the neurotropism of influenza viruses. Thus, H1N1 induces neuroinflammation, neuronal damage and cognitive dysfunction in adult mice and can result in loss of dopaminergic neurons both *in vivo* and *in vitro*. Dissemination of the viruses in the brain can be observed after intranasal inoculation of avian H5N1 viruses in mice and ferret. Our *in vitro* studies have demonstrated that avian H5N1 can directly infect microglial, astrocytes and neuronal cells. We have extended our investigations to determine the tropism and innate immune response of avian H7N9 and pdmH1N1 viruses in human brain cells.

Differentiated human astrocyte cell line, T98G, and differentiated human neuronal cell line, SH-SY5Y, were infected with avian H7N9, pdmH1N1 and avian H5N1 viruses. Distinct cytopathic effects, such as cell rounding, vacuolation and cell detachment, were observed in astrocytic and neuronal cells infected by avian H5N1 for 24 hours. Infection by avian H7N9 virus also caused cell death in both cell types, albeit to a lesser extent compared to H5N1. By contrast pdmH1N1 virus induced only mild cytopathic effects.

To examine the viral replication kinetics in influenza A virus infected cells, expression of influenza viral matrix (M) gene was measured by real time PCR. Like avian H5N1 virus, both avian H7N9 and pdmH1N1 influenza A viruses were able to infect the two cell lines as indicated by the detection of the M gene in the infected astrocytic and neuronal cells. Furthermore, the gradual increase in M gene expression along the different infection time suggests that all these influenza A viruses exhibit effective replication and viral gene transcription in both human astrocytic and neuronal cells.

To further examine the kinetics of progeny virus production from infected cells, supernatants of virus infected cells were collected to determine the titer of progeny viruses produced after multi-round of replication using $TCID_{50}$ assay. In human astrocytic cells, viral titer of avian H5N1 gradually increased from 24 to 72 h after infection. In contrast, no infectious progeny of either H7N9 or pdmH1N1 virus was detected in the supernatant at any time points. Interestingly, H7N9 virus but not pdmH1N1 virus replicated effectively in infected neuronal cells and progeny virus titer was comparable to that of avian H5N1.

Taken together, these results demonstrated that even though pdmH1N1 viruses could infect and replicate in astrocytic and neuronal cells, they are unable to produce progeny viruses efficiently in these infected cells. Of note, the two pdmH1N1 virus strains isolated from mild cases and those that caused neurological complication respectively showed no difference in the ability to infect and replicate in both brain cell types.

During influenza viral infection, signs of inflammation in the respiratory tissues are detectable and indicative of host innate immune responses. Therefore, we next investigated the cytokine expression profiles in human brain cells upon the avian H7N9 and the pdmH1N1 infection. In both human astrocytes and neuronal cells, proinflammatory cytokines as well as type-I IFN including TNFα, IL6, IL8, CCL2 and IFNβ were found to be markedly upregulated in response to avian H7N9 virus. However, when compared to highly pathogenic avian H5N1 virus, H7N9 was a less potent inducer of cytokines in both cell types.

In keeping with their inability to effectively sustain progeny virus production , both pdmH1N1 viruses only induced very low or no cytokine expression in these cells and no differential cytokine expression was observed between the two pdmH1N1 strains.

In order to understand this late cytokine response, we examined the expression of SOCS (**S**uppressor **O**f **C**ytokine **S**ignaling), whose function is to inhibit the JAK-STAT signaling pathway in a variety of cell types. We observed that the avian H7N9 and H5N1 significantly induced SOCS1 expression in infected astrocytic cells at 6 h after infection. Interestingly, there was a remarkable induction of SOCS3 expression by the two pdmH1N1 viruses as early as at 1h after infection in the astrocytic cells, whereas cells infected with the two avian viruses only showed a marginal induction of SOCS3. In infected neuronal cells, all the tested viruses did not induce SOCS1 expression. However, pdmH1N1 isolated from a patient with neurological complications induced a dramatic SOCS3 mRNA expression at 1 h after infection in neuronal cells. SOCS proteins are key negative- regulators in cytokine signaling. Recent evidence suggests that SOCS1 and SOCS3 are upregulated by seasonal and pdmH1N1 viruses and that induction of SOCS proteins in adaptive immune cells by influenza A viruses suppresses cytokine and IFN production. On the one hand, the induction of SOCS proteins in adaptive immune cells by influenza A viruses has been suggested to suppress cytokine and IFN production, thereby delaying viral clearance. On the other hand, impaired antiviral responses may serve as a feedback mechanism to trigger the host to adaptively increase cytokine induction, leading to robust cytokine expression that causes a severe cytokine storm. The present results support the hypothesis that the mRNA expression of cytokines increases significantly at the later stage of infection after SOCS1 and SOCS3 mRNA induction in infected cells, whereas excessive proinflammatory cytokine production with deficient antiviral immunity in the brain could exacerbate neuroinflammation, leading to brain injury.

Publications

1. **Lee SM**, Kok KH Jaume M, Cheung TK, Yip TF, Lai JC, Guan Y, Webster RG, Jin DY, Peiris JS (2014) Toll-like receptor 10 is involved in induction of innate immune responses to influenza virus infection. *Proc Natl Acad Sci USA* **111**:3793-3798.

Awards

Suki Lee was the recipient of the Most Promising Young Researcher Award 2014 from the Research Office of the Food and Health Bureau of Hong Kong SAR.

Collaborations (local and international)

Ben Cowling (School of Public Health, HKU, Hong Kong): Association between basal leukocyte transcriptome profile and symptom development & disease severity after influenza virus infection in humans.

RT Guo (Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, PR China): Determination of the crystal structure of TLR10.

Nancy Ip and **YP Ng** (HKUST, Hong Kong): Investigation of the neuropathogenicity of influenza A viruses and of the signaling pathways that are activated in response to virus infection in human brain cells.

Eric Perouzel (Invivogen SAS, France): Determination of TLR10 ligands

Funding

Determining the ligand and function of TLR10: a novel innate immune sensor in viral infection (**Principal Investigator**; Research Grants Council/General Research Fund – Ends: 10/2016)

Determining the ligand and function of an orphan receptor: Toll like receptor 10 (**Principal Investigator**; Area of Excellence Control of Pandemic and Inter-pandemic Influenza – Ends: 12/2015)

A potential new therapeutic option in treatment of influenza disease (**Principal Investigator**; Health and Medical Research Fund – Ends: 11/2015)

Role of Prostaglandin E2 (PGE2) in influenza A virus replication and the potential use of PGE2 receptor antagonists for the therapy of H5N1 disease (**Principal Investigator**; Health and Medical Research Fund – Ends: 10/2014)

Association between basal leukocyte transcriptome profile and symptom development & disease severity after influenza virus infection in humans (**Co-Investigator**; Health and Medical Research Fund – Ends: 2017).

Personnel

Name	Position
Suki Lee	Research Assistant Professor
Selena Yan	Technical Officer
Ping Hung Li	Research Technician
Kelvin Ip	Research Assistant
Shuting Li	Research Assistant
Tsz Fung Yip	MPhil Student
Christie Tam	Student Intern
Sharon Tse	Student Intern

3.2 CHRIS KA PUN MOK LAB

Main Objectives and Strategy

The main objective of the lab is to understand the behavior and pathogenicity of emerging viruses by combining clinical studies that span the areas of serology, epidemiology, pathogenicity and vaccination. In this context, a new research platform, the "Guangdong-Hong Kong Joint Research Centre for Clinical and Preventive Medicine against Emerging Infectious Diseases", has been set up between HKU and the First Affiliated Hospital of Guangzhou Medical University. This initiative will cover a wide range of research interests related to new emerging viruses identified in Guangzhou and laboratory space will be made available to HKU-PRP to develop collaborative research projects. We currently focus on three different pathogens including influenza H7N9, H5N6 and dengue viruses, which have recently caused outbreaks, albeit of different scale, in Guangzhou.

A related area of study is more laboratory-oriented and centers on the investigation of the role of the human importin superfamily of nuclear transporters, which recognize nuclear localization signals to mediate the movement of proteins between the cytoplasm and the nucleus, on influenza replication and pathogenicity. Human importins have been shown to be involved in the regulation of the mammalian adaptation of influenza A virus mediated by the interaction with viral polymerase basic protein 2 (PB2) and nucleoporotein (NP). Our team has been focusing on the genetic adaptation of influenza virus and has set up collaboration with the laboratory of Gülsah Gabriel (Heinrich-Pette Institute, Hamburg, Germany), who first discovered the interplay between importin and influenza proteins, and of PC Shaw (Chinese University of Hong Kong, Hong Kong SAR), who is a structural biologist working on protein-protein interaction. This project will further investigate the interplay between the newly identified mammalian adaptation mutation PB2-591K of avian influenza viruses and importins isotypes, as well as the role of importin family on the influenza B virus replication and pathogenicity. We also plan to delineate the detailed mechanisms underlying the interaction between importin and influenza proteins.

Achievements and Ongoing Research

During 2014 we have made progress in characterizing the behavior and pathogenicity of emerging viruses responsible for outbreaks in the region. We have pursued a combined clinical and laboratory approach in collaboration, which has culminated in establishing the "Guangdong-Hong Kong Joint Research Centre for Clinical and Preventive Medicine against Emerging Infectious Diseases", in which our lab has been a driving force. We have published several articles and obtained two extramural grants to fund ongoing investigations of the clinical features, serology, epidemiology and pathogenicity of emerging viruses.

Characterization of H7N9 virus pathogenicity [Funding: HMRF, AoE/M-12/16]
A novel avian H7N9 influenza A virus emerged in March of 2013, causing serious human disease with an elevated case fatality rate in China. There were 432 confirmed human cases reported until May 5 2014, leading to more than 160 deaths. Although the initial outbreak of the H7N9 in humans occurred around the Yangtze River delta in March and April 2013, the infection spread in poultry to Southern China and the second wave of the epidemic in the winter of 2013affected also Guangdong and Hong Kong. We have studied the pathogenicity of avian influenza virus H7N9 in primary human cells and mice model, and identified key mutations on the viral PB2 segment that are associated to the virulence of

the virus. We have now extended our characterization of the H7N9 outbreak on other research areas.

Clinical, virological and immunological features of patients infected with re-emergent avian-origin human H7N9 influenza disease of varying severity in Guangdong province

We have carried out extensive laboratory investigations on five patients infected with H7N9 viruses in Guangdong province, four of whom survived. Viral load in different clinical specimens was correlated with cytokine levels in plasma and broncho-alveolar fluid (BALF), therapeutic modalities used and clinical outcome. Intravenous zanamivir appeared to be more effective than peramivir as salvage therapy in patients who failed to respond to oseltamivir. Higher and more prolonged viral load was found in the sputum or endotracheal aspirates compared to throat swabs. Upregulation of proinflammatory cytokines IP-10, MCP-1, MIG, MIP-1α/β, IL-1β and IL-8 was found in the plasma and BALF samples. The levels of cytokines in the plasma and viral load were correlated with disease severity. Reactivation of herpes simplex virus type 1(HSV-1) was found in three out of five patients (60%). Together, our results demonstrate that expectorated sputum or endotracheal aspirate specimens are preferable to throat swabs for detecting and monitoring H7N9 virus. Severity of the disease was correlated to the viral load in the respiratory tract as well as the extents of cytokinemia. In addition, reactivation of HSV-1 may contribute to clinical outcome.

Development of a vaccinia-based universal influenza vaccine

Current influenza vaccines are ineffective against novel viruses and the source or the strain of the next outbreak of influenza is unpredictable; therefore, establishing universal immunity by vaccination to limit the impact of influenza remains a high priority. In collaboration with the laboratory of Leo Poon (HKU, Hong Kong SAR), we have worked on the characterization of a novel vaccine candidate developed using the immunogenic live vaccinia virus as a vaccine vector, expressing multiple H5N1 viral proteins (HA, NA, M1, M2, and NP) together with IL-15 as a molecular adjuvant. The vaccine protected mice against lethal challenge by increasing survival and significantly reducing lung viral loads against the most recent human H7N9, seasonal H3N2, pandemic-2009 H1N1, and highly pathogenic H7N7 influenza A viruses. Importantly, heterologous influenza-specific CD4(+) and CD8(+) T-cell responses that were elicited by the vaccine were effectively recalled and amplified following viral challenge in the lungs and periphery. This study illustrates the potential utility of a multivalent vaccine as universal influenza vaccine with a correlate of protective immunity that is independent of neutralizing antibodies.

