# Annual Report 2016

**HKU-Pasteur Research Pole**

**7/F Jockey Club Building for Interdisciplinary Research**

**5, Sassoon Road, Hong Kong SAR**

## Roberto Bruzzone, Co-Director

## Malik Peiris, Co-Director

# INDEX

# 4. Scientific Output



# 5. Annexes

# 1. Summary

## Mission

The HKU-Pasteur Research Pole (HKU-PRP) is a joint laboratory established by the University of Hong Kong (HKU) and the Institut Pasteur (IP). HKU-PRP was was integrated into the School of Public Health of the Li Ka Shing Faculty of Medicine of the University of Hong Kong in 2013. HKU-PRP aims to improve global health through research by confronting the challenges posed by viral infections and provide solutions to treat infectious diseases. HKU-PRP will further enhance the successful partnership between HKU and IP by expanding its current strength on respiratory and arboviral diseases to tackle basic cell biology and immunology processes that are relevant to the advancement of our understanding of pathogenetic mechanisms and rational vaccine design. HKU-PRP has been designated as one of the hubs of the Institut Pasteur International Network in recognition of its achievements and of the strategic partnership with the School of Public Health of HKU.

## Research

Our overarching goal is to generate biological knowledge to better understand and treat infectious diseases by bringing together scientists with an interdisciplinary mind. We have organized our activity around four Group Leaders who are engaged in competitive research projects on respiratory infections mosquito borne viruses (arboviruses) such as dengue and Zika. 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 and adaptive immunity for improved protection. Collaboration with the First Affiliated Hospital of Guangzhou Medical University has led to very significant publications on avian influenza and the newly identified Middle East respiratory syndrome coronavirus (MERS-CoV), and laboratory space has been made available to HKU-PRP to further develop clinical research projects. With respect to arboviruses, we are now extending our investigations on the characterization of host factors that are exploited to facilitate virus replication, biogenesis, trafficking, and egress. The scientific output of HKU-PRP has been of the highest quality, with more than 20 papers published since January 2016.

## Teaching

Our program of courses for postgraduate students and young scientists is drawing an increasing number of highly qualified applications from around the world, establishing a worldwide network of trainees. HKU-PRP will provide students, scientists and health professionals with consistent knowledge and interdisciplinary training through its international education program. In 2016 we have continued our course series in Cell Biology and Virology. In collaboration with the School of Public health at HKU, the topic of the 2016 edition of our Public Health Workshop series at the Pasteur Institute of Ho Chi Minh City (Vietnam) was "Introduction to Modeling of Infectious Diseases. This is an area in which there is still a gap between countries with different resources and public health systems. We are determined to fill the need for a dedicated course that will allow a regular training of public health staff.

## Perspectives

We have developed a strong identity to promote the agenda of HKU, IP and the Pasteur Network, through research, teaching and public health activities. We have expanded our critical mass with the recruitment of a new Group Leader that will reinforce research on basic and applied immunology. The interdisciplinary nature of our research and teaching will facilitate synergies with the new School of Biomedical Sciences established by HKU to integrate basic science departments. Thus, Sumana Sanyal has been promoted to the rank of Assistant Professor, with a joint affiliation to the School of Biomedical Sciences, with a career development award from the Croucher Foundation. Moreover, Professor James Di Santo from the Institut Pasteur has been appointed as Visiting Professor for the period 2016-8 through the "Visiting Research Professors" scheme of the University Research Committee of HKU. His appointment will stimulate research efforts and exchanges at several different levels within the LKS Faculty of Medicine. Finally, HKU and IP have signed a10-year extension of their collaborative agreement. In summary, the results obtained in 2016 are clearly in line with our strategic objective to position HKU-PRP as a cluster of excellence within the School of Public Health.

# 2. Overview of the Programs

## 2.1 Research

The scientific activity of HKU-PRP has been significantly re-organized around three core projects to become an important component of the School of Public Health at HKU. Three questions are of particular interest to us:

1. *How do viruses invade, replicate and escape infected cells?* This question encompasses both the virus point of view of the infectious process – by studying molecules and machinery of the host cells that are hijacked during the viral life cycle, as well as the cellular view – to investigate counterstrategies employed by the host in order to prevent virus infection at various steps, including replication, assembly and release.
2. *What makes a microbe pathogenic?* This question interrogates the behavior and pathogenicity of emerging viruses by combining clinical studies that span serology, epidemiology and pathogenicity to delineate 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 one hand, on innate responses and the complex strategies devised by viruses to foil them and, on the other hand, on adaptive lines of defense of the host and how they could be harnessed and optimized by vaccination to improve protection.

**Research in the Suki Lee lab** focuses on virus-host interaction and host innate immune response to viral infection, with a major objective to investigate the underlining mechanisms of innate immunity relevant to disease pathogenesis, and to explore the potential of novel therapeutic targets for the treatment. Her lab revealed a novel role of an orphan receptor, TLR10, in viral pathogenesis by showing that influenza virus infection increased TLR10 expression and providing evidence for the involvement of TLR10 in innate immune sensing of viral infection and in cytokine production. The Lee lab has now extended its investigations on TLR10 to identify its ligand and signaling pathways and has demonstrated that TLR10 is a novel nucleotide sensing receptor and that dsRNA is a ligand of TLR10 for its signaling to regulate IFN response. One MPhil student has graduated and a new MPhil candidate has joined the team. Three international students were trained in the lab for an internship.

**The lab of Chris Mok** uses a combination of clinical and experimental studies that span the areas of serology, epidemiology and molecular biology to understand the behavior and pathogenicity of emerging viruses. In this context, the Mok lab has set up with HKU and the First Affiliated Hospital of Guangzhou Medical University a research platform to cover a wide range of research initiatives related to new emerging viruses and laboratory space has been made available to HKU-PRP to develop collaborative research projects. A related effort is pursued to understand the interplay between the viral and host factors that influence the replication of influenza viruses. In particular Mok and co-workers are investigating 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 A/B replication and pathogenicity.

**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 arboviruses 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. The group 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. Among other factors, the lab is particularly interested in ubiquitin-like post-translational modifiers of protein function, such as ISG15 that play a significant role in modulating different pathways, most of which are innate signaling pathways.

**The main objectives of the group of Sophie Valkenburg** are determining the role of protective heterologous T and B cell immunity in mouse and human systems, by investigating novel vaccines and immune correlates of protection for influenza. The primary focus is to study adaptive immunity to influenza, and how this could be harnessed and optimized by vaccination to improve protection from diverse influenza virus infection. Hemagglutinin-specific antibodies can block influenza

infection, whilst T cells recognize influenza-infected cells. A vaccine which ultimately combines antibody and T cell based immunity for influenza will provide a full-proof immunological barrier to influenza infection, which our studies will ultimately help develop.

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 study interactions between viruses with host receptors, in order to have a better understanding on viral host adaptation and cellular/tissue tropism. Work on the sero-epidemiology of MERS-CoV and Ebola virus is coordinated by Malik Peiris. HKU-PRP has welcomed Barbara Gayraud-Morel as Visiting Scientist from the Institut Pasteur. Part of her research is related to skeletal muscle function, which is relevant to the subject she works on at the Institut Pasteur, in the Stem Cell and Development laboratory directed by Shahragim Tajbakhsh. This project aims to explore the consequences of respiratory virus infections on skeletal muscles and muscle stem cells in particular. The project involves collaboration between HKU-PRP and several laboratories at HKU, HKUST and Institut Pasteur. The second area of research aims to establish a human lung epithelium model to study infectious diseases. We aim to take advantage of the growing human embryonic stem cell (hESC) and iPSC fields to establish a model of human lung epithelium to investigate respiratory infectious diseases.

## 2.2 Teaching and Education

HKU-PRP pledges to extend the impact of research into the production and diffusion of knowledge by developing 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 significantly contributes to solidifying the reputation of HKU-PRP as the premier regional hub for education. The Virology course has been held for the 13$^{th}$ consecutive year and the Cell Biology course has reached the 7$^{th}$ edition.

Our Public Health Workshop series at the Pasteur Institute of Ho Chi Minh City is attracting increasing number of applications and has established a benchmark as a world-class training program for epidemiologists, researchers and public health officials in the region. The topic of the 2016 edition, organized in close partnership with the Pasteur Institute of Ho Chi Minh City, the School of Public Health at HKU and the International Network of Institut Pasteur, was "Introduction to Modeling of Infectious Diseases". We have identified mathematical modeling as an area in which there is still a gap between countries with different resources and public health systems and are determined to fill this need with a dedicated course that will be repeated in the future.

All Group Leaders are actively engaged in the Teaching and Training program. We hosted a Master student from the Netherlands and two undergraduate summer students from University College London.

## 2.3 International Activity

We retain leadership roles in a number of global projects. Roberto Bruzzone is a member of the Executive Committee and Vice-Chair of the International Severe Acute Respiratory and Emerging Infection Consortium (www.isaric.tghn.org), a network of networks which aims at ensuring that clinical researchers have the open access protocols and data-sharing processes needed to facilitate a rapid response to emerging diseases. 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-scale 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 and is the Co-Director of the WHO H5 Reference Laboratory at HKU.

# 3. Progress Report

# 3.1 Suki MY LEE Lab

## Main Objectives and Strategy

Acute respiratory viral infections remain a major cause of morbidity worldwide and of mortality in the developing world. Emerging respiratory viruses such as MERS, SARS, avian influenza H5N1 and pandemic H1N1 impacted on societies and economies in many countries. The innate immune system is the first line of host defense and is central to a patient's effort to combat such emerging infections as well as common respiratory virus diseases. My lab focuses on virus-host interaction and host innate immune response to viral infection, with a major objective to investigate the underlining mechanisms of innate immunity relevant to disease pathogenesis, and to explore the potential of novel therapeutic targets for the treatment. Our major research projects are listed below.

### *Involvement of TLR10 in induction of innate immune responses to influenza virus infection*

Our lab revealed a novel role of an orphan receptor, TLR10, in viral pathogenesis. We showed that influenza virus infection increased TLR10 expression and demonstrated the involvement of TLR10 in innate immune sensing of viral infection and the production of cytokine and interferon (IFN). We have extended our study on TLR10 to identify its ligand and signaling pathways and we have demonstrated that TLR10 is a novel nucleotide sensing receptor and dsRNA is a ligand of TLR10 for its signaling to regulate IFN response.

### *Association between basal leukocyte transcriptome profile and symptom development & disease severity after influenza virus infection in humans*

We investigate the association between leukocyte basal gene expression profile and influenza symptom development and disease severity during acute community-acquired influenza virus infection. Basal leukocyte transcriptome profile for confirmed-influenza infected household contacts and matched controls is determined by microarray analysis, and genes that are significantly differentially expressed in relation to disease severity and age are identified. Systems-level comparison on the transcriptome profile between symptomatic and asymptomatic contacts is performed.

## Achievements and Ongoing Research

We have provided different lines of evidences to confirm a novel role of TLR10, an orphan receptor without known ligand and function, as a nucleotide sensing receptor. The data arising from this study provide new mechanistic insight to understand its role in innate immunity and the functional relevance of this receptor in disease (Lee et al, submitted). One MPhil student (Tsz Fung YIP) has graduated and a new MPhil candidate (Aisha SELIM) has joined the team. Three international students trained in my lab this year for an internship period (Koen LOMMEN from Erasmus University Medical Center, The Netherlands; Elizabeth VACHER and Elaina CAYROUSE, both from University College, London, UK).

### *Novel role of TLR10 as a nucleotide sensing receptor* [Funding: GRC/GRF, Theme-based Research Scheme and RGC Seed funding for basic research]

#### (a) *Sub-cellular localization of TLR10*

We have used confocal microscopy to define the sub-cellular localization of TLR10. TLR10 was detected on the cell surface as well as intracellularly but expression was more abundant intracellularly. Markers of respective intracellular organelles were used to investigate the co-

localization of TLR10 in different cellular compartments. Our findings demonstrated that TLR10 was predominately expressed in endosomes, with the highest expression detected in RAB11A$^+$ recycling endosomes and RAB5$^+$ early endosomes. TLR10 expression level was also found to be high in endoplasmic reticulum and RAB7$^+$ late endosomes. Although TLR10 was also detected in the Golgi apparatus, it was with relatively lower expression level.

## (b) *dsRNA is a ligand for TLR10 sensing and signaling*

High expression of TLR10 in early endosomes of resting cells suggested that TLR10 may be nucleic acid sensing receptor. We screened a range of known agonists of other PRRs as potential ligands for TLR10 in WT, TLR10 OE and TLR10 KD THP-1 cells. Agonists that are known to trigger cell surface TLRs were added directly to culture medium. Pam3CSK4 and FSL-1, known agonists for TLR2/1 and TLR2/6 respectively, are synthetic lipopeptides that mimic the amino terminus of bacterial or mycoplasmal lipoproteins, which can be found on the cell wall of microorganisms. HKLM, LPS and flagellin are effective inducers of the pro-inflammatory cytokines through the activation of TLR2, 4 and 5 respectively. When these agonists were added directly to stimulate cell surface TLR10, minimal induction of *IFNβ* was detected in WT THP-1 cells and there was no significant changes in *IFNβ* expression in TLR10 OE or KD compared to WT cells. Poly(I:C), imiquimod, ssRNA40 and CpG ODN2006 are analogues of dsRNA, ssRNA or unmethylated DNA oligonucleotides, which could be recognized by TLR3/RIG-I/MDA-5, TLR7, 8 and 9 respectively. These agonists were transfected to WT, TLR10 OE or KD THP-1 cells via cationic lipid mediated delivery. Of all the PRR agonists tested, the analogue of dsRNA, poly(I:C), was the most potent in inducing type I IFN response at 4 h post stimulation in WT THP-1 cells. Interestingly, there was a significant induction of *IFNβ* in TLR10 KD cells compared to WT cells in response to poly(I:C) stimulation, whilst overexpression of TLR10 reduced the IFN response. The minimal induction of *IFNβ* by other ligands suggesting that type I IFN by imiquimod and ssRNA was un-detectable at early time points after ligand stimulation. CpG ODN2006 was a class B CpG oligonucleotide, which preferentially activates B cells rather than stimulating the type I IFN production.

Next, we further investigated if poly(I:C) stimulates TLR10 mediated IFN response in a time- and dose-dependent manner. We found that the changes in expression of type I IFN detected among WT, OE and KD THP-1 cells was consistent and showed a significant differential difference, at 3 h and 6 h post stimulation time and at various concentrations from 10 μg/ml to 40 μg/ml. In addition to poly(I:C), as specific features on nucleic acids have been reported to be crucial for the activation of certain PRRs, we have also employed other synthetic dsRNA with 5'ppp to investigate if 5'ppp could be sensed by TLR10 and enhanced its signaling. We transfected either the 5'pppdsRNA (dsRNA WT) or its structurally modified variants to improve its antiviral property (dsRNA M5) as possible ligands to stimulate TLR10 signaling. Similar to poly(I:C), we found that while *IFNβ* expression is significantly induced by these 5'pppdsRNAs in WT THP-1 cells, overexpression of TLR10 suppressed *IFNβ* expression, and knocking down of TLR10 upregulated such expression. Of note, the differential expression of *IFNβ* in OE or KD vs WT cells upon 5'pppdsRNA stimulation was found to be comparable to poly(I:C) did, suggesting that TLR10 does not have preferences to sense 5'pppdsRNA over dsRNA and 5'ppp is not an essential ligand structure for TLR10 sensing and signaling. Overall, the down-regulated IFN response in TLR10 OE cells implies that TLR10 negatively modulates IFN responses after dsRNA stimulation.

## (c) *TLR10 binds dsRNA and co-localizes in early and late endosomes*

To confirm dsRNA as ligand for TLR10, we further investigated if TLR10 could bind dsRNA using *in vitro* binding assays. Studies have been demonstrated that ligand binding and subsequent signaling of nucleic acid sensing TLRs depends on the pH environment. High affinity of nucleic acid to TLRs could only be observed at acidic pH similar to that is seen within endosomes (pH 4.5 – 6.5). We therefore chose to perform binding assay at pH 5.5. We found that TLR10 was readily pulled down *in vitro* using biotinylated poly(I:C) as bait at pH 5.5. Addition of competitive unlabelled poly(I:C) markedly decreased the amount of TLR10 being pull-down, suggesting that TLR10 specifically bound to poly(I:C). In contrast, there was a lack of detectable TLR10 pulled down when the assay was performed at pH 7.4. The pH 7.4 was chosen because it is similar to the physiological pH of

mammals and the pH at which THP-1 cells were cultured, thus resembling the condition at the cell surface. The absence of detectable TLR10 co-precipitation in suggested that binding of dsRNA to TLR10 does not occur at pH 7.4, the extracellular pH environment in mammals. Furthermore, we performed co-localization study to investigate the spatial association of TLR10 with poly(I:C). As shown, after ligand transfection, fluorescent-labelled poly(I:C) was found to co-localize with TLR10 in $RAB5^+$ early or $RAB7^+$ late endosomes, although the co-localization was barely seen in $RAB11A^+$ recycling endosome. Taken together, the data here demonstrated that TLR10 physically interacts with dsRNA, probably inside the endosomal compartment, and further supporting that dsRNA as a ligand for TLR10 sensing and signaling.