Surveillance and serology studies of H7N9 virus in outpatients with influenza-like illness and hospitalized patients with severe acute respiratory illness (SARI) in Guangzhou

Patients infected with A/H7N9 viruses have a rapidly progressive pneumonia leading to respiratory failure and acute respiratory distress syndrome (ARDS) reminiscent of the disease caused by the highly pathogenic H5N1 avian virus. However, there is still lack of information about the incidence of mild and asymptomatic infection cases. In order to provide better estimates of the percentage of severe disease and of the incidence of A/H7N9 infection (upper confidence limit), we have set up a systematic surveillance protocol (on two pre-designated days of the week) from April to August of 2014 to identify influenza A/H7N9 infection in outpatients presenting with influenza-like illness (ILI) and hospitalized patients with severe acute respiratory infection (SARI) at the First Affiliated Hospital of Guangzhou Medical University. We have recruited approximately 900 patients

with ILI and 300 patients with SARI during this period and collected throat and nasal swabs for d detection of H7N9 by qRT-PCR. In addition, to identify percentage of sero-positive cases against H7N9 virus in the normal population we have collected around 6000 serum samples (3000 during the outbreak and 3000 outside the outbreak period) at the First Affiliated Hospital of Guangzhou Medical University. Our team has established a diagnostic heamgglutinin inhibition (HI) test based on the use of lentiviral particles pseudotyped with H7N9 envelope proteins. This assay is safer as it does not rely on the use of live H7N9 virus and can be performed in a BSL-2 containment laboratory. HI positive samples will be further investigated by micro-neutralization assay, which is the gold-standard assay recommended by the WHO, using live H7N9 virus. We have just begun analyzing the collected samples. Approval for the study was obtained from the ethics committee of the First Affiliated Hospital of Guangzhou Medical University.

Identification and characterization of the novel H5N6 infection in humans

Although the infection of highly pathogenic H5N1 virus in humans has been intensively investigated, there have been no reports thus far on the pathogenicity of H5N6 virus. In collaboration with the First Affiliated Hospital of Guangzhou Medical University, we have identified a H5N6 infection case in a subject who developed ARDS. The full genome sequence of the virus has been obtained and found that it is a reassorted virus with H5N1. The clinical, virologic and immunolical features from the patients are currently being summarized. Key mutations which suspected to contribute to the virulence are identified. Further characterization by cell or animal experiments is needed to understand the pathogenicity of this novel virus.

Clinical and laboratory investigation of the 2014 dengue outbreak in Guangzhou [FUNDING: BNP-Paribas]

In mainland China, small scale outbreak of dengue fever usually occurred in the southern area especially within the Guangdong province because of the hot and humid sub-tropical weather during the summer and it provides an excellent condition to the dengue transmission by the mosquito. Phylogenetic analysis of the virus isolates from GuangDong suggested that the dengue outbreak in the region may possibly imported from the Southeast Asian countries. From July to the end of October of 2014, there was a large dengue outbreak occurred in Guangdong province. Around fifty thousands of dengue fever cases have been reported resulting in six deaths. We have studied the clinical features from 138 hospitalized patients who were infected by dengue viruses during this outbreak in Guangzhou, which is the largest metropolitan city in Guangdong province.

Investigation of the role of human importin protein family on influenza replication and pathogenicity [Funding: AoE/M-12/16]

The characterization of virus and host factors that are involved in the adaptation of avian influenza virus to mammalian cells (and mammalian transmission) remains one of the most critical questions in the field of influenza research. Mutations in any segment of the virus genome are an important mechanism of its adaptation to the mammalian host and the corresponding amino acid changes also contribute to pathogenesis. The group of Guelsah Gabriel (Heinrich-Pette Institute, Hamburg, Germany) has previously shown that avian influenza viruses undergo a switch in importin-alpha dependency upon avian–mammalian adaptation. Importin-alpha is a constituent of the classical nuclear import pathway. It acts as an adaptor protein that recognizes the nuclear localization signal (NLS) of the cargo protein, which is then transported as a ternary complex with the importin-beta receptor into the nucleus. It has been shown that importin protein can interact with PB2 and NP

proteins of influenza A virus. For example, the human-like PB2 627K virus, but not avian-like PB2 627E, displayed reduced pathogenicity and replication in importin-alpha 7 knockout mice. However, the interplay between the mammalian importins and the adaptation of avian influenza virus in mammal has not been fully mapped out. In collaboration with the Gabriel lab and the group of PC Shaw, a structural biologist at the Chinese University of Hong Kong, we aim to address several questions to delineate in mechanistic terms the interplay between influenza proteins and importins. Specifically, we will investigate the structural basis of the binding between importin and the influenza PB2/NP proteins and the interaction of the new identified PB2-Q591K mammalian adaptation and importins. Moreover, we will begin exploring the role of importins in the replication of influenza B virus, an endemic strain which only circulates in human population but not poultry.

Publications

1) Yang ZF*, **Mok CK***, Liu XQ*, Li XB, He JF, Guan WD, Xu YH, Pan WQ, Chen LY, Lin YP, Wu SG, Pan SH, Huang JC, Ding GY, Zheng K, Ke CW, Lin JY, Zhang YH, Lee HH, Liu WK, Yang CG, Zhou R, Peiris JS, Li YM, Chen RC, Chen L, Zhong NS (2015) Clinical, virological and immunological features from patients infected with re-emergent avian-origin human H7N9 influenza disease of varying severity in Guangdong province. *PLoS One*, in press (*equal contribution).

2) Guan WD*, Gong XY*, **Mok CK***, Chen TT, Wu SG, Pan SH, Cowling BJ, Yang ZF, Chen DH. (2015) Surveillance for seasonal influenza virus prevalence in hospitalized children with lower respiratory tract infection in Guangzhou, China during the post-pandemic era. *PLoS One*, in revision (*equal contribution).

3) Chin AW, Li OT, **Mok CK**, Ng MK, Peiris M, Poon LL (2014) Influenza A viruses with different amino acid residues at PB2-627 display distinct replication properties in vitro and in vivo: Revealing the sequence plasticity of PB2-627 position. *Virology*. **468-470**:545-555.

4) Chin AW, **Mok CK**, Zhu H, Guan Y, Peiris JS, Poon LL (2014) Use of fractional factorial design to study the compatibility of viral ribonucleoprotein gene segments of human H7N9 virus and circulating human influenza subtypes. *Influenza Other Respir Viruses* **8**:580-584.

5) Valkenburg SA, Li OT, Mak PW, **Mok CK**, Nicholls JM, Guan Y, Waldmann TA, Peiris JS, Perera LP, Poon LL (2014) IL-15 adjuvanted multivalent vaccinia-based universal influenza vaccine requires CD4+ T cells for heterosubtypic protection. *Proc Natl Acad Sci USA* **111**:5676-5681.

6) Leung YH, Nicholls JM, Ho CK, Sia SF, **Mok CK**, Valkenburg SA, Cheung P, Hui KP, Chan RW, Guan Y, Akira S, Peiris JS (2014) Highly pathogenic avian influenza A H5N1 and pandemic H1N1 virus infections have different phenotypes in Toll-like Receptor (TLR) 3 knock-out mice. *J Gen Virol* **95**:1870-1879.

7) **Mok CKP**, Peiris JSM, Chan MCW (2014) Anti-inflammatory and anti-viral effects of indirubin derivatives in H5N1-infected primary macrophages and pneumocytes. *Antiviral Res* **106**:95-104.

8) **Mok CK**, Lee HH, Lestra M, Nicholls JM, Chan MC, Sia SF, Zhu H, Poon LL, Guan Y, Peiris JS (2014) Amino acid substitutions in polymerase basic protein 2 gene contribute to the pathogenicity of the novel A/H7N9 influenza virus in mammalian hosts. *J Virol* **88**:3568-3576.

Seminars, Meeting Presentations

CK Mok (2014) **Pathogenicity and viral determinants of the novel A/H7N9 influenza virus in mice.** *9th Conference Louis Pasteur: Emerging Infectious Diseases*, Paris, France (Poster).

CK Mok, HH Lee, M Lestra, MC Chan, SF Sia, JM Nicholls, H Zhu, Y Guan, JS Peiris (2014) **Pathogenicity and viral determinants of the novel A/H7N9 influenza virus in mice.** *Symposium on Emerging Infectious Diseases in South East Asia*, Phnom Penh, Cambodia (Poster).

CK Mok, HH Lee, ZF Yang, JS Peiris (2014) **Host and the viral factors that contribute to the pathogenicity of the novel A/H7N9 influenza virus.** *Scientific Symposium of the Institut Pasteur International Network*, Paris, France.

CK Mok, HH Lee, ZF Yang, JS Peiris (2014) **Host and the viral factors that contribute to the pathogenicity of the novel A/H7N9 influenza virus.** *The 15th IUBMB-24th FAOBMB-TSBMB Conference*, Taipei, Taiwan.

Collaborations (local and international)

Gülsah Gabriel (Heinrich-Pette Institute, Hamburg, Germany) and **PC Shaw** (The Chinese University of Hong Kong, Hong Kong SAR): Investigation of the role of human importin protein family on influenza replication and pathogenicity.

Nan-Shan Zhong, **Ling Chen**, **Zi-Feng Yang** (State Key Laboratory of Respiratory Disease, Guangzhou, PR China): Clinical and laboratory studies on influenza and dengue virus in Guangzhou.

Funding

Infection and immunopathogenesis of avian influenza H9N2 virus in tree shrew model (**Co-Investigator**; NSFC-PR China – Potentially Fundable, in revision).

The pathogenic role of the adaptation in the polymerase basic 2 protein of the new identified duck isolated H7N9 lineage in mammalian hosts (**Principal Investigator**; Health and Medical Research Fund – Ends 2017).

Investigation on the antiviral effects of the peramivir in H7N9 infected mice model (**Principal Investigator**; Donation from GuangZhou Nanxin Pharmaceutical Co. Ltd; ENDS: 2017).

Surveillance and serology studies of influenza A H7N9 infection in Guangzhou (**Principal Investigator**; Area of Excellence "Control of Pandemic and Inter-pandemic Influenza" – ENDS: 2016).

Surveillance of outpatients with influenza-like illness and hospitalized patients with severe acute respiratory illness presenting at The First Affiliated Hospital of Guangzhou Medical University (**Co-Investigator**; Commissioned Health and Medical Research Fund – ENDS: 2015).

Risk assessing human transmission potential of H7N9 viruses using ex vivo cultures of the human respiratory tract (**Co-Investigator**; Commissioned Health and Medical Research Fund – ENDS: DATE).

Study on the interaction of influenza A virus nucleoprotein and polymerase PB2 N-terminal region (**Co-Investigator**; Research Grants Council/General Research Fund – ENDS: 2017).

The role of influenza PA-X protein on the virus replication and cytokine induction in human lung epthielial cells (**Principal Investigator**; Seed Funding Programme for Basic Research – ENDS: 2016).

Personnel

Name	Position
Chris Ka Pun Mok	Research Assistant Professor
Ying Fan	PhD student
Horace Lee	PhD student
Gannon Mak	PhD student (part-time)
Maxime Lestra	International Volunteer of the French Ministry of Foreign Affairs (until 12/14)
Jane Tse	Research Technician
Chen Xiao Kai	Research Assistant
Si Hua Pan	Research Assistant

3.3 SUMANA SANYAL LAB

Main Objectives and Strategy

The main objectives of the lab are to combine methods of cell biology and immunology to address aspects of host-pathogen interactions. Using influenza and dengue as model systems, we aim to determine the identity and function of specific host factors that are exploited by these viruses to complete their intracellular life cycle. We also investigate counterstrategies employed by the host – either through upregulation of immune signaling pathways or expression of virus restriction factors – in order to prevent virus infection at various steps, including replication, assembly and release. Amongst other factors, we are particularly interested in post-translational modifiers of protein function, especially ubiquitin and ubiquitin like small modifiers such as ISG15 that play a significant role in modulating different pathways, not the least of which are innate signaling pathways such as RIG-I, TLR7 and inflammasome activation. Our major research projects are listed below.

Host factors involved in dengue infection: role of Aup1

A molecular understanding of host cellular factors involved in dengue infection is crucial not only to provide novel insights into pathways hijacked by flaviviruses, but also for development of effective antimicrobials against the pathogen. Identification of host factors that can be targeted for developing novel anti-viral compounds has the additional benefit of avoiding potential resistance acquired in viruses by mutation and selection. The complexity of the assembly and release of dengue virus provides a potentially rich source of host targets for interference. The *modus operandi* of propagation of dengue virus (DENV), West Nile (WNV) and other members of the family appears to involve extensive remodeling of the endoplasmic reticulum (ER) to facilitate virus replication, trafficking, assembly and egress. However, we have been severely limited in our understanding of the role of fundamental biological pathways typically hijacked by flaviviruses. We recently discovered that Aup1 – a lipid droplet associated protein – is heavily expressed upon dengue infection. Preliminary results suggest that overexpression of Aup1 alone is sufficient to cause increased secretion of dengue virus like particles. The goal of this project is to investigate interactions between host factors such as Aup1 and dengue virus in order to understand their functional relevance.

Development of therapeutic strategies against viral infections by targeting the ubiquitylation machinery and its modulation of host innate immune response

Influenza virus is responsible not only for annual epidemics, but also for frequent outbreaks of pathogenic avian flu strains that have become a serious public health issue worldwide. The ubiquitylation machinery is frequently exploited by a number of pathogens either to masquerade as host proteins or to inhibit immune signaling cascades. We propose to investigate the role of deubiquitylating enzymes (DUB) specifically expressed during influenza infection. We have employed a chemoenzymatic strategy to identify DUBs that are specifically expressed upon influenza infection. Our current studies involve characterization and pharmacological intervention of these DUBs in order to attenuate influenza infection. Our preliminary data in macrophages and dendritic cells support the hypothesis that influenza takes advantage of DUBs to suppress signaling pathways such as RIG-I and inflammasome activation that require ubiquitin modification for recruitment of downstream effectors. We also propose to test small molecules that target these DUBs both in vitro and in vivo.

Role of Tsg101 and its regulation by post-translational modification during virus assembly and release

A major response of mammalian cells to viral infections is through upregulation of the interferon type I and II pathways. Viruses in turn counter this pathway through either the inhibition of IFN response or by activation of proteins that inhibit the function of interferon-stimulated genes (ISGs). The primary antagonist of the host immune response for influenza is NS1. A key interaction documented for NS1 is the dynamics of interaction with the interferon-stimulated gene 15 (ISG15). Upon type-I interferon treatment or virus infection, ISG15 is one of the immediate responders and is expressed in abundance. Based on limited proteomic analysis, the targets of ISGylation have been found to be of the order of a hundred or more genes. We have identified Tsg101 as one of the targets of ISG15 modification. We are currently exploring the functional relevance of this modification during influenza infection and how NS1 counteracts it. We find a strong correlation between the pathogenicity of the virus and the effectiveness of NS1 in preventing ISG15 mediated inhibition of Tsg101 function.

Regulation of immune signaling by deubiquitylases

Signaling cascades require tight control over activation and suppression to maintain downstream activities for appropriate durations. Such regulation is often executed by post-translational modifications such as phosphorylation and ubiquitylation. We are interested in deciphering the role of deubiquitylases (DUB) in the context of a number of innate and adaptive immune responses. We have identified DUBs that are either specifically recruited or inactivated in the T-cell receptor-signaling cascade, presumably to optimize the length and magnitude of downstream activities. Usp12, which resides in the nucleus, is redistributed to the cytosol in a TCR stimulus specific manner. In the absence of Usp12 surface expression of the TCR is drastically reduced. This phenotype is recapitulated upon inhibition of Usp12 translocation from the nucleus to the cytosol. In addition, several TCR adaptors such as LAT and Trat1 interact with Usp12 upon stimulation. We hypothesize that these are substrates of Usp12 that need to be deubiquitylated during signaling to stabilize the TCR at the cell surface.

Achievements and Ongoing Research

Since joining HKU-PRP in November 2013, we have continued working on projects that were initiated while I was a postdoctoral researcher at the Whitehead Institute/MIT. Preliminary data generated at MIT were used to submit grant applications to RGC/GRF as well as HMRF and Area of Excellence for Control of Pandemic and Inter-pandemic Influenza. We have now obtained more recent data in our current lab at HKU-PRP, in line with results obtained previously, which consolidate our findings.

Host factors involved in dengue infection: role of Aup1 [Funding: RGC/GRF]

Among the viral proteins that are known to induce protective immunity, nonstructural protein 1 (NS1) of DENV is the most promising, offering the hope of a possible vaccine against dengue. NS1 is a ~45 kDa glycoprotein expressed in infected cells and is targeted to the cell surface most likely in a dimeric form. It is also secreted in either the dimeric or a hexameric form. Our own work with dengue NS1 has uncovered a previously unrecognized role for a novel host factor Aup1, a lipid droplet associated protein that had not been identified in the published shRNA screens. We have begun to dissect the mechanism by which Aup1 is exploited during DENV assembly in an NS1-mediated fashion and a

subsequent release of virus particles. We have generated Aup1-GFP and Aup1-cherry fusion proteins stably expressed in hepatoma cells in culture in parallel with knock-out cells through the CRISPR mediated genom editing strategy. When transduced with DENV NS1 or exposed to live dengue virus, the expression level of Aup1 is significantly upregulated followed by induction of autophagy, which we can observe by confocal microscopy using the reporter constructs of Aup1. In addition we can observe induction of these lipid droplets during DENV infection or transduction by DENV NS1 through electron microscopy. In the presence of chloroquine, which blocks lysosomal degradation, we observe a dramatic increase in accumulation of lipid droplets post infection. In addition, we have engineered a number of constructs for Aup1 with either deletions or mutations in specific domains of the protein to dissect the functional relevance of Aup1 interaction with NS1 during dengue pathogenesis. A number of reagents for dengue research, such as HeLa cells stably secreting dengue virus like particles (VLP), was established as part of Dr. Peigang Wang's research at HKU-PRP. Overexpression of Aup1 in these cells results in a substantial increase in VLP secretion, whereas a knock-out attenuates it. For pathogens that regulate cellular lipid metabolism, as many flaviviruses do, the role of host lipid biosynthetic enzymes that modify properties of organelles is an aspect of viral pathogenesis that has received scant attention. Our research builds on promising preliminary data that we generated, not only to understand the role of host factors involved in dengue biogenesis in mechanistic detail, but also to explore new aspects of the immunobiology involved in the host response against this pathogen.

To this end we will track the intracellular trafficking routes of flaviviruses, which has so far focused primarily on entry pathways, relying heavily on fluorescence or electron microscopy. Notwithstanding the advantages of a visual assay, need for a quantitative biochemical technique is imperative. We have developed an enzymatic means of generating a reporter virus strain with no detectable loss in infectivity to monitor intracellular trafficking and release, both in intact and permeabilized cells. We refer to this strategy as the "sortagging" method. A wide array of proteins has been successfully engineered using this technique, which introduces minimal modification to the protein of interest that can be labeled in a robust manner by any fluorescent probe or biotin or any other tag of interest. This method has proved to be remarkably useful for generating engineered influenza viruses that are functionally identical to their wild type counterpart. This strategy has been instrumental for measuring influenza virus assembly and transport and will be adequately adapted to monitor dengue trafficking and budding.

Development of therapeutic strategies against viral infections by targeting the ubiquitylation machinery and its modulation of host innate immune response

[Funding: HMRF]

One of our research goals is to determine how ubiquitin and ubiquitin like modifiers are utilized by the host innate immune system and their modulation during viral infections. Within this context, we are investigating the role of deubiquitylating enzymes (DUB) that are expressed during influenza infection and their effect on the innate immune response. Our preliminary data indicate that upon influenza A infection in human lung epithelial cells (A549) as well as bone marrow derived macrophages in culture, the extent of ubiquitylated material recovered from whole cell lysates is dramatically reduced. We hypothesize that this is a consequence of either one or both of the following reasons: (i) upregulation of deubiquitylating enzymes (DUBs) by influenza virus that de-ubiquitylates host factors to enable a productive virus infection (ii) degradation of antiviral host restriction factors through the ubiquitin proteasome pathway to enable a productive influenza virus infection. In support of the first hypothesis we have identified DUBs, expressed during influenza

infection, which function to block the inflammasome signaling pathway as well as the RIG-I pathway and result in compromise of the host innate immune response. We anticipate that by targeting this pathway using a combination of chemoenzymatic and pharmacological methods, we will be able to restore a more robust innate immune response and restrict virus replication. Targeting specific enzymes of the ubiquitylation machinery is an emerging therapeutic strategy. We propose to test two specific small molecule inhibitors to these DUBs that are available and have shown considerable promise as drugs in phase I and II clinical trials. Questions that we are specifically focused on addressing are as follows: Are certain DUBs specifically induced during influenza infection? Our preliminary results support the conclusion that specific DUBs are induced upon H1N1 infection. Using a version of ubiquitin (Ub) modified at its C-terminus with vinyl methyl ester (Ub-VME) and a combination of large-scale immunoprecipitation and proteomic analyses by mass spectroscopy, we have successfully identified DUBs that are expressed during H1N1 infection. What is the purpose of DUBs expressed during viral infections? Do they provide an advantage to virus replication? Are there differences between DUBs expressed by different strains of influenza virus, specifically between the seasonal human influenza H1N1 and the pathogenic avian influenza H5N1 and H7N9? We hypothesize that the DUB expression profiles for seasonal H1N1 and the pathogenic avian viruses would be different and their identity would provide insights into what host factors allow avian flu to become transmissible in humans. Given our success with the seasonal influenza, we aim to use the same strategy to identify and compare DUBs expression profiles with avian influenza.

Role of Tsg101 and its regulation by post-translational modification during virus assembly and release [Funding: AoE/M-12/16]

We have shown that Tsg101, a component of the ESCRT-I complex, is required for release of influenza A/WSN/33. Tsg101 is essential for transport of hemagglutinin (HA) from the Golgi to the plasma membrane prior to release of intact virus particles from the cell surface. We want to extend these observations and will explore whether this phenomenon holds true as a general host factor necessary for influenza virus biogenesis.

We will test different influenza strains in Tsg101 deficient A549 cells we have generated by the recently developed CRISPR knock out strategy. These A549 cells will be infected with the different human H1N1 and H3N2 strains available in house as well as the avian influenza strains of H5N1 and H7N9. Differences may well exist between utilization of host proteins between the human versus the avian influenza strains. Thus, some flu strains may be better equipped at counteracting the inhibitory effect of Tsg101 deficiency through other mechanisms. We will test viral titers from supernatants of infected cells (wild-type and Tsg101 deficient A549) using plaque assays. In addition, intracellular transport of HA will be measured using FACS to quantitate HA exposure at the cell surface. We have generated epitope tagged Tsg101 (Tsg101-FLAG) for wild-type and a mutant that does not associate with the ESCRT complexes. These constructs will be expressed in Tsg101$^{-/-}$ cells to test whether they rescue influenza transport and release when infected. We anticipate that virus release will be restored to control levels with the wild-type construct. Expression of the Tsg101 variant that does not associate with the ESCRTs will indicate whether the role of Tsg101 in flu trafficking occurs independently of the ESCRT machinery. These constructs will also be used in combination with confocal microscopy to investigate the localization and intracellular trafficking characteristics upon influenza infection. In addition, we have generated BirA-ligase fusion protein constructs of Tsg101, which are ideal in identifying transient interactors through proximity based labeling strategies. Since the subcellular localization of Tsg101 undergoes drastic changes upon either virus infection or interferon treatment, these will be used to determine the interactors under different physiological conditions. We are also addressing the mechanisms of Tsg101 regulation by post-

translational modification during virus infection. Many cellular antiviral mechanisms are initiated by induction of interferon (IFN). Interferon-stimulated gene 15 (ISG15) encodes an ubiquitin dimer-like protein that is associated with defense against a number of viral pathogens. Of note, post-Golgi transport of influenza HA to the plasma membrane, which requires the protein Tsg101 as described above, is blocked upon IFN treatment and Tsg101 itself is ISG15 modified upon IFN-I induction.

Our preliminary data indicate that differential post-translational modification of Tsg101 appears to regulate its function. Our results suggest that, during a productive virus infection, Tsg101 is phosphorylated at the Y390 residue, whereas upon IFN-I induction it is ISGylated. Upon infection with ΔNS1, which fails to suppress the IFN-I response, we can isolate ISGylated but not phosphorylated Tsg101. These data suggest that phosphorylation and ISGylation are mutually exclusive modifications regulating the function of Tsg101. Phosphorylated Tsg101 facilitates virus release, whereas ISG15 modified Tsg101 blocks viral protein transport from the Golgi to the PM. Similar analyses performed for VSV infection show hyper phosphorylation of Tsg101 at Y390 (unpublished). We will generate the corresponding phosphorylation and ISGylation mutants of Tsg101 for expression in A549 cells deficient in Tsg101. These cells will be infected with different strains of influenza to test how virus trafficking and release are affected.

Together, our initial observations indicate that ISGylation of target host components interferes with virus release. Influenza virus must have co-evolved strategies to overcome the ISG15-mediated inhibition of transport, either by utilizing host de-ISGylating enzymes or inhibiting levels of ISG15 from reaching saturating levels. Chemical tools such as vinylmethylester-modified ISG15 (ISG15-VME) will be used to isolate de-ISGylases that are possibly upregulated and utilized by influenza virus during infection to circumvent the block imposed by ISG15. The nonstructural protein 1 (NS1) of influenza A is believed to inhibit the function of ISG15 through as yet unknown mechanisms. In addition, although the total number of target proteins of ISG15 is believed to be in the range of 300-1000, most of them are unknown and only a limited number of effector proteins have been identified and characterized in isolation such as TRIM25, an E3 ligase in the RIG-I mediated signaling pathway. We have identified a host protein, Tsg101 as a target for ISGylation during influenza A/WSN/33 H1N1 infection. Based on our preliminary data, we propose to scale up to immunoprecipitate ISGylated proteins upon influenza virus infection. Immunoprecipitated material will be identified by resolving on SDS-PAGE, silver staining and subjected to mass spectroscopy.