### (d) *MyD88 is recruited to TLR10 upon dsRNA stimulation*

Binding of TIR-domain containing signal-activating adaptor proteins to the TIR domains of TLRs is essential for TLR signaling transduction. The highly conserved BB-loop structure of TLRs was shown to interact with the TIR domain of the adaptor proteins. One alanine/proline residue in the BB-loop is required to confer adaptor binding specificity. All known human TLRs have a proline residue at the BB-loop, except TLR3 which contains alanine at that position. Interestingly, all human TLR members bind to MyD88, except TLR3 which binds TIR-domain-containing adaptor-inducing IFN-β (TRIF).

Sequence analysis of TLR10 revealed the presence of a proline residue in the BB loop of the TIR domain of TLR10. This suggests that MyD88 may be a potential adaptor protein candidate for TLR10 signaling. To confirm this, recruitment of MyD88 to TLR10 upon poly(I:C) stimulation was investigated by co-localization studies. As shown, unstimulated cells without poly(I:C) transfection displayed little or no co-localization of MyD88 with TLR10. When cells were transfected with poly(I:C), recruitment of MyD88 to TLR10 was observed as early as 5 min with increasing co-localization events observed at 10 min post ligand stimulation, and such co-localization decreased subsequently. Recruitment of MyD88 by TLR10 upon stimulation was further confirmed by immunoprecipitation. In accordance to these results, mock transfection without poly(I:C), MyD88 was barely detectable in samples immunoprecipitated with anti-TLR10 antibody as expected, suggesting that there was only a very trace amount of MyD88 being recruited to TLR10 in unstimulated cells. A stronger interaction between MyD88 and TLR10 was detected upon poly(I:C) stimulation. Similar to the co-localization study of MyD88 with TLR10, the strongest interaction was detected at 10 min post stimulation and gradually decreased afterwards. As TRIF could act as an adaptor protein in TLR signaling for IFN induction, immunoprecipitation of TLR10 with TRIF was also performed. However, TRIF was not pulled-down by TLR10 in the immunoprecipitation experiment. Taken together, we demonstrate that, unlike classical TLR pathway activated by poly(I:C) as of TLR3, MyD88, but not TRIF was actively recruited to TLR10 upon poly(I:C) stimulation for the regulation of IFN expression.

### (e) *TLR10 mediated IFN expression is regulated via IRF7*

IRFs play an essential role in regulating the genes of TLR signaling induced type I IFN. Among the nine members of IRFs, IRF7 is known to play a role in transcriptionally regulating virus-induced genes. In particular, IRF7-dependent amplification of type I and III IFN is shown to be essential in host defense against influenza virus infections. The activation of IRF7 is characterized by the phosphorylation of its C-terminus by the IKK-related kinases TBK-1 and $IKK_\varepsilon$, followed by IRF dimerization and nuclear translocation. To determine whether TLR10 signaling involves the reduction of phosphorylation of IRFs, subsequently leading to reduced type I IFN production, WT and TLR10 OE THP-1 cells were challenged by poly(I:C) transfection and the phosphorylation of IRF7 was examined. In WT cells, as expected, phosphorylation of IRF7 stably increased in response to poly(I:C) challenge. Whilst, phosphorylation of IRF7 was found to be markedly reduced in TLR10 OE cells compared with WT cells. In addition to IRF7, we also looked at IRF3, a well-known transcription factor involves in triggering IFN expression following TLR signaling. Phosphorylation of IRF3 was analyzed and compared between WT and TLR10 OE cells upon poly(I:C) stimulation. No difference was found in IRF3 phosphorylation with regard to TLR10 overexpression, and its degree of phosphorylation remained comparable in WT or TLR10 OE cells at all times upon poly(I:C)

challenge. Overall, data here suggests that activation of TLR10 signaling modulates the phosphorylation and expression of IRF7 to regulate IFN response.

### (f) *Increased IFN signaling in TLR10 KD cells upon poly(I:C) activation*

The correlation of type I IFN response and TLR10 expression was further analyzed upon stimulation with poly(I:C) using an inducible reporter assay. The expression of a soluble luciferase in a reporter THP-1 cell-based cell line was under the control of an IRF-inducible promoter, which is comprised of five IFN-stimulated response elements (ISRE) and an ISG54 minimal promoter. Thus quantification of the luciferase activity reflects the induction of type I IFN signaling responses in the reporter cells. To examine the role of TLR10 in type I IFN signaling, small interfering RNA (siRNA) against TLR10 was introduced to the reporter cells and their luciferase activities in response to poly(I:C) stimulation was compared to non-targeting control (NC) siRNA-treated cells. Upon poly(I:C) challenge, a significant increase in luciferase activity was observed in reporter cells transfected with siRNA against TLR10, indicating that the type I IFN response would be augmented in a TLR10 deficient environment.

In conclusion, Toll-like receptor (TLR)-10 remains an orphan receptor without well characterized ligands and functions. We demonstrated that TLR10 is predominately localized in the endosomes and involves in dsRNA stimulated interferon (IFN) expression. TLR10 physically binds dsRNA *in vitro* at pH 5.5, the pH condition found within the endosomal compartment and co-localizes with dsRNA in early and late endosomes, suggesting that dsRNA is a ligand of TLR10. Upon dsRNA stimulation, myeloid differentiation primary response gene 88 (MyD88) was recruited to activated TLR10 for signal transduction leading to suppression of interferon regulatory factor (IRF)-7 dependent IFN signaling. Our result suggested for the first time that TLR10 is a novel nucleotide sensing receptor and dsRNA is a ligand of TLR10 for its signaling to regulate IFN response.

### *Association between basal leukocyte transcriptome profile and symptom development & disease severity after influenza virus infection in humans* [Funding: HMRF]

We have obtained an HMRF grant on this project in collaboration with Ben Cowling (School of Public Health of HKU) to investigate the association between leukocyte basal gene expression profile and influenza symptom development and disease severity during acute community-acquired influenza virus infection. We have recruited subjects from a community-based household transmission study. Index with $\geq$2 symptoms of ARI within 48 hours of symptom onset and live with $\geq$2 other household members were identified in local outpatient clinics. Patients who were influenza positive by rapid test and their household members were follow-up for 6 days with daily symptom diaries. Nasal and throat swabs were collected from index and all household contacts on day 0, 3 and 6; clotted blood on day 0 and 28; and baseline whole blood on day 0. Influenza infection was confirmed by PCR or seroconversion in all influenza exposed household contacts. We have already started to optimize the RNA extraction protocol for blood samples and check for the quality and quantity of yielded RNA. Microarray study is now in pipeline and will begin in the coming year.

## Publications

1. Lee SM, Yan S, Yip TF, Peiris JS (2017) Toll-like receptor 10 is a novel nucleotide sensing receptor. *Submitted*.
2. Yan S, Ip KK, Lee SM (2017) TLR10 modulates poly(I:C) induced pro-inflammatory response. *In preparation*.

## Seminars, Invited Lectures and Oral Presentations

1. Suki Lee (2016) HMRF grant skills training workshop: Sharing session by outstanding grant applicants, City University of Hong Kong.

## Presentations at Meetings

1. Lee SMY, Yan S, Yip TF, Li ST, Yip K, Peiris JSM (2016) TLR10 as an innate receptor. *International Congress of Immunology 2016*, Melbourne, Australia (Poster).
2. Yan S, Lee SMY (2016) TLR10 is involved in regulation of dsRNA-induced proinflammatory response. *Scientific Symposium of the Institut Pasteur International Network*, Paris, France (Poster).

## Teaching

1. Suki Lee (2016) Hematology and Immunology System – Problem Based Learning (MBBS Year 2 students), The University of Hong Kong, Hong Kong SAR.
2. Suki Lee (2017) Lecturer in Institut Pasteur Massive Open Online Courses (MOOC) "Innate immunity and infectious diseases": Toll-like receptors in influenza virus infection.

## Collaborations (local and international)

1. **RT Guo** (Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, PR China): Determination of the crystal structure of TLR10.
2. **John Hiscott** (Istituto Pasteur-Fondazione Cenci Bolognetti, Italy): Effect of RIG-I agonists on TLR10 mediated signaling.
3. **Ben Cowling** (School of Public Health, The University of Hong Kong): Association between basal leukocyte transcriptome profile and symptom development & disease severity after influenza virus infection in humans.
4. **James Di Santo** (Department of Immunology, Institut Pasteur Paris): Innate lymphoid cell "adaptation" during influenza virus infection.

## Funding

1. Determining the ligand and function of TLR10: a novel innate immune sensor in viral infection (**Principal Investigator**; Research Grants Council/General Research Fund – HK$642,266.00, Ends: 09/2016).
2. Determining the ligand and function of an orphan receptor: Toll like receptor 10 (**Co-Investigator**; Area of Excellence "Control of Pandemic and Inter-pandemic Influenza" – HK$250,000.00, Ends: 12/2016).
3. Determining the involvement of TLR10-a novel innate immune sensor in influenza virus pathogenesis (**Principal Investigator**; RGC Seed Funding for basic research – HK$34,490.00, Ends: 02/2017).
4. Association between basal leukocyte transcriptome profile and symptom development & disease severity after influenza virus infection in humans (**Co-Investigator**; Health and Medical Research Fund – HK$796,778.00, Ends: 03/2017).
5. Effect of Volatile Organic Compounds (VOCs) exposure on disease severity in influenza virus infection (**Principal Investigator**; RGC Seed Funding for basic research – HK$55,400.00, Ends: 04/2018).

6. Innate lymphoid cell "adaptation" during influenza virus infection (**Principal Investigator**; Research Grants Council/Consulate General of France - PROCORE-France/Hong Kong Joint Research Scheme – HK$30,600.00, Ends: 12/2018).
7. Pathogenesis and disease severity: Role of TLR10 as an innate immune sensor (**Co-Investigator**; Theme-based Research Scheme "Viral, host and environmental determinants of influenza virus transmission and pathogenesis" – HK$800,000.00, Ends: 12/2019).

## Personnel

| Name | Position |
|---|---|
| Suki LEE | Research Assistant Professor |
| Selena YAN | Research Associate |
| Aisha SELIM | MPhil student |
| Ping Hung LI | Research Technician |
| Tsz Fung YIP | Research Assistant |
| Kelvin Ka Kay IP | Research Assistant (Until: 31-Oct-2016) |
| Shuting LI | Research Assistant (Until: 30-Aug-2016) |
| Koen LOMMEN | Student Intern |
| Ninon GAUTHIER | Student Intern (French International School) |
| Phillipp MACHER | Student Intern (French International School) |
| Emma METADIER | Student Intern (French International School) |

# 3.2 Chris Ka Pun MOK Lab

## Main Objectives and Strategy

One of the main objectives of our group is to understand the behavior and pathogenicity of emerging viruses by combining clinical and experimental studies that span the areas of serology, epidemiology and molecular biology. In this context, we have set up with HKU and the First Affiliated Hospital of Guangzhou Medical University a research platform, the "Guangdong-Hong Kong Joint Research Centre for Clinical and Preventive Medicine against Emerging Infectious Diseases". 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 second objective is to understand the interplay between the viral and host factors that influence the replication of influenza viruses. We are investigating 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 A/B 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 nucleoprotein (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, PC Shaw (Chinese University of Hong Kong, Hong Kong SAR), who is a structural biologist working on protein-protein interaction and MC Chan (The University of Hong Kong) who is an expert on studying tropism of viruses using *ex vivo* lung model. We also aim to understand the functions of the unconjugated form of an antiviral protein, ISG15 upon influenza infection. ISG15 is known as an antiviral protein induced by interferon (IFN) $\alpha/\beta$. Although its intracellular functions via protein ISGylation have been intensively investigated, the role of free, unconjugated ISG15 remains understood. Our group, in collaboration with Dr. Sumana Sanyal, another PI at HKU-Pasteur, will investigate the secretion pathways of free unconjugated ISG15 and further explore its functions.

## Achievements and Ongoing Research

During 2016, we have made progress in characterizing the pathogenicity of avian influenza viruses responsible for outbreaks in humans. We have pursued various laboratory approaches in collaboration with researchers from different labs to understand how avian influenza viruses cause disease in mammalian hosts particularly in human. Several articles describing the pathogenicity of the emerging H5N6, H7N9 and H9N2 viruses have resulted from these collaborations.

*H5N6*: [Funding: GRC/GRF, Theme-based Research Scheme and RGC Seed funding for basic research]

Clade 2.3.4.4 H5 viruses have exhibited a predilection for genetic reassortment giving rise to H5N2, H5N5, H5N6 and H5N8 virus subtypes, which have become globally widespread, causing infections in wild birds or poultry in Asia, Europe and North America. Among these recombinants, H5N6 virus is the only subtype that causes human infections. We have previously identified and characterized the clinical features as well as the evolution of the virus. This year, we have further investigated the pathogenicity of the virus using *ex vivo* and *in vivo* animal models. With the collaboration of Dr Michael Chan's group, we have compared the viral tropism and replication in ex vivo cultures of human nasopharynx, bronchus and lung. Human H5N6 virus replicated as efficiently as H1N1pdm and more efficiently than highly pathogenic avian influenza H5N1 virus, in human bronchus and

lung, and was able to replicate in nasopharynx. The avain H5N6 isolated from avian host replicated less efficient than H1N1pdm in human bronchial tissues and to similar titers as H5N1 in the lung. Whereas human H5N6 virus had affinity to avian-like receptors, the avian isolates had binding affinity for both avian- and human-like receptors. Both human and avian H5N6 viruses were less potent inducers of pro-inflammatory cytokines compared with H5N1 virus. Taken together, the novel H5N6 viruses are better adapted to infect in the human airways than H5N1virus.

In collaboration with Dr. Ron Fouchier's group (Erasmus Medical Center), we found using the ferret model that H5N6 virus has not acquired the ability of aerosol transmission. However, it caused significantly higher severity to the ferret after infection when compared to the H5N1 virus which is also isolated from human patient (data not shown). In addition, by measuring polymerase activity, we have confirmed that the PB2-E627K mutation is responsible for the increase of replication.

*H7N9*: [Funding: GRC/GRF, Theme-based Research Scheme and Health and Medical Research Fund]

In March 2013, a novel low pathogenic avian influenza A virus of the H7N9 subtype was detected in humans in eastern China for the first time. Since then, re-emergence of the H7N9 strain was repeatedly reported in China during the winter seasons in humans from 2013/14 until now, demonstrating epidemic seasonality. H7N9 strain has caused 803 laboratory-confirmed human cases and 316 deaths since February 2013. In collaboration with Dr Gülsah Gabriel (Heinrich-Pette Institute), we have analyzed whether H7N9 has already adapted to human importin-α7 usage, which is associated with high-level virus replication in mammalian cells. Using a cell-based assay, we could detect a decreased H7N9 polymerase activity when importin-α7 was silenced by siRNA. Moreover, importin-α7 knockout mice presented enhanced survival rates compared to wild type mice after infection of H7N9 virus.

Amino acid substitutions in PB2 protein were shown to influence the pathogenicity and transmissibility of H7N9 following experimental infection of ferrets and mice. We evaluated the role of amino acid substitution PB2-627K or compensatory changes at PB2-591K and PB2-701N, on the tropism and replication competence of H7N9 viruses for human and swine respiratory tracts using *ex vivo* organ explant cultures. Our results demonstrate that PB2-E627K was important for the replication of influenza H7N9 in both human and swine respiratory tracts.

We have recently identified a new lineage of H7N9 virus in duck which has a distinct genetic background to the H7N9 outbreak lineage. We found that the newly identified duck isolated H7N9 lineage causes significant pathogenesis in mice after it acquires specific adaptive mutation at PB2-E627K but not -Q591 or -D701N. This raises the concern that the viruses from this lineage will cause pathogenesis in human given that similar adaptation occurs at the PB2 gene.