Regulation of immune signaling by deubiquitylases

Amongst the myriad post-translational modifications the ubiquitin conjugation system appears to play a crucial role in regulating immune signaling cascades. When a naïve T cell encounters a foreign antigen, it undergoes clonal expansion. The strength of the interaction between the antigen and the T cell receptor is a critical determinant for activating a signal cascade and mounting an immune response. Therefore, understanding the molecular mechanism of proximal TCR signaling events following receptor engagement is critical for the purpose of modulating it.

Although some of the E3 ligases participating in the TCR signaling pathway have been identified, the ubiquitin specific proteases that carry out deubiquitylation have been less well studied. Ubiquitin is attached to a substrate by the concerted activities of a series of E1, E2 and E3 enzymes and removed by the presence of deubiquitylating enzymes (DUBs). A balance between the forward and the backward reactions determine the outcome of the strength and duration of the signal and subsequent phenotypic effects. TCR signaling to

NFκB requires assembly of large multi-protein complexes consisting of several kinases, scaffold proteins, ubiquitin ligases and deubiquitylating enzymes. The TCR forms a multisubunit complex with CD3 consisting of cytoplasmic immunoreceptor tyrosine based activation motifs (ITAMs). A series of phosphorylation steps that involves PI3K and PDK1 culminates in PKC phosphorylation. The NEMO/IkappaB (IKK) complex integrates signals from upstream stimuli and results in NFκB activation. Several studies have identified key signal mediators involved in the pathway such as Zap70, SLP-76, PLC γ, SAP, Fyn, LCK, PKCθ, Vav1, Bcl10, Malt1 and Carma1. Biochemical characterization of these effectors have suggested a putative sequence of events where PKCθ activity is followed by nucleation of the multiprotein Carma-Bcl10-Malt (CBM) complex within lipid microdomains to recruit the inhibitor of NFκB kinase (IKK). The signal initiated by these core events is then disseminated through adaptor proteins such as LAT and SLP-76 and ultimately induces global changes in gene transcription and acquisition of effector functions.

Reports on the dynamics of TCR surface expression suggest that in accordance with other receptors, the TCR is internalized and recycled rapidly with a rate constant of ~0.01min^{-1}. Engagement by an antigen-presenting cell (APC) causes an intracellular retention, although the kinetics of internalization remains unaffected. Although scant evidence exists for the mechanism of surface dynamics of TCR, available data on well-characterized receptors such as the transferrin receptor (TfR) or epidermal growth factor receptor (EGFR) indicate that internalization and recycling occurs via a dynamic interplay between monoubiquitylation and deubiquitylation process. Several enzymes of the ubiquitylation machinery have been identified that appear to play a crucial role in orchestrating maturation, differentiation and function of T cells. Amongst the well documented are TRAF6, GRAIL and the SOCS proteins of the E3 ligase family and CYLD, USP9X from the deubiquitylase (DUB) family. Adding another layer of complexity is the diverse array of ubiquitin-chain linkages that dictate the outcome of such modifications in the context of cellular responses such as localization, degradation and signaling.

We have employed a ubiquitin specific activity-based probe to target functional DUBs in the TCR signaling pathway. We used a C-terminally modified ubiquitin (Ub) with vinyl methyl ester (vme) to capture DUBs that are recruited upon TCR activation in both mouse T-lymphocytes and Jurkat cells. This approach has been successfully used previously to identify DUBs that are either cellular or expressed upon infection by chlamydia. Upon stimulation with anti-CD3 antibodies followed by large scale immunoprecipitation we identified a set of cytosolic DUBs, including CYLD and Usp9X, which have been described previously in the context of TCR signaling. We focused on those that were differentially recovered between control and stimulated cells and identified Usp12 and Usp46 that displayed enhanced recovery in the TCR stimulated cells compared to resting cells. Usp12 localizes primarily to the nucleus, but becomes enriched in the cytosol upon stimulation. Usp12$^{-/-}$ Jurkat cells generated through Cas9/CRISPR-mediated genome editing were defective in several downstream activities including NFκB, NFAT and Erk1/2 phosphorylation. In addition surface expression of TCR was severely attenuated upon stimulation in Usp12$^{-/-}$ cells. Through proximity based labeling with a promiscuous BirA-ligase fused to Usp12 (BirA*-Usp12) we identified several adaptor proteins of the TCR signaling pathway including LAT, Trat1 and SLP76. Expression of LAT and Trat1 was attenuated in Usp12$^{-/-}$ cells suggesting that Usp12 acts directly on these proteins to stabilize the TCR complex at the cell surface. We are determining the mechanism of TCR stabilization through the activity of Usp12 on these TCR adaptors.

Publications

1. Lestra M*, Jahan AS*, Swee LK*, Fan Y, Tafesse FG, Theile CS, Spooner E, Bruzzone R, Ploegh HL, **Sanyal S** (2015) Usp12 stabilizes the T cell receptor complex at the cell surface during signaling. **Under review**.
2. Claessen JHL, **Sanyal S**, Ploegh H (2014) The chaperone Bag6 captures dislocated glycoproteins in the cytosol. *PloS One* **9**:e90204.

Seminars, Meeting Presentations

Sumana Sanyal (2014) The University of Hong Kong, Hong Kong SAR.

Sumana Sanyal (2014) University of Massachusetts, Amherst, MA, USA.

Sumana Sanyal (2014) The Whitehead Institute for Biomedical Research, MIT, Cambridge, MA, USA.

Sumana Sanyal (2014) Columbia University, New York, NY, USA.

Sumana Sanyal (2014) Annual retreat, Massachusetts Institute of Technology, NH, USA.

Sumana Sanyal (2014) EMBO workshop for group leaders, Leiman, Germany.

Sumana Sanyal (2014) EMBL, Heidelberg, Germany.

S Sanyal, J Ashour, H Ploegh (2014) *Keystone Symposium on Innate immunity to viral infections*, Colorado, USA, (Oral presentation).

Y Fan, S Sanyal (2014) *Louis Pasteur Symposium on Emerging Infectious diseases*, Institut Pasteur, Paris, France (Poster presentation).

M Lestra*, AS Jahan*, LK Swee*, S Sanyal (2015) *Keystone Symposium on the Human Proteome*, Stockholm, Sweden (Oral presentation).

Sumana Sanyal (2014) EMBL, Heidelberg, Germany.

Sumana Sanyal (2015) Lecture in the Molecular Biology of the Cell course, Institut Pasteur, Paris, France.

Collaborations (local and international)

Hui-Ling Yen (School of Public Health, LKS Faculty of Medicine of HKU): Engineering influenza neuraminidase for sortase catalyzed modification.

Joseph Ashour (Mount Sinai School of Medicine, New York, NY, USA): Manipulation of host factors in influenza and dengue infections

Adolfo Garcia-Sastre (Mount Sinai School of Medicine, New York, NY, USA): Studying the function of Isg15 and its mode of restricting influenza virus trafficking, specifically, the efficacy of influenza NS1 in preventing ISG15 activity.

Hidde Ploegh (The Whitehead Institute for Biomedical Research, MIT, Cambridge, MA, USA): Studying host factors and their mechanism of function during influenza virus biogenesis centered on Tsg101.

Funding

Host factors involved in dengue infection (**Principal Investigator**; Research Grants Council/General Research Fund – Ends: 11/2016).

Development of therapeutic strategies against viral infections by targeting the ubiquitylation machinery and its modulation of the host innate immune response (**Principal Investigator**; Health and Medical Research Fund – Ends: 06/2017).

Elucidating the role of Tsg101 in influenza virus assembly and release (**Principal Investigator**; Area of Excellence Control of Pandemic and Inter-pandemic Influenza – Ends: 12/2016).

Mechanism of Influenza NS1 mediated inhibition of interferon type-I response: effect on Isg15 (**Principal Investigator**; Seed Funding for basic research – Ends: 06/2016).

Development and application of novel technologies to elucidate mechanism of influenza trafficking and assembly in vivo and in vitro (**Co-Investigator**; National Institute of Health).

Role of Tsg101 in the process of influenza virus assembly and release (**Principal Investigator**; Research Grants Council/General Research Fund - **Pending**).

Personnel

Name	Position
Sumana Sanyal	Research Assistant Professor
Ming Yuan Li	Technical Officer
Tami Zhang	Technical Officer
Lewis Siu	Research Technician
Akhee Jahan	Research Assistant
Mart Lamers	Master Student

3.4 JIMMY LAI GROUP

Main Objectives and Strategy

Our main project combines chemical, biochemical and cell biological methods to unravel the mechanisms of influenza virus-cell receptor interaction at the atomic level. In addition, we have started to collaborate in a clinical study that investigates the potential usefulness of immunotherapy as treatment of nasopharyngeal carcinoma (NPC), which is caused by a combination of environmental, genetic and viral factors, being often linked to Epstein–Barr virus (EBV) infection.

Study of influenza virus receptor

The objective of the study is to obtain a better understanding of the influenza viral tropism. Sialic acids are known to be the receptor molecules of influenza viruses, but the diversity of sialylated glycans is not equivalent in different animal species and organs. Therefore, it is likely that the interactions between influenza viral proteins and different sialylated glycans are involved in the viral adaptation to the host and one possible mechanism underlying species jump, e.g. from avian to human. In our study, we have produced influenza virions or virus-like particles of different influenza subtypes and/or different viral origin. The interactions between hemagglutinin (HA), neuraminidase (NA) and a variety of sialylated glycans were investigated using chemical methods. Functional study of the virions was also carried out on cell/tissue cultures.

Immunotherapy against nasopharyngeal carcinoma

The aim of the project is to develop an effective immunotherapy treatment against Epstein-Barr virus (EBV)-associated nasopharyngeal carcinoma (NPC), which, differently from Western countries, is endemic in southern China, including Hong Kong. EBV is present in virtually all poorly differentiated and undifferentiated nonkeratinizing NPC (type II and III, according to the WHO classification), making the viral antigens expressed by tumor cells attractive targets for immunotherapy. Our strategy is to generate LMP/EBNA1-specific T cells from PBMC isolated from NPC patients using an adenoviral vector. The safety and efficacy of expanded T cells can be assessed upon adoptive CTL infusion as immunotherapy.

Achievements and Ongoing Research

We have continued in 2014 our exploration of the basis of influenza receptor specificity and the role O-linked sialylated glycans in influenza viral infection. We have published two articles and obtained two extramural grants to fund ongoing investigations of the influenza virus receptor.

Study of influenza virus receptor [Funding: HMRF, RGC, URC]

Investigation of the binding and cleavage characteristics of influenza neuraminidase

Nuclear magnetic resonance (NMR) spectroscopy was used to investigate pH and temperature effects on binding and cleavage of NA to sialosides. We found that an acidic pH and physiological temperature are required for efficient NA enzymatic activity, although a pH change showed a minimal effect on the NA-sialic acid binding affinity. We also interrogated the selectivity of human-like or avian-like receptors for influenza neuraminidase N1 derived from a range of different influenza virus strains, including human seasonal H1N1, 2009 pandemic H1N1 and highly pathogenic avian H5N1. Our data comparing α-2,3- and α-2,6-sialyllactose indicated that the variation in

neuraminidase activity on different ligands correlated with a change in binding affinity. Epitope mapping of the sialylglycans interacting with NAs from different viral origin showed different binding profiles suggesting that different binding conformations were adopted. These findings have been published in *Influenza Other Respir Viruses*.

Characterization of sialylated glycans on human respiratory tract

Glycomic analysis of human respiratory tract tissues using MALDI-TOF mass spectroscopy demonstrates a wide range of sialylated glycans, including a high level of lactosamine repeats and biantennary sialosides. In order to look at the effect of the number of lactosamine repeats on the interaction of influenza HA with sialosides, sialylated compounds with different lactosamine length were synthesized in collaboration with Xuechen Li (Department of Chemistry, HKU), and the HA binding affinity to these compounds were tested using NMR Spectroscopy. We have observed an increase of HA binding signals with the increasing number of lactosamine repeats from 1 to 3 and we are in the progress of characterizing the interactions in order to understanding how lactosamine in the sialosides affecting the binding with HA. We hypothesize that multiple sialic acids in the biantennary sialosides may interact with multiple HA binding sites on a HA trimer, leading to a much stronger HA binding affinity from these sialylated glycans to act as efficient influenza receptor. We are now trying to obtain some biantennary sialosides to check for their binding interactions with HA.