H9N2: [Funding: The National Natural Science Foundation of China and Science research project of the Guangdong Province]

Mutations in the PB2 gene of avian influenza virus have been known as important factors to the pathogenesis as PB2 is involved in the viral replication process and also functions in the determination of host range. Recently, amino acid change at PB2-591 has been identified from the human pandemic H1N1, H5N1 and H7N9 viruses which is also related to the pathogenicity in mammalian hosts. Our previous study found that amino acid residue located at the position 591 of the H9N2 PB2 gene was mutated after serial passages in mammalian cells while all other gene segments being unaffected. In this study, we extended our investigation to the role of the PB2-Q591K mutation when it is independently introduced in the genetic background of H9N2 virus. The PB2-Q591K mutation in H9N2 virus enhanced the polymerase activity and virus replication in human NHBE cells when compared to the wild type strain. Mice infected with the PB2 mutant showed significant weight loss, higher virus replication and immune responses in the lungs. Our evidences suggest that the PB2-Q591K, in addition to the -E627K mutation in H9N2 enhanced the pathogenicity in mammalian host.

*Extracellular secretion of free ISG15 in primary human cells with influenza virus infection* [Preliminary study]

Type I interferon and its regulated interferon stimulated genes (ISGs) are important antiviral component during influenza infection. ISG15, which is a 15kDa ubiquitin-like protein from the ISGs family, is a well-studied antiviral protein through the intracellular mechanism called "ISGylation". Recent data have suggested that the free ISG15 can be secreted extracellular upon type-I interferon stimulation and subsequently regulates the immunity. However, there is very limited information on the functions and regulation of free ISG15 in disease in particular to the viral infection. Our prior results showed that influenza virus is a more potent stimulator, compared to the type I interferon, which triggers the secretion of ISG15 in primary human macrophages and dendritic cells upon infection. The goal of this proposal is to further understand the mechanisms on how the free ISG15 is directly induced and secreted in the human immune system during influenza virus infection.

## Publications


1. Li W, Lee HH, Li RF, Zhu HM, Guan Y, Peiris JS, Yang ZF, Mok CK (2017) The PB2-E627K mutation enhances the pathogenicity of an avian influenza (H7N9) virus that belongs to a non-outbreak lineage. *In preparation*.
2. Hui KP, Chan LL, Kuok DI, Mok CK, Yang Z, Li R, Luk GS, Lee EF, Lai JC, Yen HL, Zhu HM, Guan Y, Nicholls JM, Peiris JS, Chan MC (2017) Tropism and innate host responses of influenza A/H5N6 virus in the human respiratory tract. *Eur Resp J*, in press.
3. Bertram S, Thiele S, Dreier C, Resa-Infante P, Preuss A, van Riel D, Mok CK, Schwalm F, Peiris JS, Klenk HD, Gabriel G (2017) H7N9 influenza A virus exhibits importin-α7 mediated replication in the mammalian respiratory tract. Am J Pathol 187:831-840.
4. Chan RW, Chan LL, Mok CK, Tao KP, Chan MC, Peiris JS, Nicholls JM (2016) Risk assessment of human infection by H9 influenza viruses using an explant system. *Pathology* **48** (**Suppl 1**):S105.
5. Chan LL, Bui CT, Mok CK, Ng MM, Nicholls JM, Peiris JS, Chan MC, Chan RW (2016) Evaluation of the human adaptation of influenza A/H7N9 virus in PB2 protein using human and swine respiratory tract explant cultures. *Sci Rep* **6**:35401.
6. Wang C, Lee HH, Yang ZF, Mok CK*, Zhang Z* (2016) PB2-Q591K mutation determines the pathogenicity of avian H9N2 influenza viruses for mammlian species. *PLoS One* **11**:e0162163 (*Co-corresponding authors).
7. Hui KP, Li HS, Cheung MC, Chan RW, Yuen KM, Mok CK, Nicholls JM, Peiris JS, Chan MC (2016) Highly pathogenic avian influenza H5N1 virus delays apoptotic responses via activation of STAT3. *Sci Rep* **6**:28593.
8. Lin YP, Luo Y, Chen Y, Lamers MM, Zhou Q, Yang XH, Sanyal S, Mok CK, Liu ZM (2016) Clinical and epidemiological features of the 2014 large-scale dengue outbreak in Guangzhou city, China. *BMC Infect Dis* 16**:102.**
9. Lin YP, Yang ZF, Liang Y, Li ZT, Bond HS, Luo YS, Chen Y, Chen TT, Guan WD, Lai JCC, Siu YL, Pan SH, Peiris JS, Cowling BJ*, Mok CK* (2016) Population seroprevalence of antibody to influenza A(H7N9), Guangzhou, China. *BMC Infect Dis* **16**:632.
10. Blanc F, Furio L, Moisy D, Yen HL, Chignard M, Letavernier E, Naffakh N, Mok CK, Si-Tahar M (2016) Targeting host calpain proteases decreases influenza A virus infection. *Am J Physiol Lung Cell Mol Physiol* **310**:L689-L699.

## Seminars, Invited Lectures and Oral Presentations

1. Chris Mok (2016) The Wonkwang University 70th Anniversary International Symposium, Wonkwnag University, South Korea.

## Presentations at Meetings

1. Lee HHY, Yang ZF, Peiris JSM, Mok CKP (2016) Induction of proinflammatory cytokines is associated to the pathogenicity of avian H9N2 influenza viruses in mice. *Scientific Symposium of the Institut Pasteur International Network*, Paris, France (Poster).

## Teaching

1. Chris Mok (2016) Director of the 13th HKU-Pasteur Virology Course, Hong Kong, Hong Kong SAR.
2. Chris Mok (2016) Introduction to the Art and Science of Medicine– Problem Based Learning (MBBS Year 1 students), The University of Hong Kong, Hong Kong SAR

## Collaborations (local and international)

1. **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.
2. **Nan-Shan Zhong, Ling Chen, Zi-Feng Yang** (State Key Laboratory of Respiratory Disease, Guangzhou, PR China): Clinical and laboratory studies on emerging infectious diseases in Guangzhou.
3. **Michael Chan** (School of Public Health, The University of Hong Kong) and **Ron Fouchier** (Erasmus Medical Center, The Netherlands): Pathogenicity and transmissibility of H5N6 virus.

## Funding

1. Transcriptomic and serology studies of re-emergent avian-origin human H7N9 influenza disease in Guangdong province (**Principal Investigator**; Area of Excellence "Control of Pandemic and Inter-pandemic Influenza" – HK$900,000.00, Ends: 12/2016).
2. Characterization of the new identified human pathogenic avian-origin influenza (H5N6) virus (**Principal Investigator**; RGC Seed funding for basic research – HK$45,980.00, Ends: 05/2017).
3. 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 – HK$796,778.00, Ends: 06/2017).
4. Infection and immunopathogenesis of avian influenza H9N2 virus in tree shrew model (**Co-Investigator**; The National Natural Science Foundation of China – RMB1,500,000.00, Ends: 04/2018).
5. Guangdong-Hong Kong Joint Research Centre for Clinical and Preventive Medicine against Emerging Infectious Diseases (**Co-Principal Investigator**; Science research project of the Guangdong Province – RMB1,000,000.00, Ends: 09/2018).

6. Importin-alpha protein as the host determinant of influenza B virus replication in human (Principal Investigator; RGC Seed funding for basic research – HK$44,320.00, Ends: 04/2019).
7. Infection and immunopathogenesis of avian influenza H9N2 virus in tree shrew model (**Co-Investigator**; Research Grants Council – Theme-based Research Scheme "Viral, host and environmental determinants of influenza virus transmission and pathogenesis" – HK$300,000.00, Ends: 12/2019).

# Personnel

| Name | Position |
|---|---|
| Chris Ka Pun MOK | Research Assistant Professor |
| Horace Hok Yeung LEE | PhD student |
| Gannon MAK | PhD student |
| Fion Nok Lam MA | MPhil student |
| Jane Kong San TSE | Research Technician |
| Huihui TI | Research Assistant |
| Garrick YIP | Student Intern (IVE) |

# 3.3 Sumana SANYAL LAB

## Main Objectives and Strategy

The main objectives of the lab are to combine methods of molecular 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. Among other factors, we are particularly interested in ubiquitin-like post-translational modifiers of protein function, such as ISG15 that play a significant role in modulating different pathways, most of which are innate signaling pathways such as RIG-I, TLR7 and inflammasome activation. Our major research projects are listed below.

### *Characterization of host factors involved in virus infections:*

A molecular understanding of host cellular factors involved in virus infections is crucial not only to provide novel insights into pathways hijacked by them, but also for development of effective antimicrobials against such pathogens. 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.

*(a) Role of Aup1 in flavivirus infection:* The complexity of the assembly and release of dengue virus provides a potentially rich source of host targets for interference. Propagation of dengue virus (DENV), West Nile (WNV) and other members of the family appears to involve extensive membrane and lipid remodeling 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. We are also extending our findings with dengue virus to explore similarities and differences that exist in Zika.

*(b) Role of Tsg101 in influenza virus infection:* A major response of mammalian cells to viral infections is through upregulation of the interferon type I and II pathways. Viruses in turn implement counter strategies through either inhibition of IFN response or by activation of proteins that inhibit the function of interferon-stimulated genes (ISGs). The function of Tsg101 appears to be dictated by several post-translational modifications including ISG15, phosphorylation and ubiquitylation. Using a combination of CRISPR/Cas9 knockouts and protein interaction assays, we are currently exploring the functional relevance of these modifications during influenza infection, centered on (i)Tsg101 and (ii) MGRN1 - an E3-ligase that ubiquitylates Tsg101.

*(c) Mechanism of Src-family kinase (SFK)-mediated signaling during flavivirus infections:* Amongst the host factors that facilitate egress of dengue virus particles through the secretory pathway are the KDELR, class-II Arfs and several Src-family kinases. We recently screened a number of SFKs to determine their impact on intracellular transport of dengue and Zika. Deficiency of Lyn through siRNA-mediated suppression as well as pharmacological inhibition had a significant impact on release of both dengue and Zika virus particles. We are in the process of elucidating the mechanism through which these SFKs activate the signaling cascade that is necessary for transport of flavivirus particles along the host secretory pathway.

### *Targeting deubiquitylases as therapeutic strategies against viral infections*

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 have employed a chemoenzymatic strategy to identify deubiquitylating enzymes (DUBs) that are specifically expressed upon influenza infection and are currently investigating the role of these DUBs. Our ongoing studies involve characterization and pharmacological intervention of these DUBs in order to attenuate influenza infection. 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. Our current efforts are centered on OtuB1, which appears to interact with influenza NS1. Deficiency of OtuB1 results in a significant drop in release of both proinflammatory cytokines and virus particles from infected cells.

### *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. Using proximity based labeling we identified LAT and Trat1 to be substrates of Usp12. Apart from Usp12, we are also pursuing a set of DUBs identified through functional screening in mouse T-lymphocytes that function to suppress TCR signaling.

## Achievements and Ongoing Research

Since joining HKU-PRP in November 2013, we have expanded on projects that were initiated at the Whitehead Institute/MIT while creating new directions at the current setting. We have successfully submitted grant applications to RGC/GRF, HMRF and Area of Excellence for Control of Pandemic and Inter-pandemic Influenza as well as Transversal research grants available within the international network of Institute Pasteur. Results obtained in the TCR signaling project were recently accepted for publication in Proceedings of the National Academy of Sciences USA. Ongoing research to elucidate the function of Cyclin D3 in influenza infection was accepted for publication in the Journal of Biological Chemistry.

### *Characterization of host factors involved in virus infections:*

#### (a) *Role of Aup1 in dengue infection* [Funding: RGC/GRF]

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. A functional screening strategy designed to identify proteins that are differentially modified by ubiquitin upon dengue infection revealed a set of lipid droplet and autophagy associated host proteins in which Aup1 was scored as a strong candidate. Aup1 is a lipid droplet/ER associated protein whose expression is induced in dengue-infected cells in a time-dependent manner. Aup1-deficient HepG2 cells become resistant to dengue production with significant loss in DENV-E protein synthesis. Interestingly, we find that the abundance of lipid droplets in dengue-infected cells is significantly lesser compared to control cells. This phenomenon is inhibited in Aup1-deficient cells.

We have begun to dissect the mechanism by which Aup1 is exploited during DENV assembly. Our current hypothesis entails that induction of autophagy is a critical aspect to sustain dengue replication. Consumption of lipid droplets (lipophagy) generates the necessary fatty acids utilized by dengue and Aup1 is necessary for this step. Aup1$^{-/-}$ cells are unable to induce autophagy and LDs in turn are not consumed to facilitate virus replication. We have generated Aup1-GFP and Aup1-cherry fusion proteins stably expressed in hepatoma cells in culture we display increased expression and colocalization with autophagosomes by confocal microscopy. 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. 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. 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.

(b) *Role of Tsg101 and Mgrn1 in influenza infection* [Funding: RGC/GRF, PTR]:
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 aim to extend these observations and explore whether this phenomenon holds true as a general host factor necessary for influenza virus biogenesis. We are testing different influenza strains in Tsg101 and Mgrn1 deficient A549 cells that we have generated by the CRISPR genome editing strategy. These will be infected with the human H1N1 and H3N2 strains available in house as well as the avian influenza strains of H5N1 and H7N9. We will test viral titers from supernatants of infected cells (wild-type and Tsg101 deficient A549) using plaque assays. 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. The different variants 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, and identified its interactome through proximity based labeling strategies. Since the subcellular localization of Tsg101 undergoes redistribution 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. In addition subcellular localization and interacting partners of Tsg101 undergoes a drastic shift depending on the intracellular environment. Using proximity based labeling assay, we have now charted the distinct

subcellular interactome of Tsg101 under conditions of mock treatment, influenza infection and interferon treatment, which further sheds light on the differential function and tight regulation imposed on Tsg101. We are in the process of performing similar experiments with the E3-ligase Mgrn1.

### (C) *Mechanism of Src-family kinase (SFK)-mediated signaling during flavivirus infections* [Funding: HMRF]

In the absence of antiviral drugs dengue has become one of the most serious global health threats. Identification of virus-host interactions and functional characterization of their regulated signaling pathways are the primary approaches to understand molecular mechanisms responsible for disease progression and pathogenesis. Our previous results demonstrated that the specific interaction between Dengue virus 1 (DENV1) pre-membrane protein (prM) and cellular KDEL receptors (KDELRs) was required for the endoplasmic reticulum (ER)-to-Golgi transport of DENV progeny virions. KDELRs have a similar topology as that of G-proteins-coupled receptors (GPCR), which are involved in a plethora of cellular signaling events. Recent studies revealed that KDELRs can recruit Gαq/11 (Gq) proteins to activate Src family Kinases (SFKs) in the Golgi apparatus and regulate intra- and post-Golgi transport. Our current data show that inhibitors of SFKs, such as PP2, can effectively decrease the secretion of DENV1 recombinant subviral particles (RSPs), suggesting the involvement of activated SFKs in DENV egress. Among the different subtypes, egress of DENV4 is independent of an interaction with KDELRs; however, secretion of DENV4 RSPs was also attenuated in PP2 treated cells. Moreover, compared to parental cells, recovery of DENV1 RSP production after serum starvation resulted in delayed phosphorylation of SFKs. This delay was not observed in cells either stably expressing DENV4 prME, or expressing a DENV1 prME mutant which is impaired prM/KDELRs interaction. Taken together, these results imply that KDELRs may play multiple roles in DENV secretion along the cellular secretory pathway. The prM/KDELs interaction in ER might sequester KDELRs, thus reducing the KDELRs-dependent SKFs activation in Golgi complex. There are nine members of SFKs expressed in various combinations in different cell types. We immunoprecipitated phosphorylated SKFs from DENV infected cells by using antibody recognizing phosphorylated SKFs, specifically at tyrosine 416 in the activation loop. We identified five members by mass spectroscopy of which displayed increased phosphorylation in the DENV infected cells. Amongst those, Lyn was determined as a host kinase that plays a significant role in egress of newly formed dengue virus particles.