The role of O-linked sialylated glycans in influenza viral infection

Cell surface sialosides contain both N-linked and O-linked glycans. N-linked sialosides were found to be important in the influenza viral infection whereas the role of O-glycans as influenza receptor remains unclear. In our work using glycosylation mutant cell-lines, efficient influenza infections were observed in GnT1-deficient cells in which maturation of N-glycans is blocked, indicating that O-glycans might also be used as the virus binding receptor. Influenza infections were significantly reduced while using benzyl-N-acctyl-galactosaminide as a blocker of O-glycan synthetic pathway, further supporting the involvement of O-linked sialylated glycans in influenza virus entry. NMR spectroscopy study on influenza HA interacting with O-glycans analogues also reflected significant binding of HA to these glycans. However, we observed a variation in binding affinity to the O-glycans when different influenza subtypes were included in the study. A manuscript describing these finding is in preparation.

Molecular determinants of HA and NA affecting H9N2 virus tropism

H9N2 infection is one of the most widespread influenza viruses in poultry in Asia and has transmitted occasionally to swine and human. We hypothesize that poor transmission between humans by H9N2 viruses may depend on NA not being well adapted for human airway. We will utlize H9N2 viruses isolated from human cases and avian species to compare infection and tropism using *ex vivo* cultures of human respiratory tract. Our role in this project is to investigate the binding and cleavage activities of N2 neuraminidase using enzymatic methods coupled with NMR spectroscopy, using the above described approach. Different sialylated glycans will be included in the study especially those with the two different sialic acid species, Neu5Ac and Neu5Gc. Neu5Ac/Gc sialiosides expression varies in different animals. Neu5Gc sialiosides is predominant in swine, with human expressing only Neu5Ac sialiosides, whereas avian species present both types of sialic acid on their cell surface. Virus-like particles containing N2 have been engineered but the yield was unsatisfactory so far therefore we are now optimizing the methodology.

Study of the emerging H7N9 influenza

Zoonotic H7N9 disease is a lurking threat to public health but the major question is whether this virus poses a risk of acquiring transmission potential in humans (pandemic potential). We would like to perform a risk assessment of the H7N9 virus. Viral *in vitro* and *ex vivo* tropism is being addressed and the ability of viral replication in mammalian cells by H7N9 viral polymerase complex will also be investigated. Pseudotyped viruses containing H7 hemagglutinin were successfully constructed for the study of virus-receptor interactions. Potential gain-of-function or loss-of-function amino acid mutations were introduced into the H7 hemagglutinin to investigate the risk posed by such mutation in the process off viral adaptation to humans. Regarding the safety concerns on generating a high risk influenza virus, pseudotyped viruses were utilized instead of replicative virions, therefore eliminating any ethical consideration linked to performing experiments that could fall in the category of Dual Use of Research Concern. Preliminary studies using *in vitro* pseudotyped viral infections suggest an increased infectivity upon the potential gain-of-function single amino acid mutations. These wildtype and mutant pseudotyped viruses will then be tested for infections in *ex vivo* human cultures. Studies using NMR spectroscopy for their binding affinity to avian and human sialic acid receptor analogues will also be included if further funding is available.

Comparison of native influenza virus and virus-like-particles in their receptor-binding properties

Our group has previously developed non-infectious influenza virus-like-particle (VLP) containing influenza HA or NA as tools for influenza study (reviewd in Garcia and Lai, 2011). Together with the NMR technology, these VLPs have been applied to address HA/NA functions, resulting in a series of publications. However, the similarity between native virons and VLPs in virus-receptor interaction has not been thoroughly investigated. In order to validate previous and prospective data obtained using VLP, experimental evidence from a systematic study comparing VLP and native virons on their receptor interactions is necessary. In addition, it will be interesting to test the possibility of using inactivated virus for the receptor study as the data obtained will be closely comparable to natural infections. Native H9N2 and H1N1 virions will be inactivated by UV treatment or fixation with either formaldehyde or glutaraldehyde. NMR experiments will be performed using these inactivated virions or VLP engineered with the corresponding HA to compare their interactions with sialylated glycans.

Immunotherapy against nasopharyngeal carcinoma [Funding: Ester Lee and Chew Pik Foundation, Croucher Foundation]

NPC is endemic in China and Southeast Asia where it is tightly associated with infections by EBV. The role of tumor-associated viral antigens in NPC renders makes them promising candidates for cellular immunotherapy. In earlier preclinical studies, a novel adenoviral vector–based vaccine termed AdE1-LMPpoly has been generated; it encodes EBV nuclear antigen-1 (EBNA1) fused to multiple CD8+ T-cell epitopes from the EBV latent membrane proteins, LMP1 and LMP2. Our group has previously reported data of an early phase I clinical trial using AdE1-LMPpoly as an immunotherapeutic tool for EBV-associated NPC (*Cancer Res* 72:1116–1125). Twenty-four NPC patients were selected and EBV-specific T cells were successfully expanded from 16 patients. Transient increase in the frequencies of LMP1&2- and EBNA1-specific T-cell responses was observed after adoptive CTL transfer and the median overall survival compared with patients who did not receive T-cell therapy increased from 220 to 523 days. We are now in the late phase I clinical trial with the target to expand the number of NPC patients involved to a total of 50. At present there is no facility present in Hong Kong that allow the expansion of T-cells for adoptive therapy in a

GMP accredited standard. As a preparation for the phase II clinical trial, a physical facility will be established to initiate the process of expansion of T-cells here in Hong Kong and I will be trained in the laboratory of Rajiv Khanna (Queensland Institute of Medical Research, Australia) to (*i*) establish criteria to identify a location where T-cells can be expanded in a safe environment; (*ii*) provide training in compliance with Australian/Hong Kong regulations regarding cell based therapy; (*iii*) transfer know-how and protocols to expand donor T-cells and test for CTL efficiency.

Publications

1. Lee SM, Kok KH, Jaume M, Cheung TK, Yip TF, **Lai JC**, Guan Y, Webster RG, Jin DY, Peiris JS (2014) Toll-like receptor 10 is involved in induction of innate immune responses to influenza virus infection. *Proc Natl Acad Sci USA* **111**:3793–3798.
2. Garcia JM, **Lai JC**, Haselhorst T, Choy KT, Yen HL, Peiris JS, von Itzstein M, Nicholls JN (2014) Investigation of the binding and cleavage characteristics of N1 neuraminidases from avian, seasonal and pandemic influenza viruses using saturation transfer difference nuclear magnetic resonance. *Influenza Other Respir Viruses* **8**:235-242.

Seminars, Meeting Presentations

Study of Influenza Virus-host Receptor Interactions using Pseudotyped Virus. Department of Pathology, The University of Hong Kong, Hong Kong SAR.

Collaborations (local and international)

Xuechen Li (Department of Chemistry, HKU, Hong Kong): Molecular determinants of influenza virus tropism and binding; expertise in glycan synthesis, to produce glycans of interest as influenza receptor analogues.

Guang Zhu (Division of Life Science, HKUST, Hong Kong): Access to equipment and technical support regarding NMR spectroscopy.

Mark von Itzstein and Thomas Haselhorst (Institute for Glycomics, Griffith University, Australia): Study of O-linked sialylated glycans and synthesis of O-glycans analogue.

Renee Chan (School of Public Health, HKU, Hong Kong): Comparison of native influenza virus and virus-like-particles in their receptor-binding properties using the *ex vivo* human culture model.

Dora Kwong and Janice Tsang (Department of Clinical Oncology, HKU, Hong Kong): Clinical trials of immunotherapy against EBV-associated NPC.

Rajiv Khanna (Department of Immunology, Queensland Institute of Medical Research, Australia): Immunotherapy against EBV and technology transfer to develop methods of T cells expansion.

Funding

Molecular determinants of H9N2 virus haemagglutinin and neuraminidase affecting virus tropism for the human and swine respiratory tract (**Co-Investigator**; Health and Medical Research Fund – Ends: 10/2015).

Risk assessing human transmission potential of H7N9 viruses using ex vivo cultures of the human respiratory tract (**Co-Investigator**; Commissioned Studies on Emerging Influenza A Viruses with Epidemic Potential RRG-03 – Ends: 05/2015).

Comparison of native influenza virus and virus-like-particles in their receptor-binding properties (**Principal Investigator**; Small Project Funding – University Research Committee – Ends: 10/2015).

Immunotherapy against nasopharyngeal carcinoma (**Co-Investigator**; Ester Lee and Chew Pik Foundation, Croucher Foundation and other donors – Ends: open).

Personnel

Name	Position
Jimmy Lai	Postdoctoral Fellow (Joint Appointment with the Department of Pathology in the Nicholls Lab)
Wong Ho Him	Research Assistant

3.5 ROBERTO BRUZZONE and MALIK PEIRIS GROUPS

This section summarizes the main results of research work done by PhD students under the supervision of Roberto Bruzzone and by HKU-PRP staff on MERS coronavirus under the supervision of Malik Peiris.

Main Objectives and Achievements

Several projects were completed in 2012-2014 and four manuscripts were published in the process (see Annual Report 2012-2013). In addition, we published a paper that has provided conclusive evidence for antibody-dependent enhancement of infection human monocyte-derived macrophages by replication-competent SARS coronavirus as well as Spike-pseudotyped lentiviral particle (Yip et al, 2014). We focus here on current projects investigating the dynamics of host-pathogen interactions with dengue and influenza viruses. Work on MERS coronavirus in camels and humans has led to the development of a safe diagnostic test for sero-epidemiological studies and has resulted in the publications of six manuscripts since 2013.

Molecular dissection of intracellular transport of dengue virus [Funding: BNP-Paribas]

The life cycle of enveloped viruses is a complex process relying on specific interactions with host factors that, in turn, represent potential targets for functionally interfering with viral replication and pathogenesis. Although the molecular identity of cellular receptors involved in virion entry has been revealed for many types of viruses, few studies have investigated whether host proteins on intracellular compartments also function as receptors to facilitate viral trafficking and release from infected cells. Thus, viral-host interactions during dengue virus egress are still poorly characterized and most cellular targets identified in high-throughput screens have not been mapped to the secretory pathway. DENV has two structural glycoproteins: pre-membrane (prM) and envelope (E); E mediates interaction with cellular receptor(s) for viral attachment and entry, whereas prM assists E in its correct folding and protects it from pre-fusion in the acidic environment of the secretory pathway. Assembly of DENV occurs at the endoplasmic reticulum (ER), and requires interaction of prM and E. Nascent virions bud into the lumen of the ER, accumulating in dilated cisternae oriented towards the cis-Golgi, and are translocated to the Golgi via trafficking vesicles. In the trans-Golgi network (TGN), prM protein is cleaved by the cellular protease furin, resulting in the release of the pr peptide and formation of infectious DENV. Besides mature virions, non-infectious recombinant subviral particles (RSP) can be produced by cells expressing DENV prME proteins. Dengue RSP traffic along the same compartments as infectious DENV, and represent a safe and convenient tool for the study of virus-host interactions during secretion.

We have obtained several lines of evidence demonstrating that dengue virus 1 (DENV1) requires host KDEL receptors (KDELRs), which cycle between the endoplasmic reticulum (ER) and Golgi apparatus to retrieve resident ER proteins, for transport from budding sites at the ER to the Golgi. Depletion of KDELRs by siRNA reduced egress of both DENV1 progeny (70%) and of Recombinant Subviral Particles (RSPs) produced in stable cell lines (90%) expressing the structural protein prME. Co-immunoprecipitation revealed that KDELRs interact with prM through three positively charged amino acids at the N-terminus, whose mutation disrupted this interaction and inhibited both trafficking of newly formed RSPs from ER to Golgi and their release from cells. Finally, perturbation of KDELR cycle by siRNA depletion of class II Arfs, which results in KDELRs accumulation in the Golgi, phenocopied results obtained with both prME triple mutant and knockdown of KDELR.

Taken together, these data indicate that loss of interaction with KDELRs reduced DENV transport from ER to Golgi and, consequently, release from infected cells. Our results, therefore, have uncovered a novel function for KDELRs as an internal receptor required for DENV trafficking and have identified a rate-limiting molecular step in the late stages of DENV lifecycle. This work has been accepted for publication in *Cell Reports*.

Role of Cyclin D3-influenza A Virus M2 Ion Channel Interaction in Influenza Virus Infection and Pathogenesis [Funding: Research Fund for the Control of Infectious Diseases and AoE/M-12/06]

We had previously performed a genome-wide yeast two-hybrid screen using as a bait the cytoplasmic tail (CT) of viral matrix protein 2 (M2), which has the longest CT among the three virus envelope proteins. The screen revealed a high-score interaction of M2with cyclin D3, a key regulator of cell cycle G1/S transition. We hypothesized that this interaction may perturb cell cycle and/or represent a host defence mechanism to counteract viral infection. The M2/cyclin D3 interaction was confirmed by both GST pull-down and co-immunoprecipitation experiments. Infection of synchronized A549 cells with influenza A/WSN/33 virus led to a dramatic down-regulation of cyclin D3 protein level, which was associated to a cell cycle arrest in G0/G1 phase, as quantified by BrdU incorporation. Cyclin D3 knockdown by small interfering RNA (siRNA) significantly enhanced virus progeny titers in cell culture supernatant. This increase was due to the absence of cyclin D3 protein *per se*, as concomitant retinoblastoma knockdown, which rescued cell cycle progression into the S phase, did not normalize virus production. Moreover, cyclin E knockdown, which induced G1 cell cycle arrest without appreciably modifying cyclin D3 protein levels, did not increase virus progeny titers. Collectively, this study has identified cyclin D3 as a novel interactant of influenza A virus M2 protein, and has provided several lines of evidence to suggest that cyclin D3 acts as a restriction factor of influenza life cycle, independently of its classical role in cell cycle regulation. This work is carried out by Ying Fan, a final year PhD student. A manuscript describing the results of this project is in preparation.

Invovlement of C-type lectin receptor CLEC5A in influenza virus pathogenesis

Human infections with influenza A virus (IAV) may exhibit mild to severe clinical outcomes as a result of differential virus-host interactions. C-type lectin receptors (CLRs) are pathogen recognition receptors expressed on monocytes or Natural Killer cells that mediate immune response via different mechanisms. Among the transmembrane human CLRs identified so far, only DC-SIGN and DC-SIGNR have been shown to increase influenza viral entry in a sialic acid independent manner; however, the potential interactions between other CLRs with influenza virus have not yet been systematically investigated. We utilized lentiviral based pseudoparticles expressing influenza hemagglutinin (HA) to examine the binding potential between HA and a panel of human soluble CLRs highly expressed in cells of the immune system. CLEC5A was identified as a potential interacting target as positive binding was observed with HA derived from a highly pathogenic avian H5N1 viruses A/VN/1203/04 (VN1203) or a human seasonal H1N1 virus A/HK/54/98 (HK5498), albeit at different intensity. We first evaluated the potential of CLEC5A in mediating influenza entry either by siRNA knockdown of endogenously expressed protein in U937 cells or by over-expressing it in CHO and CHO-Lec2 cells. No significant difference in the viral nucleoprotein (NP) was observed in all conditions tested, compared to control, following infection with recombinant A/PR/8/34 virus expressing HA and NA derived from either VN1203 (H5) or HK5498 (H1) viruses, suggesting no role for CLEC5A in mediating viral entry. To investigate downstream signaling upon engagement of CLEC5A, we used M-CSF macrophages because we observed high expression levels of the adaptor protein DAP12

that associates with CLEC5A to initiate signal transduction, whereas U937 did not. Knockdown of CLEC5A in M-CSF macrophages led to a significant reduction of cytokine (TNF-α and IFN-α) and chemokines (IP-10, MCP-1, MIG and MIP-1α) secretion after infection with H5, whereas significant suppression of IP-10 expression was observed with H1 infection. In order to assess the relative contribution of HA and neuraminidase (NA) to the observed effects, we infecting M-CSF macrophages with recombinant A/PR/8/34 virus expressing all combinations of HA or NA derived from either VN1203 or HK5498 viruses. We found that, following CLEC5A knockdown, VN^{HA} expressing IAV showed significant reduction of cytokines and chemokines secretion, whereas HK^{HA} expressing IAV exhibited significant suppression of chemokines only, suggesting that cytokines induction via CLEC5A was dependent on the influenza HA subtype. Since DAP12 phosphorylation is known to activate Syk, we further treated M-CSF macrophages with a Syk inhibitor and observed a significant reduction of TNF-α and IP-10 after H5 infection whereas, as expected, this treatment only inhibited IP-10 in H1 infected macrophages. However, $CLEC5A^{-/-}$ mice exhibited increased in mortality rate against VN1203 challenge compared to wild-type, which indicated that CLEC5A is playing a protective role in secreting cytokines and chemokines after IAV infection. In conclusion, we have identified CLEC5A as a novel host factor implicated in the modulation of IAV pathogenesis. This project was part of the PhD work of Ooiean Teng, who was jointly supervised by Roberto Bruzzone and Hui-Ling Yen, Assistant Professor in the School of Public Health, and successfully defended her thesis in October 2014. A manuscript is in preparation.

Characterization of MERS coronavirus in camels and humans [Funding: National Institute of Allergy and Infectious Diseases, National Institutes of Health; European Community Seventh Framework Program]

Middle East respiratory syndrome (MERS) is an emerging respiratory disease of global public health concern. As of 5 February 2015, 971 laboratory-confirmed cases of human infection with Middle East respiratory syndrome coronavirus (MERS-CoV) have been reported to WHO, including at least 356 deaths. Overall, 63.5% of cases reporting gender (n=949) are male and the median age is 48 years (range 9 months–99 years; n=964). The current epidemiology of MERS is one of zoonotic transmission, sometimes followed by chains of limited human-to-human transmission for limited periods of time within families or healthcare facilities. This is reminiscent of the emergence of severe acute respiratory syndrome (SARS) in late 2002. It is therefore critically important to identify the sources of zoonotic transmission, so that evidencebased interventions to minimise such infections can be implemented. Seroepidemiology is an invaluable tool in such investigations. We had previously reported a MERS-CoV pseudoparticle neutralisation test (ppNT) that can be used to detect antibody to MERS-CoV without the need for Biosafety Level-3 (BSL-3) containment that is required for conventional MERS-CoV microneutralisation (MN) tests. We have now systematically investigated potential cross-reactions that may confound the use of this and of the standard microneutralization assay in seroepidemiological studies in animals. Our data demonstrate that these two serological assays are free of cross-reaction with Bovine coronavirus and other coronavirusesand, therefore, can be used with confidence in seroepidemiological studies to identify animal species that may serve as reservoirs or vectors of MERSCoV. We have also confirmed that MERS-CoV, or a very closely related virus, was circulating in dromedaries in Saudi Arabia as early as 1993. In contrats, sera from adult dromedary camels in Australia, which were imported into Australia between 1840 and 1907 to serve as means of transport, were uniformly seronegative, although Bovine coronavirus was as common as in Middle East animals. Given the small number of sera tested in our study, a larger number of samples is needed to confirm that Australia is indeed MERS-CoV free.

In separate studies we have been able to isolate MERS-CoV from the nose and feces, but more frequently from the nose, of dromedary camels in Saudi Arabia and Egypt. Preexisting neutralizing antibody did not appear to protect against infection. The full-genome sequence of MERS-CoV from dromedaries in these study was very similar to genomes of human clade B MERS-CoV. This finding implies that viruses genetically very similar to human MERS-CoV are infecting dromedaries beyond the Arabian Peninsula, where human MERS-CoV infections have not yet been detected. Taken together our findings confirm that MERS-CoV infection is common in dromedaries camels and that this virus is genetically very similar to a MERS-CoV that is infecting humans, thus lending further support to the contention that dromedaries may be a potential source of human infection. In contrast, serum from 179 persons working in the dromedary abattoirs was negative for antibody to MERS-CoV. This finding includes 114 persons working in the 2 abattoirs from which the MERS-CoV–positive animal swab specimens were obtained. Although these observations suggest that transmission of this virus to humans is uncommon, it is plausible that cases may occur in humans beyond the Arabian Peninsula. MERS-CoV diagnostic tests should be considered for all patients with unexplained severe pneumonia in Egypt, northeastern Africa, and beyond.

Publications

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2. Chu DK, Poon LL, Gomaa MM, Shehata MM, Perera RA, Abu Zeid D, El Rifay AS, Siu LY, Guan Y, Webby RJ, Ali MA, Peiris M, Kayali G (2014) MERS coronaviruses in dromedary camels, Egypt. *Emerg Infect Dis* **20**:1049-1053.
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10. Memish ZA, Alsahly A, Masri MA, Heil GL, Anderson BD, **Peiris** M, Khan SU, Gray GC (2015) Sparse evidence of MERS-CoV infection among animal workers living in Southern Saudi Arabia during 2012. *Influenza Other Respir Viruses*, **in press**.

Collaborations (local and international)

Marc Grandadam (Institut Pasteur-Laos): Effect of cellular factors on replication competent dengue viruses of different serotypes.

Jean-Christophe Olivo-Marin (Institut Pasteur, France): Quantitative imaging of intracellular dengue transport.

Peter Sicinski (Harvard Medical School, USA): Interactions of influenza virus M2 proton channel with cyclin D3.

Ziad A Memish (College of Medicine, Alfaisal University, Riyadh, KSA), **Gazi Kayali** (St. Jude Children's Research Hospital, Memphis, USA) and **Maged G Hemida** (King Faisal University, Al Hofuf, KSA): MERS coronavirus in animals and humans.

Funding

Molecular dissection of intracellular transport of dengue virus (BNP Paribas, Hong Kong – Ends: 12/2015).

Role of Cyclin D3-influenza A Virus M2 Ion Channel Interaction in Influenza Virus Infection and Pathogenesis (AoE/M-12/06).

Characterization of MERS coronavirus in camels and humans (NIAID, National Institutes of Health and FLUPIG, 7th Framework Program of the European Union).

Personnel

Name	Position
Roberto Bruzzone	Professor
Malik Peiris	Professor
Ranawaka A.P.M. Perera	Postdoctoral Fellow (Peiris Lab)
Ming Yuan Li	Technical Officer (Sanyal Lab)
Ying Fan	PhD student (Mok Lab)
Sebastien Tremolet	Scientific Officer (International Volunteer of the French Ministry of Foreiogn Affairs)
Lewis Siu	Research Technician (Sanyal Lab)
Ooiean Teng	Research Assistant (Yen lab)

3.6 Teaching and Education

HKU-Pasteur Courses

The main objective of our educational pillar is to further develop an advanced teaching program in life sciences that will train a highly selected group of students who will be at the forefront of biomedical research in their countries. Our courses are unique in that they are designed to train postgraduate students (MPhil and PhD) during their formative years; furthermore they are extremely competitive and comparable in quality to that of established benchmarks, such as EMBO and Cold Spring Harbor courses and, therefore, are solidifying the reputation of HKU-PRP and Hong Kong as the premier regional hub for education. Some figures help to illustrate the overall impact of the program in Hong Kong. If we consider the past 4 years during which the complete series of three courses has been operational (2010-2013), we note that we received more than 750 applications from over 25 countries; 303 students with the same global geographic representation were selected for participation. Each course includes a full-time senior faculty member of HKU, as well as of Institut Pasteur in the organizing committee, thereby strengthening the ties between the two institutions. During the same period of time (2010-2013), we have assembled a stellar faculty of more than 200 scientists from over 20 countries, including several Nobel Prize winners, as well as numerous members of the National Academy of Sciences USA and/or EMBO, HHMI Investigators, Professors at College de France and Fellows of the Royal Society. These courses have, therefore, allowed a group of leading scientists to discover Hong Kong and their research programs, leading to collaborations. It should be noted that an added value of this series is the possibility to offer many seminars open to the scientific community of HKU and HK (**see Annex 5.8 for a complete list of the 2014 speakers**). More than 100 lectures were given as open seminars during the 2010-2013 period analyzed. Thus, through this program we were able to organize on average 25 public seminars per year, or two per month, figures that compare very favorably to those of much larger departments in the LKS Faculty of Medicine at HKU.

HKU-PRP teaching approach is unique and unrivalled in Hong Kong and in the region. Our courses rely on the excellence of our international faculty of leading scientists, including several Nobel Prize winners, who concretely prove their generosity by taking time out of their busy schedule to travel to Hong Kong while forfeiting their honorariums. HKU-Pasteur courses bring together students from all over the world, coming from countries with markedly different resources to support their students. This heterogeneity is one of the key components of our success as it promotes awareness of the inequalities in resource allocations and the fundamental Pasteurian values that scientific knowledge knows no frontiers but belongs to mankind and can be efficiently shared with an altruistic behavior. HKU-PRP offers great opportunities, through the Institut Pasteur International Network, by providing access to basic science laboratories and field projects where trainees can apply their knowledge collaborate with leading researchers and start building their careers through hands-on experience. This combination of unsurpassed quality and altruism sets an important example that will no doubt influence the future paths of our alumni.

HKU-Pasteur courses are supported with external grants that are received, on a competitive basis, from Institut Pasteur International Network, the Li Ka Shing Faculty of Medicine at HKU, the Croucher Foundation, the French Consulate and other private donations. Our funds cover advertising costs, travel and accommodation for all lecturers and students (except from industry). Selected students are expected to pay for their travel costs, but a small number of travel grants may be available at the discretion of the course directors. These basic guidelines have been modeled after those set by EMBO Global Exchange Courses.