## *Targeting deubiquitylases as therapeutic strategies against viral infections* [Funding: HMRF, PTR]

We have taken a functional proteomic approach to elucidate the mechanism of DUB-mediated regulation during virus infections and activation of immune signaling pathways. We performed two separate functional screens to identify host proteins that play a significant role in the process of influenza virus infection. The first screen was designed to identify deubiquitylases (DUBs) that are specifically upregulated upon influenza infection as shown in schematic. This was performed with an activity based reporter, HA-tagged ubiquitin modified at the C-terminus with vinylmethylester (HA-Ub-vme). This probe specifically recognizes and reacts with the active site cysteines of DUBs to form an irreversible covalent thio-ether linkage. The HA-tag provides a handle to isolate all the DUBs that reacted with the probe. HA-Ubvme was delivered to permeabilized cells that were either mock or influenza A virus infected, using a method we described previously. DUBs that reacted irreversibly with HA-Ub-vme were then isolated on anti-HA conjugated beads and identified by mass spectrometry. The second screen was designed to identify those DUBs that interacted with influenza virus PB2 as shown in the schematic.

To employ this approach in a high throughput manner, a split luciferase assay developed in the laboratory of Sylvie Van der Werf at the Institut Pasteur (Paris) was applied using the PB2 subunit as bait. Five distinct strains of influenza virus with varying pathogenicity were used to map this interaction. This method enables detection of interacting protein pairs among a matrix of exploratory proteins and allows us to monitor the pair-wise association of the host protein with the viral factor. Identified interactors were further validated through co-immunoprecipitation and immunoblotting. OtuB1 belongs to the family of Ovarian-tumor domain-containing (OTU)

deubiquitylating enzymes. Our results suggest that the physiological role of OtuB1 is very likely in the production of pro-inflammatory cytokine and chemokine. This function however, is hijacked by influenza virus (depending on its pathogenicity) to instead remove ubiquitin molecules from viral proteins. This in turn, stabilizes the viral proteins that are otherwise degraded and subsequently facilitates assembly of intact progeny virions. Our preliminary data also confirms that the activity of OtuB1 can be regulated by post-translational modification with phosphorylation. We aim to explore the mechanism through which influenza virus hijacks the function of OtuB1 to its own advantage in further detail and extend to a larger repertoire of viruses to establish whether or not it is a universal phenomenon during virus infection.

## *Regulation of immune signaling by deubiquitylases* [Funding: AoE/M-12/16, Seed Funding]

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 kinase for IκB (IKK). The signal initiated by these core events is disseminated through adaptor proteins such as LAT and SLP-76; proteasomal degradation of IκB is followed by nuclear translocation of NFκB 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 in the process of characterizing the other TCR regulators we identified in the initial screen, especially the role of Usp4 which appears to be a negative regulator.

## Publications


1. Fan Y, Mok CK, Chan MCW, Zhang Y, Nal B, Kien F, Bruzzone R, Sanyal S (2017) Cell-cycle independent role of CyclinD3 in host restriction of influenza infection. *J Biol Chem* **292**:5070-5088.
2. Sanyal S (2016) Reply to Rodriguez: Mechanism of nuclear-cytosol shuttling of Usp12 *Proc Natl Acad Sci USA* **113**: E3317-E3318.
3. Jahan AS, Lestra M, Swee LK, Fan Y, Lamers MM, Tafesse FG, Theile CS, Spooner E, Bruzzone R, Ploegh HL, Sanyal S (2016) Usp12 stabilizes the T cell receptor complex at the cell surface during signaling. *Proc Natl Acad Sci USA* **113**:E705-E714.
4. Lin YP, Luo Y, Chen Y, Lamers MM, Zhou Q, Yang XH, Sanyal S, Mok CKP, Liu ZM (2016) Clinical and epidemiological features of the 2014 large-scale dengue outbreak in Guangzhou city, China. *BMC Infect Dis* **16**:102.
5. **Zhang J**, Sze DM, Yung BY, Tang P, Chen WJ, Chan KH, Leung PH (2016) Distinct expression of interferon-induced protein with tetratricopeptide repeats (IFIT) 1/2/3 and other antiviral genes between subsets of dendritic cells induced by dengue virus 2 infection. *Immunology* **148**:363-376.


## Seminars, Invited Lectures and Oral Presentations

1. Sumana Sanyal (2016) The University of Oxford, UK
2. Sumana Sanyal (2016) Scientific Symposium of the Institut Pasteur International Network, Paris, France
3. Sumana Sanyal (2016) Department of Biochemistry, University of Lausanne, Switzerland
4. Sumana Sanyal (2016) Proteomics in Cell Biology and Human Disease, EMBL, Heidelberg
5. Sumana Sanyal (2016) Innate Immunity to Host Pathogen Interactions, EMBL, Heidelberg
6. Sumana Sanyal (2016) EMBO workshop on characterization of post translational modifications, Denmark
7. Sumana Sanyal (2017) Department of Immunology Annual Retreat, Institut Pasteur

8. Sumana Sanyal (2017) Molecular Biology of the Cell, Institut Pasteur

## Presentations at Meetings

1. Zhang JT, Sanyal S (2016) Role of Tsg101 in influenza virus assembly and release. *Options meeting for the control of influenza*, Chicago, USA (Oral).
2. Zhang JT, Sanyal S (2016) Role of Aup1 in the assembly and egress of dengue virus. *12th GERLI International Lipidomics meeting*, Toulouse, France (Oral).
3. Jahan AS, Sanyal S (2016) Role of deubiquitylating enzymes in influenza virus infection. *European Society of Clinical Microbiology and Infectious Diseases*, Amsterdam, The Netherlands (Poster).

## Teaching

1. Sumana Sanyal (2017) Lecturer and practical tutor in the Molecular Biology of the Cell Course, Institut Pasteur, Paris, France.
2. Sumana Sanyal (2016) Lecturer and Tutor in the 7th HKU-Pasteur Cell Biology Course, Hong Kong, Hong Kong SAR.

3. Sumana Sanyal (2017) Cardiopulmonary and Renal Systems – Problem Based Learning (MBBS Year 1 students) The University of Hong Kong, Hong Kong SAR.

## Collaborations

1. **Caroline Demeret** (Institut Pasteur, Paris): Role of deubiquitylases in influenza virus infections.
2. **Lee Kim Swee** (BiomedX Innovation Center, Heidelberg, Germany): Ubiquitin-mediated regulation of the T-cell receptor-signaling pathway
3. **Joseph Ashour** (Mount Sinai School of Medicine, New York, NY, USA): Manipulation of host factors in influenza and dengue infections
4. **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.
5. **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

1. Host factors involved in dengue infection (**Principal Investigator**; Research Grants Council/General Research Fund – HK$654.557.00, Ends: 11/2016).
2. Elucidating the role of Tsg101 in influenza virus assembly and release (**Principal Investigator**; Area of Excellence Control of Pandemic and Inter-pandemic Influenza – HK$550,000.00, Ends: 12/2016).
3. 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 – HK$981,120.00, Ends: 06/2017).

4. Role of Tsg101 in influenza virus infection (**Principal Investigator**; Research Grants Council/General Research Fund – HK$769,020.00, Ends: 10/2017).
5. Deciphering influenza viral polymerase interplay with host ubiquitin proteasome system in correlation with pathogenesis (**Co-principal Investigator**; Institut Pasteur – Programme Transversaux de Recherche – Euro 143,000.00, Ends: 12/2017)
6. Role of Usp4 in T Cell Receptor-signaling (**Principal Investigator**; Seed Funding for basic research – HK$80,470.00, Ends: 05/2018).
7. Regulation of host factors in influenza virus infections through ubiquitin and ubiquitin like modifiers (**Co-Investigator**; Research Grants Council – Theme-based Research Scheme "Viral, host and environmental determinants of influenza virus transmission and pathogenesis" – HK$450,000.00, Ends: 12/2019).
8. Targeting lipid droplet metabolism as therapeutic intervention during dengue virus infections (**Principal Investigator**; Health and Medical Research Fund – HK$1,200,000.00, Ends: 06/2019).
9. Regulation of dengue virus life cycle by KDEL receptor-dependent signaling pathway: a new target to interfere with viral infection and pathogenesis (**Co-Investigator**; Health and Medical Research Fund – HK$1,170,000.00, Ends: 08/2019)

## Personnel

| Name | Position |
|---|---|
| Sumana SANYAL | Assistant Professor |
| Ming Yuan LI | Post doctoral fellow |
| Tami Jingzhu ZHANG | Postdoctoral fellow |
| Yun LAN | Technical Officer |
| Sabiha Jahan AKHEE | PhD Student |
| Joao POMBO | MPhil student |
| Lewis SIU | Research Technician |
| Agathe LE QUANG | Student Intern |
| Clotilde WICART | Student Intern |
| Mei Lam CHAN | Student Intern (IVE) |
| Hon Lam NG | Student Intern (IVE) |

# 3.4 Sophie VALKENBURG Lab

*NB: Dr. Sophie Valkenburg returned from maternity leave from April 2016 and relocated laboratory space to the HKU-Pasteur in September 2016.*

## Main Objectives and Strategy

The main objectives of the lab are determining the role of protective heterologous T and B cell immunity in mouse and human systems, by investigating novel vaccines and immune correlates of protection for influenza. Our primary focus is to study adaptive immunity to influenza, and how this could be harnessed and optimized by vaccination to improve protection from diverse influenza virus infection. HA-specific antibodies can block influenza infection, whilst T cells recognize influenza-infected cells. A vaccine which ultimately combines antibody and T cell based immunity for influenza will provide a full-proof immunological barrier to influenza infection, which our studies will ultimately help develop. Our major research projects, which aim to elucidate how cross-reactive T and B cell responses to influenza provide broad immunity, are listed below.

### *Broadly reactive influenza vaccines in mouse models*

A vaccine that is broadly protective against different strains and subtypes of influenza is needed in lieu of the current seasonal vaccine which requires yearly update and is not protective against pandemic or outbreak strains.

#### (a) *A T cell based universal vaccine*

In collaboration with Liyange Perera and Thomas Waldmann at NIH, our group is determining the mechanism of protection of a Vaccinia Wyeth vaccine vector encoding 5 influenza proteins, HA, NA, NP and Matrix 1 and 2 proteins, with a molecular IL-15 adjuvant to enhance vaccine memory responses, termed Wyeth/5Flu/IL-15. The vaccine has been highly effective in mice providing protection against avian, pandemic and seasonal strains of influenza. The vaccine elicits effective influenza-specific T cell memory responses that establish early local T cell responses upon influenza challenge, significantly reducing viral lung titers and thus survival. Importantly, depletion of T cell subsets showed that memory CD4 T cell responses were necessary for vaccine mediated protection, an under appreciated role of helper subset. The vaccine is currently being investigated for use in a sequential combination regime with inactivated influenza viruses by Scarlett Yan and Olive Li within the Poon laboratory at HKU.

#### (b) *An HA-stem based vaccine*

The HA protein contains a stem region with conserved conformational epitopes that are relatively conserved between different influenza strains, leading to the induction of broadly neutralizing antibodies that recognize influenza viruses of different subtypes, in some cases groups (group 1 or 2) or even both influenza A and B viruses. Using a protein minimization technique, the Varadarajan lab at the Indian Institute of Science developed a HA-mini stem recombinant protein vaccine that mimics the pre-fusion native form of the HA protein by utilizing a trimerization motif, Foldon. Both an H5 and H1 form of the vaccine, H5-Foldon and H1-Foldon, have been assessed in the mouse influenza challenge model, and found to induce broadly HA binding antibodies for different subtypes and groups. Vaccine antibodies were able to mediate protection from heterologous H5N1 challenge. The mechanism of action of HA-stem specific antibodies is being investigated and will be assessed in the context of antibody dependent cellular cytotoxicity (ADCC) and its effect on the virus life cycle.

## *Human correlates of protection from influenza*

### (a) *Protective pre-existing T cell responses*

Whilst T cell responses have been shown to be highly effective in mediating protection in mouse models, corresponding data in human influenza infection is not as robust. All adults have established influenza-specific memory T cell responses; however we have repeated infection during our lifetime that can range from mild to life threatening infection. The half-life of T cell memory and cross reactivity may explain the variability in protection from repeated infection. In collaboration with Benjamin Cowling at HKU, we aim to determine the correlation between higher baseline early effector T cell memory responses and protection from influenza infection or reduced symptom severity and viral shedding in a household transmission setting. Blood samples are obtained from infected index cases, and uninfected household contacts that are monitored for influenza transmission, at day 0 and day 28. The aim of the study is to find if there is an immunological difference between contacts positive for influenza transmission during sampling and contacts negative for influenza transmission. Sample collection has been ongoing since June 2013 due to the limited and specific nature of cases and intensity of monitoring households.

### (b) *ADCC avian cross-reactivity*

H5N1-specific ADCC antibodies have been found from the blood of healthy unexposed adults, and therefore ADCC antibodies must target conserved epitopes of the HA protein. In collaboration with Joe Wu at HKU, the level of H1 and H7-specific ADCC antibodies is being probed in a large community cohort study, using archived serum from Red Cross blood collection. This study will also determine the effect of the 2009 pandemic on the level of HA-stem antibodies and their relationship with ADCC responses, as well as the pandemics relationship with increasing breadth of responses against potential outbreak strains like H7N9.

### (c) *H3N2 vaccine in elderly cohort*

The 2015 Northern hemisphere winter influenza season had excess mortality in over 65 year olds due to vaccine mismatch between the H3N2 circulating strain and vaccine strain (A/Texas/50/2012). The updated vaccine containing the A/Switzerland/9715293/2013 virus became available after the peak of the influenza season. In collaboration with Yat Hang Tam at HKU, the benefit of re-vaccinating the elderly in establishing H3N2-Switzerland specific T and B cell and ADCC responses is being assessed between subjects who received one dose of the updated vaccine versus two doses of vaccine.

# Achievements and Ongoing Research

## *Broadly reactive influenza vaccines in mouse models* [Funding: Health and Medical Research Fund]

### (a) *A T cell based universal vaccine*

The vaccine is being further investigated by Maireid Bull (MPhil student) for DC trafficking, immune mediated pressure by Next Generation Sequencing and innate lymphoid cell recruitment to determine the impact of T cell activated vaccines.

### *(b) An HA-stem based vaccine*

The H1-Foldon and H5-Foldon vaccine were assessed in the BalbC mouse model. HA-Stem vaccine antibodies provided heterologous protection against avian, seasonal and pandemic influenza viruses, and also were protective in an adoptive serum transfer experiment. Vaccine antibodies bound a broad array of recombinant HA proteins. Whilst the HA-mini stem vaccine was clearly protective in mice with significant gains in survival, the vaccine did not reduce viral loads. This has lead us to further studies on the mechanism of action of the HA-stem antibodies and whether they

provide ADCC action is currently being determined using alpha-test kit from Promega and further in vitro experiments.

### *Human correlates of protection from influenza* [Funding: Health and Medical Research Fund]

#### (a) *Protective pre-existing T cell responses*

Patient recruitment is ongoing for the household study for the winter and summer influenza seasons in Hong Kong. An 11-colour flow cytometry assay to measure influenza-specific IFN□ producing early effector T cell responses is under development.

#### (b) *ADCC avian cross-reactivity*

A databank of age-stratified serum from the Red Cross was used to probe ADCC activity in the Hong Kong community. The level of ADCC responses towards the Avian H7N9 HA protein before and after the H1N1 2009 pandemic showed an age related increase in ADCC activity, whilst ADCC activity towards the relatively conserved internal NP protein remained unchanged.

#### (c) *H3N2 vaccine in elderly cohort*

In collaboration with Yat Hang Tam (HKU) and his network of field nurses, we recruited over 300 donors at three time-points (d0, d7 and d30 post-vaccination), over two vaccine seasons (May-August and October-December 2015). Peripheral blood mononuclear cells and serum were cryobanked for future T cell and ADCC assays. An NK cell line was obtained from Conkwest and Fox Chase Cancer Centre to improve the ADCC assay. The NK cell line significantly increases the number of serum samples and proteins that can be assessed in parallel whilst also streamlining the assay initially developed by Jegaskanda and Kent (J Immunol, 2013). Initial results by Isabella Chan in the Poon lab at HKU show a high level of H3-specific ADCC responses before and after vaccination with no effect of vaccination on ADCC responses. The impact of repeated vaccination in the elderly upon T cell immunity is currently being determined.

## Publications


1. Teng O, Chen ST, Hsu TL, Sia SF, Cole S, Valkenburg SA, Hsu TY, Zheng JT, Tu W, Bruzzone R, Peiris JS, Hsieh SL, Yen HL (2017) CLEC5A-mediated enhancement of the inflammatory response in myeloid cells contributes to influenza pathogenicity in vivo. *J Virol* **91**: e01813-16.
2. Grant EJ, Josephs TM, Valkenburg SA, Wooldridge L, Hellard M, Rossjohn J, Bharadwaj M, Kedzierska K, Gras S (2016) Lack of heterologous cross-reactivity toward HLA-A*02:01 restricted viral epitopes is underpinned by distinct αβT cell receptor signatures. *J Biol Chem* **291**:24335-24351.
3. Valkenburg SA, Zhang Y, Chan KY, Leung K, Wu JT, Poon LL (2016) Preexisting antibody-dependent cellular cytotoxicity-activating antibody responses are stable longitudinally and cross-reactive responses are not boosted by recent influenza exposure. *J Infect Dis* **214**:1159-1163.
4. Valkenburg SA, Josephs TM, Clemens EB, Grant EJ, Nguyen TH, Wang GC, Price DA, Miller A, Tong SY, Thomas PG, Doherty PC, Rossjohn J, Gras S, Kedzierska K (2016) Molecular basis for universal HLA-A*0201-restricted CD8+ T-cell immunity against influenza viruses. *Proc Natl Acad Sci USA* **113**:4440-4445.
5. Chan MC, Kuok DI, Leung CY, Hui KP, Valkenburg SA, Lau EH, Nicholls JM, Fang X, Guan Y, Lee JW, Chan RW, Webster RG, Matthay MA, Peiris JS (2016) Human mesenchymal stromal cells reduce influenza A H5N1-associated acute lung injury in vitro and in vivo. *Proc Natl Acad Sci USA* **113**:3621-3626.
6. Valkenburg SA, Mallajosyula VV, Li OT, Chin AW, Carnell G, Temperton N, Varadarajan

R, Poon LL (2016) Stalking influenza by vaccination with pre-fusion headless HA mini-stem. *Sci Rep* **6**:22666.

## Presentations at Meetings

1. Valkenburg SA, Mallajosyula VVA, Li OTW, Chin AWH, Carnell G, Temperton N, Varadarajan R, Poon LLM (2016) Targeting influenza by vaccination with prefusion headless HA ministem. *Victorian Infection and Immunity*, Lorne, Australia (Oral).
2. Valkenburg SA, Mallajosyula VVA, Li OTW, Chin AWH, Carnell G, Temperton N, Varadarajan R, Poon LLM (2016) Influenza vaccination with HA mini stem for broad antibody immunity. *International Congress of Immunology*, Melbourne, Australia (Oral).
3. Valkenburg SA, Mallajosyula VVA, Li OTW, Chin AWH, Carnell G, Temperton N, Varadarajan R, Peiris JSM, Perera LP, Poon LLM (2016) Universal correlates of immune protection during influenza infection and vaccination in mouse and humans. *2nd International forum on Influenza and other Respiratory viruses, Guangzhou*, PR China (Oral).
4. Valkenburg SA, Li OTW, Peiris JSM, Perera LP, Poon LLM (2016) Protection by universal influenza vaccine from the immunological back line- CD4 T cell mediated protection. *Scientific Symposium of the Institut Pasteur International Network*, Paris, France (Oral).

## Teaching

1. Sophie Valkenburg (2016) Tutor in the 9th HKU-Pasteur Immunology Course The University of Hong Kong, Hong Kong SAR.
2. Sophie Valkenburg (2016) Tutor for the Croucher Summer Course "Vaccinology for Public Health and Clinical Practice in the 21st century", The University of Hong Kong, Hong Kong SAR.

## Collaborations

1. **Leo LM Poon** (The University of Hong Kong): Key collaborator and contributor to ongoing projects.
2. **Ragahavan Varadarajan** (Indian Institute of Science, Bangalore, India): Characterization of a headless-trimeric pre-fusion conformation HA recombinant protein vaccine in a mouse model to generate broadly reactive HA-stem antibodies.
3. **Benjamin Cowling** (The University of Hong Kong): Determining the correlation between baseline T cell responses and protection from transmission in a household transmission setting.
4. **Joseph Wu** (The University of Hong Kong): Probing ADCC antibody responses towards avian influenza viruses in the community.
5. **Yat Hang Tam** (The University of Hong Kong): Two-dose vaccine immune effect in elderly for the H3N2-mismatch.
6. **Liyange Perera and Thomas Waldmann** (NIH, NAID, USA): Vaccinia vector H5N1 vaccine for broad T cell responses, with an emphasis on CD4 mediated heterologous protection.
7. **Katherine Kedzierska** (The University of Melbourne, Australia): Mutation rates in T cell epitopes during infection and human T cell responses towards influenza.

## Funding

1. Understanding vaccine induced T cell protection from influenza viruses (**Principal Investigator**; Health and Medical Research Fund – HK$997,164.00, Ends: 06/2016).
2. Probing community susceptibility to influenza infection by measuring alternate antibodies (**Principal Investigator**; RGC Seed Funding for basic research – HK$48,048.00, Ends: 09/2016).
3. Understanding alternate immune correlates of protection in household transmission of influenza (**Principal Investigator**; Health and Medical Research Fund – HK$999,828.00, Ends: 06/2017).
4. Vaccination scheme development to stimulate both B and T cell dependent heterosubtypic protection against Influenza A viruses in mice (**Co-Investigator**; Health and Medical Research Fund – Ends: 06/2017).
5. Detection and characterization of antibody-dependent cell-mediated cytotoxicity (ADCC) responses against human H7N9 virus in humans and mice (**Co-Investigator**; Health and Medical Research Fund – Ends: 04/2018).
6. Influenza virus escape is the double edged sword of effective T cell immunity (**Principal Investigator**; Seed Funding for basic research – HK$150,000.00, Ends: 10/2018).
7. Research on the Epidemiology, Vaccine Effectiveness and Treatment of Influenza and Other Respiratory Viruses in Southeast Asia and the Western Pacific (**Co-Investigator**; Center for Disease Control – Ends: 07/2021).

## Personnel

| Name | Position |
|---|---|
| Sophie VALKENBURG DOAK | Research Assistant Professor |
| Maireid BULL | MPhil Student |
| Lik Yan FAN | Research Assistant (until: September-2016) |
| Athena LI | Research Assistant (until: May-2016)<br>PhD Student (starts: June-2016) |
| Yizhuo WANG | Research Assistant |
| Raphelle De SAINT GERMAIN | Student Intern (French International School) |

# 3.5 Jimmy Chun Cheong LAI GROUP

## Main Objectives and Strategy

Our group aim to study the interactions between viruses with the host receptors, in order to have a better understanding on viral host adaptation and cell/tissue tropism. Main projects include the study of influenza virus-cell receptor interactions at the atomic level by combination of chemical, biochemical and cell biological methods; and the investigation of the interplay between different influenza surface proteins during viral infection. We are also interested in the effect of human B4GALNT2 gene expression on influenza virus infection; and the role of dipeptidyl peptidase-4 (DPP4) as the host-receptor for Middle East respiratory syndrome coronavirus (MERS-CoV). In addition, in collaboration with the department of clinical oncology and QIMR in Australia, we are performing a clinical trial to evaluate effectiveness of adoptive 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-host 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 (VLP) of different influenza subtypes and/or different viral origin. The interactions between hemagglutinin (HA), neuraminidase (NA) and a variety of sialylated glycans are being investigated using chemical methods. Functional studies of the virions are also carried out on cell/tissue cultures.

### *Interplays between influenza surface proteins in cell receptor interactions*

Influenza HA and NA are two major glycoproteins both interacting with sialic acids receptor on cell surface. It has been long recognized that a balance between HA receptor-binding and NA receptor-destroying functions is important for the influenza virulence and transmission. However, interplays between the two viral proteins were not clearly studied. In this project we aim to investigate the role of HA-receptor binding properties on the NA functions. The effect of HA inactivation on NA enzymatic activity will be tested in native virions. VLPs containing NA with or without corresponding HA will also be produced for the comparison of their NA activities.

### *Effect of human B4GALNT2 gene expression on influenza virus infection*

B4GALNT2 is involved in the biosynthesis of human $Sd^a$ blood group antigen by adding a terminal GalNAc side chain to glycoprotein containing NeuAc2,3-Gal1,4-GlcNAc moiety. Published data suggested that $Sd^a$ glycotope is expressed in both N- and O-linked glycans and the present of $Sd^a$ in animal tissue is a potential protective mechanism in against avian influenza viruses. Our objective is to over-express B4GALNT2 gene in different cell lines and test for their susceptibility against avian and human influenza viruses.

### *The role of DPP4 in MERS viral infection*

MERS-CoV emerged in 2012 and DPP4 was identified as the functional receptor for the virus spike protein. Although infection was blocked in cells pre-incubated with DPP4 antiserum,it remain unclear whether DPP4 is the only membrane receptor needed for effective MERS-CoV infection. In our study expression of DPP4 in MERS-CoV susceptible cells will be knocked down or knocked out, for the examination of MERS-CoV infection and replication. If viral infection were detected in DPP4 deficient cells, we will also attempt to identify the cellular factor which contributes to the DPP4-independent infection.

### *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

In 2016, we have continued our exploration of the basis of influenza receptor specificity and the role O-linked sialylated glycans in influenza viral infection. Our data shown that O-glycans are important receptors for some influenza strains, which overturn the dogma that N-glycans are the predominant cell receptor for influenza viruses. Using our VLPs system together with the HA inactivation methodology we developed, we further characterized the enhancement of NA activities in present of HA bindings to sialic acid receptor.

### *Study of influenza virus receptor* [Funding: RGC, URC, ARC]

(a) *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, histochemical analysis using lectins and antibodies indicating the presence of sialylated-O-glycans in MDCK and A549 cell-lines, as well as human lung and bronchial tissues. Efficient influenza infections were observed in GnT1-deficient cells in which maturation of N-glycans is blocked, while reduced infection were observed in cells pretreated with GalNAc-O-Bn that inhibit the O-glycan synthesis. These data indicate the biologically relevant role of sialylated O-linked glycans in influenza virus infections. 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. To investigate the role of sialyl-O-glycans during influenza virus cell attachment and infection, virus neutralization assays were performed in the presence of sialyl-N- and O-glycans at various concentrations. Our data reveal a significant difference between H1N1 and H3N2 influenza virus subtypes in their IC50 values against sialylated O-glycans. MD modelling was also performed for the binding of O-glycans to influenza HA. A manuscript describing these finding was submitted to Proceeding of the National Academy of Sciences USA.

### *(b) 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. 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. Virus-like particles containing N2 have been engineered but the yield was unsatisfactory therefore native virions were used instead. In order to eliminated the HA binding to sialic acids during the study of NA, a novel methodology using acidic pH to inactivated HA receptor-binding function has been developed. At low pH, HA undergo irreversible conformational change and no longer binds to sialic acids. Different sialylated glycans were included in the study especially those contain the two different sialic acid species, Neu5Ac and Neu5Gc, and those with the two different sialic acid linkage, alpha2-3 and alpha2-6. We found that NA from H9N2 has weak binding and enzymatic activity against Neu5Gc, indicates a poor adaptation for the mammalian system. These NA also display lower sialidase activity against alpha2-6 linked sialylated glycans with compared to human seasonal influenza viruses, reflected the H9N2 virus remains avian-like influenza. However, sialic acid binding affinity to the hemadsorption site (which is conserved in avian influenza viruses) of the tested H9N2 is lower than other avian influenza. In summary, our data suggest that the NA of H9N2 viruses is not well adapted for mammalian system, and the HA inactivation methodology developed during this study could become a useful tool in influenza research. These data were included in a manuscript submitted to *Respiratory Research*.

### *(c) Study of the emerging H5N6 influenza*

The recently human infection of novel avian H5N6 virus is causing a global public health threat. The first human H5N6 infection was reported in 2014 and zoonotic infections continues. H5N6 infected patients presented symptoms similar to the highly pathogenic H5N1. In this study, we investigated the tropism and innate immune response in ex vivo cultures of human respiratory tract. The data using human bronchus and lung tissues showed that human H5N6 virus replicated as efficiently as the pandemic H1N1 virus, and with higher replication competence than HPAI H5N1 virus. Receptor specificity study suggested that some H5N6 isolates bind to both avian- and human-like receptors. These results suggested the novel H5N6 viruses are better adapted to the human system than the HPAI H5N1 virus, which may pose a significant threat to the public health. The findings were accepted for publication in the *European Respiratory Journal*.

### *Interplays between influenza surface proteins in cell receptor interactions* [Funding: RGC, URC]

In a preliminary study of neuraminidase inhibition antibodies in patient sera, we observed a difference in NA activity level between NA proteins on VLP surface and native virons. Using fetuin as a substrate, NA on VLP surface displayed a lower enzymatic activity when compared to the same amount of NA on the whole virus. However, this phenomenon was not observed when using simple NA-Star substrate. We hypothesize that HA binding to complexes sialylated glycans (e.g. fetuin) promotes the NA-substrate recognition, thereby enhancing its enzymatic activity. NA activity against fetuin in enyme-linked lectin assay (ELLA) was largely reduced after HA inactivation by acid treatment but only minor reduction was detected using NA-Star substrate. VLPs containing NA with or without corresponding HA were also included in the study which showed that the presence of HA significantly enhanced NA cleavage of fetuin. We further characterized the phenomenon and we found that HA induce NA function on to both N- and O-linked sialyl-glycans. Our data also showed that NA activity enhancement by HA is prominent in immobilized fetuin compared to immobilized 3'SLN while no effect was detected using soluble SLN. A manuscript describing these finding was submitted for publication.

### *Effect of B4GALNT2 gene expression on influenza virus infection*

B4GALNT2 catalyzes the last step in the biosynthesis of the human Sda blood group antigen through the addition of an N-acetylgalactosamine residue via a beta-1,4 linkage to a subterminal galactose residue substituted with an alpha-2,3-linked sialic acid. Glycan array data shown that influenza viruses could not bind to Sda antigens therefore we hypothesized that high expression of B4GALNT2 acts a protective mechanism against avian influenza virus infection. MDCK cells stably expressing human B4GALNT2 gene were derived and a decrease of infection activities was obtained upon influenza virus infection, when compared to parental MDCK cells. Despite the lower infection rates and viral titres observed using viral protein staining and plague assay respectively, influenza viruses could infect the B4GALNT2-overexpression cells. It is possible that viruses attach and entre

the B4GALNT2-overexpression cells through glycosphingolipids (GSLs). To test the involvement of GSLs in influenza infections, UDP-glucose ceramide glucosyltransferase (UGCG) was knock-out in MDCK cells using CRISPR/ Cas9 system to ablate glucosylceramide biosynthesis. Significant drop of infections was observed in the UGCG-ko cells, indicated a role of GSLs in influenza infection.

### *The role of DPP4 in MERS viral infection*

To address the question of whether DPP4 receptor is necessary for MERS-CoV infection, VeroE6 cells transfected with siRNAs targeting DPP4 were challenged with MERS-CoV. Although DPP4 was reported as the functional receptor for MERS, in our preliminary data, no significant differences were observed between the DPP4-knockdown(~90% protein reduction) and control cells. DPP4-knockout VeroE6 cells were constructed using CRISPR/Cas9 to identify the cellular factor which contributes to the DPP4-independent infection. However, MERS infection was totally abolished in DPP4 deficient cells indicating that MERS infection requires only low amount of DPP4 receptors (<10% of normal cellular expression).

### *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 sixteen 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 finished the phase I clinical trial in2015 with a total of fifty-two NPC patients involved in which the CTL infusion is shown to be safe and well tolerated. Phase II clinical trial was approval and recruitment of patient is on-going. During Phase I trial, patient blood samples were shipped to Queensland Institute of Medical Research and frozen CTLs were shipped back to Hong Kong after T-cell differentiation, expansion and characterization. These process led to a lower quality of patient PBMC and requires longer processing time before infusion. In order to enhance the effectiveness and efficiency, we aim to initiate the process of T-cells expansion in Hong Kong and a 3-months training in QIMR were scheduled (Feb-May).

## Publications


1. Hui KP, Chan LL, Kuok DI, Mok CK, Yang ZF, Luk GS, Lee, EF, **Lai JC**, Yen HL, Zhu HC, Guan Y, Nicholls JN, Peiris JS, Chan MC (2017) Tropism and innate host responses of influenza A/H5N6 virus: an analysis of *ex-vivo* and *in-vitro* cultures of the human respiratory tract. *Eur RespJ*, in press.
2. Chan RW, Chan LL, Mok CK, **Lai JC**, Tao KP, Chan MC, Perez DR, Peiris JS, Nicholls JN (2017) Replication of avian and human H9 viruses in the human ex vivo respiratory tract, and the influence of neuraminidase on this replication. *Submitted*.
3. **Lai JC**, Herath MT, Wong HH, Zhu G, Peiris JS, Nicholls JN (2017) Neuraminidase activity of Influenza A virus is enhanced by Hemagglutinin-receptor binding. *Submitted*.
4. Mayr J, Lau K, **Lai JC**, Gagarinov I, Chan RW, von Itzstein M, Nicholls JN, Haselhorst T (2017). Unraveling the role of O-glycans in influenza virus infections. *Submitted*.