Additional teaching and training

All HKU-PRP faculties are teaching in the Problem-Based Learning modules for MBBS students. Robero Bruzzone started a new course for MPH students, entitled "Biological basis of diseases" (CMED6227). Malik Peiris teaches "Emerging infectious diseases: the One Health concept" (CMED6104). HKU-PRP regularly hosts undergraduate/postgraduate students from overseas institutios for summer internships. For the past three years Chris Mok has supervised each summer, from June to August, two internship students from the Institute of vocational education (IVE) received laboratory training from 2014.

We have completed supervision of two students who defended their PhD thesis in 2014: Dr. Mingyuan Li and Dr. Ooiean Teng. They have remained at HKU-PRP with the aim of completing their research projects. Overall, these results attest of the quality of research supervision and career mentoring that have been a core value of HKU-PRC.

We have established in 2013, with the support of L'Oreal Hong Kong Ltd, an exchange program of short-term scholarships at Institut Pasteur for Hong Kong students (http://loreal-scholarship.com/). The Scholarship is offered to postgraduate students who are permanent residents of Hong Kong or Macau and demonstrate a keen and devoted capability and intention to pursue research studies in France at the doctoral or postdoctoral level. This initiative is showing the potential to create a very solid bridge between Hong Kong and Institut Pasteur. The salient points of the first calls are the very good quality of the applicants, their enthusiasm in preparing for the internship (a short cycle of French lessons is also offered), the professional support from Pasteur administration and, last but certainly not least, the interest shown by top scientists at Institut Pasteur to take part in this scheme.

Public Health Workshop Series at Pasteur Institute of Ho Chi Minh City (Vietnam): Surveillance of Influenza-like Illness

In line with the regional training needs in the Asian-Pacific Region, we have launched with the Pasteur Institute of Ho Chi Minh City and the support of the Institut Pasteur International Network and the French Regional Scientific Cooperation a series of international workshops for epidemiologists and public health personnel involved in surveillance activities. Facing current and future global health challenges requires a comprehensive understanding of health issues. Researchers and public health staff must become familiar with the various dimensions of global health issues in order to efficiently organize teamwork in an interdisciplinary environment and build groundbreaking multidimensional projects. The 2014 workshop has been devoted to analyze and discuss methods and impact of surveillance programs of influenza-like illness (ILI). The course allowed participants to examine in critical fashion the rationale and purpose of ILI surveillance, acquire theoretical and practical skills to assess the burden of disease during epidemics, and use evidence-based data for prediction, forecasting and information to public policy makers. The practical sessions in the afternoon were devoted to the use of the statistical software package "R", which is freely available from http://www.r-project.org/ for Windows, Mac and Linux operating systems, allowing trainees to go through some example analyses of ILI data. This course was prepared in collaboration with Ben Cowling and the School of Public Health at HKU, with a top quality faculty from HKU and leading international universities. The success of this course represents a strong foundation to further develop the impact of the International Training Center of the Pasteur Institute in Ho Chi Minh City, in order to promote evidence-based public health principles and practices in the control of infectious diseases and to improve human health (**see Annex 5.7**). Another workshop will be organized in the second half of 2015.

3.7 International Activity

HKU-PRP exerts a leadership role in a number of network projects with a major focus on viral respiratory infections.

International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC)

Roberto Bruzzone has been on the Executive Committee of ISARIC since its official launching in 2012. ISARIC - is a global initiative aiming to ensure that clinical researchers have the open access protocols and data-sharing processes needed to facilitate a rapid response to emerging diseases that may turn into epidemics or pandemics. ISARIC has become a Consortium of over 70 networks and individuals involved in research related to the outbreaks of diseases such as avian influenza, SARS, and MERS-CoV. ISARIC grew from the recognition that we have to do things differently, in the light of our experience during the epidemics of SARS, H5N1, and the 2009-10 influenza pandemic, but also regional epidemics of EV71, dengue, viral haemorrhagic fevers, and even during the rapid emergence of drug resistant malaria. In 2014 ISARIC has laid down the foundations for more challenging co-ordinated studies, including clinical trials of pathogen-specific therapies with pragmatic endpoints. Unlike the existing model that prioritises independence, we have argued that effective collaboration should be rewarded. The core materials required to enrol patients must be freely available, making it as easy as possible for investigators at the front line. The core materials of clinical research – protocols, information sheets, consent forms, and case report forms – are analogous to the source code of computer software. In open-source software projects contributors receive recognition that builds their reputation within the software community. ISARIC proposes a similar approach to clinical research, in parallel with the drive towards open access in academic publishing.

ISARIC is playing a major role in the Ebola virus crisis and has urged the deployment of alternative trial designs to fast-track the evaluation of new Ebola treatments. In a letter to *The Lancet*, we argued that although randomized controlled trials provide robust evidence in most circumstances, the lack of effective treatment options for Ebola, the high mortality with the standard of care and the paucity of effective health care systems in the affected regions means that alternative trial designs needed to be considered. We suggested that one viable approach would be to try different treatments in parallel and at different sites, following observational studies that document mortality under standard care. These trials can be designed adaptively, meaning that patient enrollment can be altered as efficacy data emerges, minimizing the numbers who get ineffective treatments and increasing the numbers getting those that show benefits. ISARIC is now involved in the coordination of two clinical trials in West Africa.

7th Framework Programme: Pathogenesis and transmission of influenza in pigs (FLUPIG)

HKU-PRC has entered into a collaborative project (Funding scheme: Large-scale integrating project) supported through the 7th Framework Program of the European Union. FLUPIG aims at a better understanding of the role of pigs in influenza pandemics. Pandemic influenza viruses come from wild birds, but they must adapt to efficient replication and transmission in humans to cause a pandemic. Malik Peiris is coordinating the involvement of the Centre in this project.

Theme-based Research Scheme "Viral, host and environmental determinants of influenza virus transmission and pathogenesis"

Malik Peiris is the Coordinator of the Theme-based Research Scheme (TRS): "Viral, host and environmental determinants of influenza virus transmission and pathogenesis", which has been awarded a HK$75 million grant. The program addresses two outstanding "grand-challenge" research questions in influenza: i) the biological determinants of influenza virus transmission from animals-to-humans and from human-to-humans; and ii) the pathogenesis of severe influenza disease. The specific goals of the TRS are to:

- Understand the viral, host and environmental determinants of influenza virus transmission between humans, and from animals to humans;
- Understand the viral and host determinants of pathogenesis of severe influenza;
- Develop evidence based interventions to reduce transmission and novel therapeutic strategies targeting the host.

The TRS will promote the implementation of the "One Health" concept to manage influenza risks, with a strong educational component embedded in the program.

The Pasteur Center for Global Health Research & Education

Institut Pasteur and the Institut Pasteur International Network are developing a Center for Global Health Research and Education (CGH) that aims to improve the health of populations worldwide. The grand challenge consists in setting up a new paradigm that will be able to enhance human, animal and environmental health by developing multi-disciplinary research projects of international excellence through the integration of basic, clinical and epidemiological research spanning the animal-human-environment interface, i.e. an inclusive One Health approach.

The CGH will develop interdisciplinary international research projects to improve knowledge on diseases and promote transformative discoveries in the very countries where the research is being conducted and where populations are the most affected. Besides strengthening synergy between research and education by establishing a comprehensive training program, the CGH will reinforce scientific knowledge and technical skills in the five continents in order to train and equip the next generation of researchers and global health leaders.

HKU-PRP has been designated as the Asian the hub of the CGH to promote the One-Health approach of infectious diseases, thereby providing the opportunity to further develop our teaching mission by aligning our educational program with the overarching objective to improve human, animal and environmental health through research and public health. The Global Teaching Program should be viewed as an extension and development of core activities (that is, research projects) into capacity building, to confront the biggest challenges in global health, from research, to public health and translational medicine. An Educational Advisory Committee has been formed and an Educational Forum to discuss and confront the scope and design of the Global Teaching Program (under the suggested designation Pasteur Global Health Academy) with other major institutions involved in global education will be organized by Roberto Bruzzone, who has been appointed Direcotr of the International Teaching and Training Progam of Institut Pasteur, during the first half of 2015. The educational activity organized in the Asia-Pacific region of the Institut Pasteur International Network has been described in the previous section (see 3.6).

4. Scientific Output

4.1 Publications cited in PubMed

1. Adebamowo C, Bah-Sow O, Binka F, Bruzzone R, Caplan A, Delfraissy JF, Heymann D, Horby P, Kaleebu P, Muyembe Tamfum JJ, Olliaro P, Piot P, Tejan-Cole A, Tomori O, Toure A, Torreele E, Whitehead J (2014) Randomised controlled trials for Ebola: practical and ethical issues. *Lancet* **384**:1423-1424.
2. Baranovich T, Burnham AJ, Marathe BM, Armstrong J, Guan Y, Shu Y, Peiris JM, Webby RJ, Webster RG, Govorkova EA (2014) The neuraminidase inhibitor oseltamivir is effective against A/Anhui/1/2013 (H7N9) influenza virus in a mouse model of acute respiratory distress syndrome. *J Infect Dis 209*:**1343**-1353.
3. Chan KP, Wong CM, Chiu SS, Chan KH, Wang XL, Chan EL, **Peiris** JS, Yang L (2014) A robust parameter estimation method for estimating disease burden of respiratory viruses. *PLoS One* **9**:e90126.
4. Chin AW, Li OT, Mok CK, Ng MK, Peiris M, Poon LL (2014) Influenza A viruses with different amino acid residues at PB2-627 display distinct replication properties in vitro and in vivo: Revealing the sequence plasticity of PB2-627 position. *Virology* **468-470**:545-555.
5. Chin AW, Mok CK, Zhu H, Guan Y, Peiris JS, Poon LL (2014) Use of fractional factorial design to study the compatibility of viral ribonucleoprotein gene segments of human H7N9 virus and circulating human influenza subtypes. *Influenza Other Respir Viruses* **8**:580-584.
6. Chu DK, Poon LL, Gomaa MM, Shehata MM, Perera RA, Abu Zeid D, El Rifay AS, Siu LY, Guan Y, Webby RJ, Ali MA, Peiris M, Kayali G (2014) MERS coronaviruses in dromedary camels, Egypt. *Emerg Infect Dis* **20**:1049-1053.
7. Claessen JHL, Sanyal S, Ploegh H (2014) The chaperone Bag6 captures dislocated glycoproteins in the cytosol. *PloS One* **9**:e90204.
8. Dunning J, Merson L, Rohde GG, Gao Z, Semple MG, Tran D, Gordon A, Olliaro PL, Khoo SH, Bruzzone R, Horby P, Cobb P, Longuere KS, Kellam P, Nichol A, Brett S, Everett D, Hien TT, Yu H, Zambon M, Ruiz-Palacios G, Lang T, Akhvlediani T, ISARIC Working Group 3, ISARIC Council, Hayden F, Marshall J, Webb S, Angus DC, Shindo N, van der Werf S, Openshaw PJ, Farrar J, Carson G, Baillie JK (2014) Open source clinical science for emerging infections. *Lancet Infect Dis* **14**:8-9.
9. Garcia JM, Lai JC, Haselhorst T, Choy KT, Yen HL, Peiris JS, von Itzstein M, Nicholls JN (2014) Investigation of the binding and cleavage characteristics of N1 neuraminidases from avian, seasonal and pandemic influenza viruses using saturation transfer difference nuclear magnetic resonance. *Influenza Other Respir Viruses* **8**:235-242.
10. Hemida MG, Chu DK, Poon LL, Perera RA, Alhammadi MA, Ng HY, Siu LY, Guan Y, Alnaeem A, Peiris M (2014) Seroepidemiology of Middle East respiratory syndrome (MERS) coronavirus in Saudi Arabia (1993) and Australia (2014) and characterisation of assay specificity. *Euro Surveill* **19**(23). pii:20828.
11. Hemida MG, Chu DK, Poon LL, Perera RA, Alhammadi MA, Ng HY, Siu LY, Guan Y, Alnaeem A, Peiris M (2014) MERS coronavirus in dromedary camel herd, Saudi Arabia. *Emerg Infect Dis* **20**:1231-1234.
12. Lee SM, Kok KH, Jaume M, Cheung TK, Yip TF, Lai JC, Guan Y, Webster RG, Jin DY, Peiris JS (2014) Toll-like receptor 10 is involved in induction of innate immune responses to influenza virus infection. *Proc Natl Acad Sci USA* **111**:3793-3798.
13. Leung YH, Nicholls JM, Ho CK, Sia SF, Mok CK, Valkenburg SA, Cheung P, Hui KP, Chan RW, Guan Y, Akira S, Peiris JS (2014) Highly pathogenic avian influenza A H5N1 and pandemic H1N1 virus infections have different phenotypes in Toll-like Receptor (TLR) 3 knock-out mice. *J Gen Virol* **95**:1870-1879.