## Collaborations

1. **Xuechen Li** (Department of Chemistry, The University of Hong Kong): Molecular determinants of influenza virus tropism and binding; expertise in glycan synthesis, to produce glycans of interest as influenza receptor analogues.
2. **Guang Zhu** (Division of Life Science, Hong Kong University of Science and Technology): Access to equipment and technical support regarding NMR spectroscopy.
3. **Mark von Itzstein and Thomas Haselh**orst (Institute for Glycomics, Griffith University, Australia): Study of O-linked sialylated glycans and synthesis of O-glycans analogue.
4. **Michael Chan** (School of Public Health, The University of Hong Kong): Comparison of native influenza virus and virus-like-particles in their receptor-binding properties using the *ex vivo* human culture model.
5. **Dora Kwong** (Department of Clinical Oncology, The University of Hong Kong ong): Clinical trials of immunotherapy against EBV-associated NPC.
6. **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

1. 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).
2. Towards an influenza virus glycan interaction map (Glycointeractome). (**Co-Investigator**; Discovery Projects DP110104028, Australian Research Council – Ends: 2016).
3. Structural insights of virus-glycan interactions. (**Collaborator**, ARC Future Fellowships FT120100419, Australian Research Council – Ends: 2017).
4. Viral, Host and Environmental Determinants of Influenza Virus Transmission and Pathogenesis (**Co-Investigator**; RGC Theme-based Research Scheme – Ends: 12/2019).
5. Immunotherapy against nasopharyngeal carcinoma (**Co-Investigator**; Ester Lee and Chew Pik Foundation, Croucher Foundation and other donors – Ends: open).

# Personnel

| Name | Position |
|---|---|
| Jimmy CHUN CHEONG LAI | Postdoctoral Fellow (Joint Appointment with the Department of Pathology in the Nicholls Lab) |
| Herath M. THUSITHA KUMARA K. | MPhil student (Graduated February 2017) |
| Ho Him WONG | MPhil student |

# 3.6 Barbara GAYRAUD-MOREL (Visiting Scientist from the Institut Pasteur, Paris)

## Main Objectives and Strategy

*Consequences of influenza infection on skeletal muscles*

Part of my research is related to skeletal muscle function, which is relevant to the subject I work on at the Institut Pasteur, in the Stem Cell and Development laboratory directed by Shahragim Tajbakhsh. This project aims to explore the consequences of respiratory virus infections on skeletal muscles and muscle stem cells in particular. This work is at the interface between my expertise about skeletal muscle biology and infectious diseases studied at HKU-PRP. The project involves collaboration between HKU-PRP and several laboratories: Leo Poon (The University of Hong Kong), Tom Cheung (Hong Kong University of Science and Technology), Shahragim Tajbakhsh (Institut Pasteur, Paris).

Respiratory virus infections are primarily damaging the respiratory tract, but also induce several other symptoms that are not life threatening but remain highly uncomfortable for patients, like fever, headache, cough, nasal congestion and skeletal muscle pain, referred as myalgia. Most respiratory viruses (with the exception of some highly pathogenic ones like influenza H5N1) infecting the lung do not reach the systemic circulation and are therefore unlikely to affect directly other organs. However, inflammatory molecules released during viral pulmonary infection and circulating in the bloodstream throughout the body are considered to induce myalgia by stimulating the peripheral nervous system. Several cytokines, such as Interleukin 6 (Il6), Il1$\beta$ and TNF$\alpha$, generate E2 prostaglandins, which trigger dorsal root ganglion (DRG) stimulation and pain. Cytokines acting on skeletal muscles are called myokines. Among the numerous cytokines released in the body several of them are known to have certain biological effects on muscle and muscle stem cells, particularly during muscle regeneration. Most of these cytokines have a dose-dependent effect and can have a beneficial or detrimental effect on muscle cells. For example, IFN$\gamma$ has a positive effect on myoblast proliferation and regeneration at low doses but interferes with regeneration if present in high concentration. Similarly, a low level of IL6 improves regeneration but high levels are linked to muscle wasting, chronic inflammation in mdx mice (muscle dystrophic mice).

Skeletal muscle cells, named myofibers, are elongated and multinucleated. Satellite cells are the principal cell type ensuring muscle growth and regeneration and are therefore identified as the stem cell of the skeletal muscle. They are located to the periphery myofibers, between the sarcolemma and the basement membrane surrounding the fiber. The precise composition of their niche is still poorly defined, but involves multiple other cell types. For example, capillaries running along the fibers are found in proximity to satellite cells. Mesoangioblasts, or pericytes, surrounding the capillaries and small vessels establish some cross talk with satellite cells trough signaling molecules. Fibroblasts, adipocytes and resident macrophages are also present in the vicinity of satellite cells and could contribute to its homeostasis. A previous study has shown that influenza virus can directly infect muscle cells in culture but are not able to sustain the infection due to lack of cleavage of the viral surface receptor required to be infectious. Consequently, very few viral particles have been identified in skeletal muscle of infected mice. Nevertheless, myalgia occurring during influenza infection suggests that cytokines reach the skeletal muscle and the consequences of this high amount of inflammatory molecules on the skeletal muscle have not been addressed. We are particularly interested in potential damages that could occur on muscle stem cells, which are essential for homeostasis and regeneration of skeletal muscles. In addition, it has not been explored whether the skeletal

muscle itself contributes to inducing or sustaining muscle pain, for example via resident macrophages. We chose to address these questions *in vivo* with a mouse model of viral infection, during homeostasis and during regeneration after a muscle injury.

### *Establishment of a human lung epithelium derived from hESCs to study infectious diseases*

The second area of research aims to establish a human lung epithelium model to study infectious diseases. For now, most experiments with influenza and other respiratory viruses are performed on cell lines more or less related to human epithelial lung cells. We aim to take advantage of the growing human embryonic stem cell (hESC) and iPSC fields to establish a model of human lung epithelium to investigate respiratory infectious diseases. These past years, few laboratories have succeeded to generate efficient *in vitro* lung and airway epithelial cells from human pluripotent cells for applications in regenerative medicine, modeling lung diseases, or drug screening.

To differentiate hESCs into pulmonary cells the Snoeck lab developed a protocol which consist in recapitulating embryonic stages of lung development by providing key signaling molecules (Activin A, BMP, FGF, Wnt...) in a sequential and controlled timing. Briefly, hESCs are induced into Definitive Endoderm (DE), and then specified to a more anterior foregut endoderm (AFE) fate. They are further directed to produce lung progenitors before being finally differentiated into mature epithelial cells (mostly distal type II alveolar epithelial cells). To monitor differentiation, expression of several genetic markers, like transcription factors or cell surface receptors, is used to validate the sequential cell types generated. Once differentiated epithelial cell cultures will be established in the laboratory, we will validate our model by performing viral infection. Several respiratory viruses (influenza, dengue) will be tested in this in vitro system to evaluate their infectiousness. This paradigm should be very useful to translate experiments from cell lines to a more physiological human lung model, which presents several advantages, including an unlimited and homogeneous source of hESC, contrary to primary explant cultures, which rely on availability of human lung biopsies. hESCs are also easily manipulated to perform editing (mutation, deletion, tagging) of genes relevant to biological questions concerning host-virus mechanisms of infection. The first attempts will be performed with hESCs which are considered to be more homogeneous in their ability to proceed through differentiation. However, we aim to apply such protocol to hiPSC lines that could be generated in the lab from patients more resistant or susceptible to influenza infection.

## Achievements and ongoing research

### *Consequences of influenza infection on skeletal muscles*

Consequences of viral infection on skeletal muscles are studied in mice to model an organismal response to infection. Mice were infected by intra nasal inoculation with a high dose of a mouse-adapted PR8 influenza A strain, which causes acute lung infection, rapid weight loss within 5 days and severe sickness. We performed our analysis on day 5 post-infection before animals reached the critical end point of 30% weight loss after which ethical regulations impose that they be euthanized. The first analysis aims to quantify the different cell populations present in infected and control muscles. Briefly, hindlimb muscles were dissected and subsequently digested enzymatically by collagenase and dispase into a single cell suspension prior to FACS analysis. A specific set of antibodies for cell surface receptors was used to identify distinct cell populations, i.e., fibroblast, endothelial, hematopoietic, inflammatory, and muscle stem cells. Non-muscle cells were discarded with a set of antibodies (CD45- CD31- Sca1-) and muscle stem cells were labeled with Vcam+ cell surface receptor. Interestingly, muscles from PR8 infected mice showed around 50% loss of their Vcam+ muscle stem cells compared to PBS-treated control mice.

Vcam, the α4ß1 integrin cell surface receptor, is present in cell types other than muscle stem cells, including some endothelial cell s. However, no general loss of the Vcam marker was observed in the total cell population of the muscle (37% of total Vcam+ cells in control vs. 39% in infected muscles; data not shown). Thus, the observed decrease in Vcam+ cells is specific to the muscle stem cell population and does not reflect an overall loss of this receptor in muscles.

Therefore, two possibilities could account for the observed reduction of Vcam+ muscle stem cells: a) this cell population has been lost by a mechanism that remains to be determined (apoptosis, differentiation) or b) the Vcam+ receptor (or at least the epitope recognized by the antibody) has been downregulated at the cells surface and can no longer be detected by FACS after immunostaining. A combination of the two mechanisms cannot be excluded. To further substantiate this result, we used the muscle stem cell marker Pax7 to detect satellite cells by immunohistochemistry on muscle frozen sections. Pax7 is a transcription factor specifically expressed on quiescent or activated muscle stem cells. Quantification of the Pax7+ cells in *soleus* muscle (calf muscle) showed also a 50% reduction of satellite cells (data not shown). However, a different muscle, the *tibialis anterior* (lower front leg) showed only a limited reduction in Vcam+ cells, suggesting that variation in biological response to viral infection may exist between muscles. Interestingly, patients with influenza have reported muscle pain or fatigue especially in the calf. For example, cases reported in the literature of myolysis in children, affected by influenza A or B, indicate that the *gastrocnemius* muscle was preferentially affected. Using the same FACS sorting strategy, our preliminary data indicate that the loss of Vcam+ cells occurs progressively from day 3 post-infection when mice are inoculated with a high dose of virus (data not shown).

We cannot exclude that, following viral infection, Pax7 protein, like VCam, could be down regulated and, therefore, become undetectable by immunodetection assays. To exclude this possibility, we will use genetically labeled satellite cells to perform our quantification after viral infection, leaving out cell surface markers. We will use *TgPax7CreERT2:RosaYFP* transgenics to generate mice, which after tamoxifen treatment, will have permanently labeled YFP+ satellite cells. These mice will be subjected to a PR8 viral infection and YFP+ satellite cells will be quantified by FACS without the need of antibody labeling. The disappearance of a large part of satellite cells during an acute viral infection has never been reported and would open the field for further investigations. The alternative possibility, viz., downregulation of Vcam at the surface of muscle stems, is less likely as it has been reported that, in the lung, Vcam is upregulated during viral infection to facilitate binding of virus to cells. Although influenza does not reach muscles directly during infection, Vcam clearance would be unexpected and raise questions about its functional consequences. Using the same paradigm, we could study the time course of these changes and their reversibility. Therefore, it seems important to evaluate how these molecular or cellular changes could affect the tissue regeneration if the viral infection is concomitant to a muscle injury.

We have also analyzed cells contributing to the muscle stem cell niche to determine if they were also compromised during viral infection. Hematopoietic cells (CD45+), endothelial cells (CD45-CD31+) and fibroblasts (CD31-CD45-Sca1+) present in the muscle were quantified by FACS analysis. None of these populations showed significant changes. This result suggests that the loss of Vcam+ cells does not follow a more general loss of cells or cell surface markers on these mice undergoing acute viral infection.

Our preliminary data analysis of total muscle cell suspension show an increase of inflammatory cells, monocytes and macrophages, labeled by CD11b or Ly6G/C, in infected muscles compared to controls. However, further experiments are required to determine if this higher number of inflammatory cells is present in blood vessels or in the muscle interstitium. This study will determine whether the skeletal muscle is also subjected to a local inflammation during viral infection. Interestingly, if inflammatory cells are detected within the muscle fascia, it will raise the question of which signals are attracting the granulocytes in absence of direct injury from the muscle.

## *Establishment of a human lung epithelium derived from hESCs to study infectious diseases*

To generate human lung epithelial cells, Dr. Ronald Li (Dr. Li Dak-Sum Research Centre, The University of Hong Kong) provided us with TE-HES2 and H9 hESC lines to set up the differentiation

protocol. These two cell lines present the advantage to be grown on a feeder free culture system. TE-HES2 is additionally adapted to trypsin for passaging but is known to be instable and susceptible to genomic alterations. The first step of the differentiation protocol induces hESC to adopt an endodermal fate. This is achieved through the formation of embryonic bodies treated with Activin A, bFGF and BMP4 to induce a Definitive Endoderm (DE) signature within 4 to 5 days. This step should generate more than 80-90% of cells positives for DE markers like CXCR4+, cKit, and Epcam. A weak DE induction is impacting the final percentage of lung epithelial cells generated. Using these markers as readout, we have been able thus far to orient only 50% of initial TE-HES2 or H9 cells towards the DE fate (data not shown). Such cells, can however differentiate further and express some lung progenitors markers such NKX2.1+, FoxA2+ or Sox2+, albeit in a low proportion (data not shown). To improve the efficiency of DE formation, we decided to switch to the RUES2 hESC line used in the original publication of the Snoeck lab. This line has been established in the Brivanlou laboratory (The Rockefeller University, USA) and we have completed the administrative procedures to obtain it. In addition, we will be able to use a second cell line, RUES2-Sox17 hESC recently generated by the Brivanlou lab. This cell line expresses the YFP-Sox17 marker on cells adopting a DE fate, which would allow assaying directly by immunofluorescence microscopy or FACS, the efficiency of our differentiation protocol. Upon embryonic body maturation, we will try to sort YFP-SOX17 cells to enrich the DE population before proceeding with downstream steps of differentiation. The establishment of the differentiated lung epithelial cells is a long multi-steps protocol lasting over 50 days, which requires several adjustments but represent an appropriate human model for a reductionist study of lung infectious diseases.

## Publications

1. **Gayraud-Morel B**, Pala F, Sakai H, Tajbakhsh S (2016) Isolation of muscle stem cells from mouse skeletal muscle. In: Muscle Stem Cells – Methods and Protocols (**Perdiguero** E and **Cornelison** D, Eds.), pp. 23-39.

## Collaborations (local and international)

1. **Leo Poon** (School of Public Health, The University of Hong Kong)
2. **Tom Chang** (Division of Life Sciences, Hong Kong University of Science and Technology)
3. **Shahragim Tajbakhkh** (Stem Cell and Development, Institut Pasteur)

# 3.7 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 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 biomedical education. In 2016 we organized two courses in Hong Kong (Cell Biology and Virology) and one in Ho Chi Minh City, Vietnam (Introduction to Modeling of Infectious Diseases). We received more than 200 applications from over 30 countries; 78 students with global geographic representation were selected for participation. The 9th HKU-Pasteur Immunology course was postponed to the Spring of 2017. In 2016 we have assembled an outstanding faculty, including members of the Chinese Academy of Sciences, of the National Academy of Sciences USA, of EMBO, and a recipient of the Lasker Award. More than 20 lectures were open to the scientific community of HKU (**see Annex 5.7 for a complete list of the 2016 lectures**).

HKU-PRP has pioneered a unique method in Hong Kong and in the region as we provide state of the art lectures and practical workshops in a "Master class" setting to outstanding students at the postgraduate and early postdoctoral level coming from countries with markedly different resources. Our alumni network, demonstrates that this educational program helps intensify human and scientific links between HKU-PRP, the School of Public Health at HKU and the Institut Pasteur International Network, and will continue to attract to Hong Kong top scientists and highly motivated students. The Cell Biology course has reached the 7th edition (**see Annex 5.4**) and the Virology course has been held for the 13th consecutive year (**see Annex 5.5**). Both programs are included in the coursework curriculum for research postgraduate studies of the University of Hong Kong. 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 budget cover advertising costs, travel and accommodation for all lecturers (except from industry). Selected students are expected to pay for their travel costs. Registration fees (HKD 1,000) include tuition, all course materials, accommodation (on sharing twin basis for overseas participants) and food (lunch and coffee breaks). Full reports of all events have been separately provided to the Advisory Committee.