14. Mok CK, Peiris JS, Chan MC (2014) Anti-inflammatory and anti-viral effects of indirubin derivatives in H5N1-infected primary macrophages and pneumocytes. *Antiviral Res* **106C**:95-104.

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18. Wang XL, Wong CM, Chan KH, Chan KP, Cao PH, Peiris JM, Yang L. (2014) Hospitalization risk of the 2009 H1N1 pandemic cases in Hong Kong. *BMC Infect Dis* **14**:32.

19. Wu JT, Leung K, Perera RA, Chu DK, Lee CK, Hung IF, Lin CK, Lo SV, Lau YL, Leung GM, Cowling BJ, Peiris JS (2014) Inferring influenza infection attack rate from seroprevalence data. *PLoS Pathog* **10**:e1004054.

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23. Guan WD*, Gong XY*, Mok CK*, Chen TT, Wu SG, Pan SH, Cowling BJ, Yang ZF, Chen DH (2015) Surveillance for seasonal influenza virus prevalence in hospitalized children with lower respiratory tract infection in Guangzhou, China during the post-pandemic era. *PLoS One*, **in revision** (*equal contribution)**.**

24. Lestra M*, Jahan AS*, Swee LK*, Fan Y, Tafesse FG, Theile CS, Spooner E, Bruzzone R, Ploegh HL, Sanyal S (2015) Usp12 stabilizes the T cell receptor complex at the cell surface during signaling. **Under review** (*equal contribution)**.**

25. Li MY, Grandadam M, Kwok K, Lagache T, Siu YL, Zhang JS, Sayteng K, Kudelko M, Qin CF, Olivo-Marin JC, Bruzzone R, Wang PG (2015) KDEL receptors assist dengue virus exit from the endoplasmic reticulum. *Cell Rep*, **in press**.

26. Memish ZA, Alsahly A, Masri MA, Heil GL, Anderson BD, Peiris M, Khan SU, Gray GC (2014) Sparse evidence of MERS-CoV infection among animal workers living in Southern Saudi Arabia during 2012. *Influenza Other Respir Viruses*, **in press**.

27. Yang ZF*, Mok CK*, Liu XQ*, Li XB, He JF, Guan WD, Xu YH, Pan WQ, Chen LY, Lin YP, Wu SG, Pan SH, Huang JC, Ding GY, Zheng K, Ke CW, Lin JY, Zhang YH, Lee HHY, Liu WK, Yang CG, Zhou R, Peiris JS, Li YM, Chen RC, Chen L, Zhong NS (2015) Clinical, virological and immunological features from patients infected with re-emergent avian-origin human H7N9 influenza disease of varying severity in Guangdong province. *PLoS One*, **in press** (*equal contribution).

4.2 Presentations at Meetings

1. Y Fan, N Lagarde, R Bruzzone, F Kien (2014) **Cyclin D3 restricts influenza infection by interacting with M2 ion channel protein.** *Keystone Symposium: Innate immunity to viral infections*, Keystone, CO, USA (Poster).
2. M Lestra*, AS Jahan*, LK Swee*, S Sanyal (2015) *Keystone Symposium on the Human Proteome*, Stockholm, Sweden (Oral).
3. MY Li, M Grandadam, KTH Kwok, T Lagache, LYL Siu, K Sayteng, M Kudelko, JC Olivo-Marin, R Bruzzone, PG Wang (2014) **Dengue virus requires KDEL receptors to exit the endoplasmic reticulum**. *33th Meeting of the American Society for Virology*, Fort Collins, CO, USA (Oral).
4. CK Mok (2014) **Pathogenicity and viral determinants of the novel A/H7N9 influenza virus in mice**. *9th Conference Louis Pasteur: Emerging Infectious Diseases*, Paris, France (Poster).
5. CK Mok, HH Lee, M Lestra, MC Chan, SF Sia, JM Nicholls, H Zhu, Y Guan, JS Peiris (2014) **Pathogenicity and viral determinants of the novel A/H7N9 influenza virus in mice.** *Symposium on Emerging Infectious Diseases in South East Asia*, Phnom Penh, Cambodia (Poster).
6. CK Mok, HH Lee, ZF Yang, JS Peiris (2014) **Host and the viral factors that contribute to the pathogenicity of the novel A/H7N9 influenza virus.** *Scientific Symposium of the Institut Pasteur International Network*, Paris, France.
7. CK Mok, HH Lee, ZF Yang, JS Peiris (2014) **Host and the viral factors that contribute to the pathogenicity of the novel A/H7N9 influenza virus.** *The 15th IUBMB-24th FAOBMB-TSBMB Conference*, Taipei, Taiwan.
8. S Sanyal, J Ashour, H Ploegh (2014) **Viral exploitation of host intracellular trafficking pathways upon interferon induction**. *Keystone Symposium: Innate immunity to viral infections*, Keystone, CO, USA (Oral).
9. Y Fan, S Sanyal (2014) **Role of Tsg101 in influenza virus assembly and release**. *9th Conference Louis Pasteur: Emerging Infectious Diseases*, Paris, France (Poster).
10. O Teng, SL Hsieh, TL Hsu, R Bruzzone, M Peiris, HL Yen (2014) **CLEC5A is involved in the influenza virus pathogenesis by modulating the cytokine secretion**. *33th Meeting of the American Society for Virology*, Fort Collins, CO, USA (Oral).

4.3 Seminars, Invited Lectures and Oral Presentations

1. Roberto Bruzzone (2014) The First Affiliated Hospital of Guanzhou Medical University, Guanzhou, PR China.
2. Roberto Bruzzone (2015) International Conference on Scientific Insight and Response of Ebola Virus Disease, Beijing, PR China.
3. Jimmy Lai (2014) Department of Pathology, The University of Hong Kong, Hong Kong SAR.
4. Sumana Sanyal (2014) The University of Hong Kong, Hong Kong SAR.
5. Sumana Sanyal (2014) University of Massachusetts, Amherst, MA, USA.
6. Sumana Sanyal (2014) The Whitehead Institute for Biomedical Research, MIT, Cambridge, MA, USA.
7. Sumana Sanyal (2014) Keystone Symposium "Innate immunity to viral infections", Keystone, CO, USA.
8. Sumana Sanyal (2014) Columbia University, New York, NY, USA.
9. Sumana Sanyal (2014) Annual retreat, Massachusetts Institute of Technology, NH, USA.
10. Sumana Sanyal (2014) EMBO workshop for group leaders, Leiman, Germany.
11. Sumana Sanyal (2014) EMBL, Heidelberg, Germany.
12. Sumana Sanyal (2015) Lecture in the Molecular Biology of the Cell course, Institut Pasteur, Paris, France.
13. Sumana Sanyal (2015) Department of Cell Biology and Infectiont, Institut Pasteur, Paris, France.
14. Sumana Sanyal (2015) Keystone Symposium "The Human Proteome", Stockholm, Sweden.

4.4 Active Grants 2014-Present

Area of Excellence, Control of Pandemic and Inter-pandemic Influenza

Principal Investigator: Suki Lee
Amount: HK$248,400.00
Period: 01/May/2014 to 31/Dec/2015

Area of Excellence, Control of Pandemic and Inter-pandemic Influenza

Principal Investigator: Suki Lee
Amount: HK$250,000.00
Period: 01/May/2015 to 31/Dec/2016

Area of Excellence, Control of Pandemic and Inter-pandemic Influenza

Principal Investigator: Chris Mok
Amount: HK$250,000.00
Period: 01/May/2014 to 31/Dec/2015

Area of Excellence, Control of Pandemic and Inter-pandemic Influenza

Principal Investigator: Chris Mok
Amount: HK$250,000.00
Period: 01/May/2015 to 31/Dec/2016

Area of Excellence, Control of Pandemic and Inter-pandemic Influenza

Principal Investigator: Sumana Sanyal
Amount: HK$300,000.00
Period: 01/May/2014 to 31/Dec/2015

Area of Excellence, Control of Pandemic and Inter-pandemic Influenza

Principal Investigator: Sumana Sanyal
Amount: HK$250,000.00
Period: 01/May/2015 to 31/Dec/2016

BNP-Paribas

Principal Investigator: Roberto Bruzzone
Amount: HK$400,000.00
Period: 01/Jan/2014 to 31/Dec/2015

Commissioned Health and Medical Research Fund (HMRF)

Co-Investigator: Chris Mok (PI: Malik Peiris)
Amount: HK$360,000.00
Period: 01/Jun/2014 to 31/May/2015

Commissioned Health and Medical Research Fund (HMRF)

Co-Investigators: Chris Mok and Jimmy Lai (PI: Malik Peiris)
Amount: HK$882,580.00
Period: 01/Jun/2014 to 31/May/2015

European Commission (FP7)

Principal Investigator: Malik Peiris/ John Nicholls
Amount: €375,150.00
Period: 01/Jul/2010 to 31/Dec/2014

Ester Lee and Chew Pik Foundation, Croucher Foundation and other donors

Co-Investigator: Jimmy Lai (PI: John Nicholls)

GuangZhou Nanxin Pharmaceutical Co Ltd

Principal Investigator: Chris Mok
Amount: HK$124,082.84
Period: ends 31/Dec/2017

Health and Medical Research Fund (HMRF)

Principal Investigator: Suki Lee
Amount: HK$991,404.00
Period: 01/Jan/2012 to 31/Oct/2014

Health and Medical Research Fund (HMRF)

Principal Investigator: Suki Lee
Amount: HK$998,544.00
Period: 01/Jan/2013 to 30/Nov/2015

Health and Medical Research Fund (HMRF)

Co-Investigator: Suki Lee (PI: Ben Cowling)
Amount: HK$796,778.00
Period: 24 months

Health and Medical Research Fund (HMRF)

Principal Investigator: Chris Mok
Amount: HK$696,067.00
Period: 01/Oct/2012 to 30/Sep/2014

Health and Medical Research Fund (HMRF)

Co-Investigator: Chris Mok (PI: Michael Chan)
Amount: HK$978,704.00
Period: 01/Mar/2013 to 28/Feb/2015

Health and Medical Research Fund (HMRF)

Co-Investigators: Chris Mok and Jimmy Lai (PI: John Nicholls)
Amount: HK$794,000.00
Period: 01/Nov/2013 to 31/Oct/2015

Health and Medical Research Fund (HMRF)

Principal Investigator: Chris Mok
Amount: HK$638,340.00
Period: 01/Jul/2015 to 30/Jun/2017

Health and Medical Research Fund (HMRF)

Principal Investigator: Sumana Sanyal
Amount: HK$981,120.00
Period: 01/Jul/2015 to 30/Jun/2017

L'Oreal Scholarship

Principal Investigator: Roberto Bruzzone
Amount: HK$700,000.00
Period: 01/Jan/2014 to 31/Dec/2015

National Institute of Health

Co-Investigator:	Sumana Sanyal (PI: Hidde Ploegh, MIT)
Amount:	US$500,000.00
Period:	ends 31/Dec/2016

Research Grants Council

Principal Investigator:	Suki Lee
Amount:	HK$632,266.00
Period:	01/Oct/2014 to 30/Sep/2016

Research Grants Council

Co-Investigator:	Chris Mok (PI: PC Shaw, CUHK)
Amount:	HK$650,000.00
Period:	2015 to 2017

Research Grants Council

Principal Investigator:	Sumana Sanyal
Amount:	HK$664,557.00
Period:	01/Dec/2014 to 30/Nov/2016

Seed Fund, University of Hong Kong

Principal Investigator:	Chris Mok
Amount:	HK$120,000.00
Period:	01/Apr/2014 to 31/Mar/2016

Seed Fund, University of Hong Kong

Principal Investigator:	Sumana Sanyal
Amount:	HK$120,000.00
Period:	01/Jul/2014 to 30/Jun/2016

Small Project Funding, University Research Committee

Principal Investigator:	Jimmy Lai
Amount:	HK$40,536.00
Period:	01/May/2014 to 31/Oct/2015

UGC-Matching Fund Scheme (6th Phase)

Principal Investigator:	Roberto Bruzzone
Amount:	HK$723,027.00
Period:	01/Apr/2014 to 31/Mar/2016

4.5 Pending Grant Applications

NSFC – PR China (Potentially fundable)

Co-Principal Investigator:	Chris Mok (PI: Yang Zi Feng)
Amount:	CNY 1,500,000.00
Period:	5 years

Health and Medical Research Fund (HMRF)

Principal Investigator:	Jimmy Lai
Amount:	HK$900,000.00
Period:	24 months

Health and Medical Research Fund (HMRF)

Principal Investigator:	Chris Mok
Amount:	HK$1,000,000.00
Period:	24 months

Research Grants Council

Principal Investigator:	Chris Mok
Amount:	HK$1,000,000.00
Period:	24 months

Research Grants Council

Principal Investigator:	Sumana Sanyal
Amount:	HK$1,033,296.00
Period:	24 months