Our Public Health Workshop series at the Pasteur Institute of Ho Chi Minh City is attracting increasing number of applications and has established a benchmark as a world-class training program for epidemiologists, researchers and public health officials in the region. The topic of the 2016 edition, organized in close partnership with the Pasteur Institute of Ho Chi Minh City,the School of Public Health at HKU and the International Network of Institut Pasteur, was "Introduction to Modeling of Infectious Diseases" (**see Annex 5.6**). The course discussed modeling logistics of epidemic interventions, optimizing allocation of intervention measures, cost and effectiveness evaluation of different interventions, practical applications of modeling, including predicting the impact of control strategies against pandemic influenza and other infections, as well as interpreting outbreak data and modeling in real-time to describe the course of outbreaks. We have identified mathematical modeling as an area in which there is still a gap between countries with different resources and public health systems. We are determined to fill the need for a dedicated course that will allow a regular training of public health staff and researchers, who have no modeling background but are interested in learning the basics of infectious

disease modeling and also recent advances in the field. We would like to express our deepest gratitude to the Regional Health Cooperation Office of the French Ministry of Foreign Affairs for their unflagging support of the program since its inception in 2012; their generous contribution has been instrumental to build the reputation of these courses in ASEAN countries and beyond.

## Additional teaching and training

Malik Peiris has set up with Maria Van Kerkhove (Head of the Outbreak Investigation Task Force at Institut Pasteur's Center for Global Health) a course on "**Infectious Disease Outbreak Investigation**", an intensive 2-week program (4-15 April 2016) intended for medical and public health professionals involved in field investigations of infectious disease outbreaks. This international course, held at the Institut Pasteur, provided professionals with a thorough approach of the methods of outbreak investigation of emerging and re-emerging pathogens including Ebola Virus Disease, Middle East Respiratory Syndrome Coronavirus, Plague, Avian Influenza subtype H5N1, pandemic influenza, enterovirus 71, and others. Participants were trained to the methods and processes used to detect, investigate, interpret and report on infectious disease outbreaks. Trainig includes wide ranging topics from preparing to respond to an emerging threat, implementing multi-disciplinary field investigations, analyzing and interpreting results from field studies, to communicating results to different partners.

Directors and Group Leaders at HKU-PRP are teaching in the Problem-Based Learning modules for MBBS students and are all actively involved in the HKU-Pasteur course series. HKU-PRP regularly hosts undergraduate/postgraduate students from overseas institutions for internships. In 2016 we welcomed four international trainees for an internship period:

- Dr. Ndongo DIA from Institut Pasteur Dakar
- Agathe LE QUANG from University of Montpellier, France;
- Clotide WICART from McGill University, Canada;
- Koen LOMMEN from Erasmus University Medical Center, The Netherlands.

Despite the success of the first three editions (two of the awardees have been hired as postdoctoral fellows at the Institut Pasteur and a third one has submitted a similar application), the L'Oreal Research Scholarship scheme has been discontinued due to the pull out of the sponsor.

## Complete list of taught courses

1. Roberto Bruzzone (2016) CMED6227 – Biological Basis of Disease (Master of Public Health), The University of Hong Kong, Hong Kong SAR.
2. Roberto Bruzzone (2016) Course Director, Molecular Biology of the Cell Course, Institut Pasteur, Paris, France.
3. Roberto Bruzzone (2016) Course Director, Introduction to Modeling of Infectious Diseases, Pasteur Institute of Ho Chi Minh City, Vietnam.
4. Roberto Bruzzone (2016) Course Director, 13$^{th}$ HKU-Pasteur Virology Course, The University of Hong Kong, Hong Kong SAR.
5. Suki Lee (2016) Hematology and Immunology System – Problem Based Learning (MBBS Year 2), The University of Hong Kong, Hong Kong SAR.
6. Suki Lee (2017) Lecturer in Institut Pasteur Massive Open Online Courses (MOOC) "Innate immunity and infectious diseases": Toll-like receptors in influenza virus infection.
7. Chris Mok (2016) Course Director, 13$^{th}$ HKU-Pasteur Virology Course, The University of Hong Kong, Hong Kong SAR.
8. Chris Mok (2016) Introduction to the Art and Science of Medicine– Problem Based Learning (MBBS Year 1), The University of Hong Kong, Hong Kong SAR.

9. Mailik Peiris (2016) Course Director, 13th HKU-Pasteur Virology Course, The University of Hong Kong, Hong Kong SAR.
10. Mailik Peiris (2016) Course Director, Infectious Disease Outbreak Investigation, Institut Pasteur, Paris, France.
11. Malik Peiris (2016) CMED6104 – Emerging infectious diseases: the "One Health" concept (Master of Public Health), The University of Hong Kong, Hong Kong SAR.
12. Sumana Sanyal (2017) Lecturer and practical tutor in the Molecular Biology of the Cell Course, Institut Pasteur, Paris, France.
13. Sumana Sanyal (2016) Lecturer and Tutor in the 7th HKU-Pasteur Cell Biology Course, The University of Hong Kong, Hong Kong SAR.
14. Sumana Sanyal (2017) Cardiopulmonary and Renal Systems – Problem Based Learning (MBBS Year 1) The University of Hong Kong, Hong Kong SAR.
15. Sophie Valkenburg (2016) Tutor for the Croucher Summer Course "Vaccinology for Public Health and Clinical Practice in the 21st century", The University of Hong Kong, Hong Kong SAR.
16. Sophie Valkenburg (2017) Tutor in the 9th HKU-Pasteur Immunology Course, The University of Hong Kong, Hong Kong SAR.

## 3.8 International Activity

HKU-PRP exerts a leadership role in a number of research and educational programs of global scope.

### 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 and has been Vice-Chair since 2014. 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 health research. In 2014 ISARIC has laid down the foundations for more challenging co-ordinated studies, including clinical trials of pathogen-specific therapies with pragmatic endpoints. ISARIC has played 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. It has been involved in the coordination of two clinical trials in West Africa. ISARIC is now assisting with the deployment of research on Zika virus and has set up a web site for shared resourced and information.

### 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.

### Visiting Research Professors Scheme

Professor James Di Santo from the Institut Pasteur has been appointed as Visiting Professor for the period 2016-8 through the "Visiting Research Professors" scheme of the University Research Committee of HKU. It is expected that having Professor James Di Santo as a Visiting Research Professor will stimulate research efforts and exchanges at several different levels within the LKS Faculty of Medicine. A discussion concerning establishing the Institut Pasteur Healthy Human Global Project (HHGP; http://www.milieuinterieur.fr/en) in Hong Kong, where cohorts have been established for many years by the School of Public Health, has been initiated; HHGP aims to understand the genetic and environmental determinants of immune responsiveness in normal individuals and provides a framework for future disease-related investigations that involve

the immune system. Professor Di Santo will have an active role in mentoring postgraduate students and early stage investigators involved in the projects.

We have obtained additional funds through the PROCORE – France/Hong Kong Joint Research Scheme supported by the RGC and are generating preliminary data that will be used to submit a grant proposal to **The French National Research Agency (ANR)/Research Grants Council (RGC) Joint Research Scheme and to the** Transversal Research Program, an intramural scheme funded by **the Institut Pas**teur. HKU-PRP is interested to further explore the functions of human ILCs during the course of infection by respiratory pathogens. Several questions will be addressed: How are ILC numbers and effector functions modified during influenza infection? Does this result from changes in ILC subset structure? How is ILC functional heterogeneity influenced? Are ILCs targets for influenza infection? The complementary expertise of Professor Di Santo and research scientists at HKU should create the perfect conditions to address these and other questions. The influenza research team in HKU has established a robust cell infection system to study the responses of virus-infected cells. We will investigate whether these human ILCs can be infected by influenza A virus, including the highly pathogenic influenza virus H5N1 as well as the lately emerging H7N9 virus and compared to low pathogenic viruses, including seasonal H3N2 and H1N1 viruses. We will then characterize whether ILC effector functions are modified by influenza infection and the underlying mechanisms for these changes. These studies will provide a new view of how different types of influenza virus infection might alter host immune response. Results in this area could help explain mechanisms of virus pathogenesis.

These research proposals will investigate the role of innate lymphoid cells (ILCs), which is one of the areas of expertise of James Di Santo, during microbe infection, and in particular, influenza infection. This and related projects also complement well the recently approved Theme-based Research Scheme entitle "Viral, host and environmental determinants of influenza virus transmission and pathogenesis" under the "Promoting Good Health" theme, which is coordinated by Malik Peiris. The appointment of James Di Santo will be an invaluable asset in building an even stronger program in human immunology, a strategic area of research that will reinforce the partnership between LKS Faculty of Medicine of HKU and Institut Pasteur.

## Other key initiatives

A symposium on "Transmission and Control of Respiratory Pathogens" was held on June 14, 2016 to mark the official signining of a Memorandum of Understanding Signing Ceremony between the School of Public Health and State Key Laboratory of Respiratory Disease at the First Affiliated Hospital of Guangzhou Medical University (PR China). This agreement will further strengthen the scientific partnership and academic exchange between the two institutions in the context of the research platform that we have established in Guanzhou to cover a wide range of research interests related to new emerging viruses.

# 4. Scientific Output

# 4.1 Publications cited in PubMed – 2016 to present


1. Blanc F, Furio L, Moisy D, Yen HL, Chignard M, Letavernier E, Naffakh N, Mok CK, Si-Tahar M (2016) Targeting host calpain proteases decreases influenza A virus infection. *Am J Physiol Lung Cell Mol Physiol* **310**:L689-L699.
2. Bertram S, Thiele S, Dreier C, Resa-Infante P, Preuss A, van Riel D, Mok CK, Schwalm F, Peiris JS, Klenk HD, Gabriel G (2017) H7N9 influenza A virus exhibits importin-α7 mediated replication in the mammalian respiratory tract. *Am J Pathol* **187**:831-840.
3. Chan LL, Bui CT, Mok CK, Ng MM, Nicholls JM, Peiris JS, Chan MC, Chan RW (2016) Evaluation of the human adaptation of influenza A/H7N9 virus in PB2 protein using human and swine respiratory tract explant cultures. *Sci Rep* **6**:35401.
4. Chan MC, Kuok DI, Leung CY, Hui KP, Valkenburg SA, Lau EH, Nicholls JM, Fang X, Guan Y, Lee JW, Chan RW, Webster RG, Matthay MA, Peiris JS (2016) Human mesenchymal stromal cells reduce influenza A H5N1-associated acute lung injury in vitro and in vivo. *Proc Natl Acad Sci USA* **113**:3621-3626.
5. Chan RW, Chan LL, Mok CK, Tao KP, Chan MC, Peiris JS, Nicholls JM (2016) Risk assessment of human infection by H9 influenza viruses using an explant system. *Pathology* **48** (**Suppl 1**):S105.
6. Chan RW, Chan LL, Mok CK, Lai JC, Tao KP, Chan MC, Perez DR, Peiris JS, Nicholls JN (2017) Replication of avian and human H9 viruses in the human ex vivo respiratory tract, and the influence of neuraminidase on this replication. *Submitted*,
7. Fan Y, Mok CK, Zhang Y, Nal B, Kien F, Bruzzone R, Sanyal S (2017) Cell cycle independent role of cyclin D3 in host restriction of influenza virus infection. *J Biol Chem*, **292**:5070-5088.
8. Gayraud-Morel B, Pala F, Sakai H, Tajbakhsh S (2017) Isolation of muscle stem cells from mouse skeletal muscle. In: Muscle Stem Cells – Methods and Protocols (**Perdiguero** E and **Cornelison** D, eds.), pp. 23-39.
9. Grant EJ, Josephs TM, Valkenburg SA, Wooldridge L, Hellard M, Rossjohn J, Bharadwaj M, Kedzierska K, Gras S (2016) Lack of heterologous cross-reactivity toward HLA-A*02:01 restricted viral epitopes is underpinned by distinct αβT cell receptor signatures. *J Biol Chem* **291**:24335-24351.
10. Hui KP, Chan LL, Kuok DI, Mok CK, Yang ZF, Luk GS, Lee, EF, **Lai JC**, Yen HL, Zhu HC, Guan Y, Nicholls JN, Peiris JS, Chan MC (2017) Tropism and innate host responses of influenza A/H5N6 virus: an analysis of *ex-vivo* and *in-vitro* cultures of the human respiratory tract. *Eur RespJ*, in press.
11. Hui KP, Li HS, Cheung MC, Chan RW, Yuen KM, Mok CK, Nicholls JM, Peiris JS, Chan MC (2016) Highly pathogenic avian influenza H5N1 virus delays apoptotic responses via activation of STAT3. *Sci Rep* **6**:28593.
12. Jahan AS, Lestra M, Swee LK, Fan Y, Lamers MM, Tafesse FG, Theile CS, Spooner E, Bruzzone R, Ploegh HL, Sanyal S (2016) Usp12 stabilizes the T cell receptor complex at the cell surface during signaling. *Proc Natl Acad Sci USA* **113**:E705-714.
13. Lai JC, Herath MT, Wong HH, Zhu G, Peiris JS, Nicholls JN (2017) Neuraminidase activity of Influenza A virus is enhanced by Hemagglutinin-receptor binding. *Submitted*.
14. Lee SM, Yan S, Yip TF, Peiris JS (2017) Toll-like receptor 10 is a novel nucleotide sensing receptor. *Submitted*.

15. Li W, Lee HH, Li RF, Zhu HM, Guan Y, Peiris JS, Yang ZF, Mok CK (2017) The PB2-E627K mutation enhances the pathogenicity of an avian influenza (H7N9) virus that belongs to a non-outbreak lineage. *In preparation*.

16. Lin YP, Luo Y, Chen Y, Lamers MM, Zhou Q, Yang XH, Sanyal S, Mok CKP, Liu ZM (2016) Clinical and epidemiological features of the 2014 large-scale dengue outbreak in Guangzhou city, China. *BMC Infect Dis* **16**:102.

17. Lin YP, Yang ZF, Liang Y, Li ZT, Bond HS, Luo YS, Chen Y, Chen TT, Guan WD, Lai JCC, Siu YL, Pan SH, Peiris JS, Cowling BJ, Mok CK (2016) Population seroprevalence of antibody to influenza A(H7N9), Guangzhou, China. *BMC Infect Dis* **16**:632.

18. Mayr J, Lau K, **Lai JC**, Gagarinov I, Chan RW, von Itzstein M, Nicholls JN, Haselhorst T (2017). Unraveling the role of O-glycans in influenza virus infections. *Submitted*.

19. Miguel E, Perera RA, Baubekova A, Chevalier V, Faye B, Akhmetsadykov N, Ng CY, Roger F, Peiris M (2016) Absence of Middle East Respiratory Syndrome Coronavirus in Camelids, Kazakhstan, 2015. *Emerg Infect Dis* **22**:doi:10.3201.

20. Sanyal S. Reply to Rodriguez: Mechanism of nuclear-cytosol shuttling of Usp12 (2016) *Proc Natl Acad Sci USA* **113**: E3317-E3318.

21. Teng O, Chen ST, Hsu TL, Sia SF, Cole S, Valkenburg SA, Hsu TY, Zheng JT, Tu W, Bruzzone R, Peiris JS, Hsieh SL, Yen HL (2017) CLEC5A-mediated enhancement of the inflammatory response in myeloid cells contributes to influenza pathogenicity in vivo. *J Virol* **91**: e01813-16.

22. Valkenburg SA, Josephs TM, Clemens EB, Grant EJ, Nguyen TH, Wang GC, Price DA, Miller A, Tong SY, Thomas PG, Doherty PC, Rossjohn J, Gras S, Kedzierska K (2016) Molecular basis for universal HLA-A*0201-restricted CD8+ T-cell immunity against influenza viruses. *Proc Natl Acad Sci USA* **113**:4440-4445.

23. Valkenburg SA, Mallajosyula VV, Li OT, Chin AW, Carnell G, Temperton N, Varadarajan R, Poon LL (2016) Stalking influenza by vaccination with pre-fusion headless HA mini-stem. *Sci Rep* **6**:22666.

24. Valkenburg SA, Zhang Y, Chan KY, Leung K, Wu JT, Poon LL (2016) Preexisting antibody-dependent cellular cytotoxicity-activating antibody responses are stable longitudinally and cross-reactive responses are not boosted by recent influenza exposure. *J Infect Dis* **214**:1159-1163.

25. Wang C, Lee HH, Yang ZF, Mok CK, Zhang Z (2016) PB2-Q591K mutation determines the pathogenicity of avian H9N2 influenza viruses for mammlian species. *PLoS One* **11**:e0162163.

26. Yan S, Ip KK, Lee SM (2017) TLR10 modulates poly(I:C) induced pro-inflammatory response. *In preparation*.

27. Zhang J, Sze DM, Yung BY, Tang P, Chen WJ, Chan KH, Leung PH (2016) Distinct expression of interferon-induced protein with tetratricopeptide repeats (IFIT) 1/2/3 and other antiviral genes between subsets of dendritic cells induced by dengue virus 2 infection. *Immunology* **148**:363-376.

## 4.2 Presentations at Meetings

1. Jahan AS, Sanyal S (2016) Role of deubiquitylating enzymes in influenza virus infection. *European Society of Clinical Microbiology and Infectious Diseases*, Amsterdam, The Netherlands (Poster).
2. Lee HHY, Yang ZF, Peiris JSM, Mok CKP (2016) Induction of proinflammatory cytokines is associated to the pathogenicity of avian H9N2 influenza viruses in mice. *Scientific Symposium of the Institut Pasteur International Network*, Paris, France (Poster).
3. Lee SMY, Yan S, Yip TF, Li ST, Yip K, Peiris JSM (2016) TLR10 as an innate receptor. *International Congress of Immunology 2016*, Melbourne, Australia (Poster).
4. Valkenburg SA, Li OTW, Peiris JSM, Perera LP, Poon LLM (2016) Protection by universal influenza vaccine from the immunological back line- CD4 T cell mediated protection. *Scientific Symposium of the Institut Pasteur International Network*, Paris, France (Oral).
5. Valkenburg SA, Mallajosyula VVA, Li OTW, Chin AWH, Carnell G, Temperton N, Varadarajan R, Poon LLM (2016) Targeting influenza by vaccination with prefusion headless HA ministem. *Victorian Infection and Immunity*, Lorne, Australia (Oral).
6. Valkenburg SA, Mallajosyula VVA, Li OTW, Chin AWH, Carnell G, Temperton N, Varadarajan R, Poon LLM (2016) Influenza vaccination with HA mini stem for broad antibody immunity. *International Congress of Immunology*, Melbourne, Australia (Oral).
7. Valkenburg SA, Mallajosyula VVA, Li OTW, Chin AWH, Carnell G, Temperton N, Varadarajan R, Peiris JSM, Perera LP, Poon LLM (2016) Universal correlates of immune protection during influenza infection and vaccination in mouse and humans. *2nd International forum on Influenza and other Respiratory viruses, Guangzhou*, PR China (Oral).
8. Yan S, Lee SMY (2016) TLR10 is involved in regulation of dsRNA-induced proinflammatory response. *Scientific Symposium of the Institut Pasteur International Network*, Paris, France (Poster).
9. Zhang JT, Sanyal S (2016) Role of Tsg101 in influenza virus assembly and release. *Options meeting for the control of influenza*, Chicago, USA (Oral).
10. Zhang JT, Sanyal S (2016) Role of Aup1 in the assembly and egress of dengue virus. *12th GERLI International Lipidomics meeting*, Toulouse, France (Oral).

## 4.3 Seminars, Invited Lectures and Other Oral Presentations

1. **Roberto Bruzzone** (2016) Mae Fah Luang University International Conference, Chiang Rai, Thailand.
2. **Suki Lee (2016)** HMRF grant skills training workshop: Sharing session by outstanding grant applicants, City University of Hong Kong.
3. **Chris Mok** (The Wonkwang University 70th Anniversary International Symposium, Wonkwnag University, South Korea (2016).
4. **Malik Peiris** (2016) FAO of the United Nations, Rome, Italy.
5. **Malik Peiris** (2016) Institut Pasteur International Network – Asia Regional Meeting, Shanghai, PR China.
6. **Malik Peiris** (2016) 16th Conference of the SCA , Science Council of Asia (SCA), Sri Lanka
7. **Malik Peiris** (2016) 48th Council of Directors, Institut Pasteur International Network Meeting, Paris, France.
8. **Malik Peiris** (2016) CEIRS Annual Meeting and CEIRS Surveillance Meeting, Memphis, USA.
9. **Malik Peiris** (2016) ISIRV, Options for the Control of Influenza IX Conference , Chicago, USA.
10. **Malik Peiris** (2016) Qatar Workshop on Emerging Pathogens at Human-Animal-Environment Interface, Qatar.
11. **Malik Peiris** (2016) Nature Conference on Viral Infection and Immune Response, Wuhan, PR China.
12. **Malik Peiris** (2016) Infectious Diseases week 2016, New Orleans, USA.
13. **Malik Peiris** (2016) 2016 World Life Science Conference, Beijing, PR China .
14. **Malik Peiris** (2016) Institut Pasteur – Global Virome Project Meeting, Paris France.
15. **Malik Peiris** (2016) One Health EcoHealth 2016, Melbourne, Australia.
16. **Malik Peiris** (2016) Infectious Disease Outbreak Course, Institut of Pasteur, Qatar, Doha.
17. **Malik Peiris** (2016) Infectious Disease Outbreak Investigation Course, Paris, France.
18. **Malik Peiris** (2016) 8th WHO meeting on development of influenza vaccines that induce broadly protective and long-lasting immune responses, Chicago, USA.
19. **Malik Peiris** (2016) WHO meeting for launching the Tool for Influenza Pandemic Risk Assiessment (TIPRA). Malik Peiris (2016) MERS Mission to KSA, Riyadh, Saudi Arabia.
20. **Sumana Sanyal** (2016) The University of Oxford, UK.
21. **Sumana Sanyal** (2016) Scientific Symposium of the Institut Pasteur International Network, Paris, France.
22. **Sumana Sanyal** (2016) Department of Biochemistry, University of Lausanne, Switzerland.
23. **Sumana Sanyal** (2016) Proteomics in Cell Biology and Human Disease, EMBL, Heidelberg.
24. **Sumana Sanyal** (2016) Innate Immunity to Host Pathogen Interactions, EMBL, Heidelberg.
25. **Sumana Sanyal** (2016) EMBO workshop on characterization of post translational modifications, Denmark.
26. **Sumana Sanyal** (2017) Department of Immunology Annual Retreat, Institut Pasteur.
27. **Sumana Sanyal** (2017) Molecular Biology of the Cell, Institut Pasteur.

# 4.4 Active Grants

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

| | |
|---|---|
| Principal Investigator: | Dr 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: | Dr Chris Mok |
| Amount: | HK$900,000.00 |
| Period: | 01/May/2015 to 31/Dec/2016 |

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

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

***Australian Research Council***

| | |
|---|---|
| Principal Investigator: | Dr Thomas Haselhorst (Institute for Glycomics, Austraila) |
| Co-Investigator: | Dr Jimmy Lai |
| Amount: | AU$ 320,000.00 |
| Period: | 01/Jan/2012 to 31/Dec/2016 |

***Australian Research Council***

| | |
|---|---|
| Principal Investigator: | Dr Thomas Haselhorst (Institute for Glycomics, Australia) |
| Collaborator : | Dr Jimmy Lai |
| Amount: | AU$ 696,000.00 |
| Period: | 24/Aug/2015 to 22/Aug/2017 |

***Center for Disease Control***

| | |
|---|---|
| Principal Investigator: | Prof Ben Cowling |
| Co-Investigator: | Dr Sophie Valkenburg |
| Period: | ending 31/Jul/2021 |

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

| | |
|---|---|
| Principal Investigator: | Prof Rajiv Khanna (QIMR Berghofer Medical Research Institute, Australia); |
| Co-Investigatorss: | Prof John Nicholls; Prof Dora Kwong (HKU) |

***Health and Medical Research Fund (HMRF)***

| | |
|---|---|
| Principal Investigator: | Dr Sophie Valkenburg |
| Amount: | HK$999,828.00 |
| Period: | 01/Jul/2015 to 31/Dec/2017 |

***Health and Medical Research Fund (HMRF)***

| | |
|---|---|
| Principal Investigator: | Prof Leo Poon |
| Co-Investigator: | Dr Sophie Valkenburg |
| Period: | 01/May/2016 to 30/Apr/2018 |

***Health and Medical Research Fund (HMRF)***

| | |
|---|---|
| Principal Investigator: | Dr Ben Cowling |
| Co-Investigator: | Dr Suki Lee |
| Amount: | HK$796,778.00 |
| Period: | 01/Apr/2015 to 30/Mar/2017 |

***Health and Medical Research Fund (HMRF)***

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

***Health and Medical Research Fund (HMRF)***

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

***Health and Medical Research Fund (HMRF)***

| | |
|---|---|
| Principal Investigator: | Dr Sumana Sanyal |
| Amount: | HK$1,200,000.00 |
| Period: | 01/Jul/2017 to 30/Jun/2019 |

***Health and Medical Research Fund (HMRF)***

| | |
|---|---|
| Principal Investigator: | Dr Roberto Bruzzone/Dr Mingyuan Li |
| Amount: | HK$1,170,000.00 |
| Period: | 01/Sep/2017 to 31/Aug/2019 |

***IPP-PTR***

| | |
|---|---|
| Principal Investigator: | Dr Sumana Sanyal |
| Amount: | EUR143,000.00 |
| Period: | 01/Jul/2015 to 31/Dec/2017 |

***National Natural Science Foundation of China***

| | |
|---|---|
| Principal Investigator: | Dr Zi Feng Yang |
| Co-Investigator: | Dr Chris Mok |
| Amount: | RMB1,500,000.00 |
| Period: | ending 30/Apr/2018 |

***Research Grants Council***

| | |
|---|---|
| Principal Investigator: | Dr Suki Lee |
| Amount: | HK$64,266.00 |
| Period: | 01/Oct/2014 to 30/Sep/2016 |

***Research Grants Council***

| | |
|---|---|
| Principal Investigator: | Dr Sumana Sanyal |
| Amount: | HK$654,557.00 |
| Period: | 01/Dec/2014 to 30/Nov/2016 |

***Research Grants Council***

| | |
|---|---|
| Principal Investigator: | Dr Sumana Sanyal |
| Amount: | HK$769,020.00 |
| Period: | 01/Nov/2015 to 31/Oct/2017 |

***Research Grants Council/Consulate General of France – PROCORE – France/Hong Kong Joint Research Scheme***

| | |
|---|---|
| Principal Investigator: | Dr Suki Lee |
| Amount: | HK$30,600.00 |
| Period: | 01/Jan/2017 to 31/Dec/2018 |

***RGC Seed Funding for Basic research***

| | |
|---|---|
| Principal Investigator: | Dr Suki Lee |
| Amount: | HK$34,490.00 |
| Period: | 01/Mar/2016 to 28/Feb/2017 |

***RGC Seed Funding for Basic research***

| | |
|---|---|
| Principal Investigator: | Dr Suki Lee |
| Amount: | HK$55,400.00 |
| Period: | 01/May/2017 to 30/Apr/2018 |

***RGC Seed Funding for Basic research***

| | |
|---|---|
| Principal Investigator: | Dr Chris Mok |
| Amount: | HK$45,980.00 |
| Period: | 01/Jun/2016 to 31/May/2017 |

***RGC Seed Funding for Basic research***

| | |
|---|---|
| Principal Investigator: | Dr Chris Mok |
| Amount: | HK$44,320.00 |
| Period: | 01/May/2017 to 30/Apr/2019 |

***RGC Seed Funding for Basic research***

| | |
|---|---|
| Principal Investigator: | Dr Sumana Sanyal |
| Amount: | HK$80,470.00 |
| Period: | 01/Jun/2016 to 31/May/2018 |

***RGC Seed Funding for Basic research***

| | |
|---|---|
| Principal Investigator: | Dr Sophie Valkenburg |
| Amount: | HK$48,048.00 |
| Period: | 01/04/2015 to 30/09/2016 |

***Science research project of the Guangdong Province***

| | |
|---|---|
| Principal Investigator: | Dr Zi Feng Yang |
| Co-Investigator: | Dr Chris Mok |
| Amount: | RMB1,000,000.00 |
| Period: | 01/Jul/2016 to 30/Jun/2018 |

***Seed Funding for Basic research***

| | |
|---|---|
| Principal Investigator: | Dr Sophie Doak |
| Amount: | HK$150,000.00 |
| Period: | 14/Oct/2016 to 13/Oct/2018 |

***Viral, Host and Environmental Determinants of Influenza Virus Transmission and Pathogenesis***

| | |
|---|---|
| Principal Investigator: | Dr Suki Lee |
| Amount: | HK$800,000.00 |
| Period: | 01/Jan/2015 to 31/Dec/2019 |

***Viral, Host and Environmental Determinants of Influenza Virus Transmission and Pathogenesis***

| | |
|---|---|
| Principal Investigator: | Dr Chris Mok |
| Amount: | HK$300,000.00 |
| Period: | 01/Jan/2015 to 31/Dec/2019 |

***Viral, Host and Environmental Determinants of Influenza Virus Transmission and Pathogenesis***

| | |
|---|---|
| Principal Investigator: | Dr Sumana Sanyal |
| Amount: | HK$450,000.00 |
| Period: | 01/Jan/2015 to 31/Dec/2019 |

***Viral, Host and Environmental Determinants of Influenza Virus Transmission and Pathogenesis***

| | |
|---|---|
| Principal Investigator: | Prof Malik Peiris |
| Co-Investigator: | Prof John Nicholls/Dr Jimmy Lai |
| Period: | 01/Jan/2015 to 31/Dec/2019 |

***WHO***

| | |
|---|---|
| Principal Investigator: | Prof Malik Peiris |
| Amount: | US$19,444.99 |
| Period: | 01/Nov/2016 to 30/Sep/2017 |

## 4.5 Pending Grant Applications

***Health and Medical Research Fund (HMRF) – 2017 Submission***
Principal Investigator: Dr Roberto Bruzzone/Dr Yun Lan
Amount: HK$ 1,161,424.00

***Health and Medical Research Fund (HMRF) – 2017 Submission***
Principal Investigator: Dr Sophie Valkenburg
Amount: HK$ 1,187,554.00

***Health and Medical Research Fund (HMRF) – 2017 Re-Submission***
Principal Investigator: Dr Suki Lee
Amount: HK$1,160,048.00

***Health and Medical Research Fund (HMRF) – 2017 Submission***
Principal Investigator: Dr Suki Lee
Amount: HK$1,198,262.00

***Health and Medical Research Fund (HMRF) – 2017 Submission***
Principal Investigator: Dr Mingyuan Li
Amount: HK$ 1,189,424.00

***Health and Medical Research Fund (HMRF) – 2017 Submission***
Principal Investigator: Dr Chris Mok
Amount: HK$ 996,376.00

***Health and Medical Research Fund (HMRF) – 2017 Submission***
Principal Investigator: Dr Sumana Sanyal
Amount: HK$ 1,200,000.00

***Health and Medical Research Fund (HMRF) – 2017 Submission***
Principal Investigator: Dr Selena Yan
Amount: HK$ 1,196,424.00

***Health and Medical Research Fund (HMRF) – 2017 Submission***
Principal Investigator: Dr Tami Zhang
Amount: HK$ 1,179,424.00

***Research Grants Council – 2016 Submission***
Principal Investigator: Dr Sophie Valkenburg
Amount: HK$ 1,425,612.00

***Research Grants Council – 2016 Submission***
Principal Investigator: Dr Suki Lee
Amount: HK$ 1,125,112.00

***Research Grants Council – 2016 Submission***
Principal Investigator: Dr Chris Mok
Amount: HK$ 985,112.00

***Research Grants Council – 2016 Submission***
Principal Investigator: Dr Sumana Sanyal
Amount: HK$1,030,408.00

***IPP – PTR 2017***
Principal Investigator: Dr Sophie Valkenburg
Amount: EUR 249,715.00

# 5. Annexes

# 5.1 List of Staff

| *Name* | *Position* | |
|---|---|---|
| BRUZZONE, Roberto | Co-Director | |
| PEIRIS, Malik | Co-Director | |
| DI-SANTO, James | Visiting Research Professor | Start: 12-Dec-2016 |
| SANYAL, Sumana | Research Assistant Professor<br>Assistant Professor (non-Clinical) | Start: 01-Feb-2017 |
| LEE, Man Yan Suki | Research Assistant Professor | |
| MOK, Ka Pun Chris | Research Assistant Professor | |
| VALKENBURG, Sophie | Research Assistant Professor | |
| LAI, Chun Cheong Jimmy | Post-Doctoral Fellow | (Joint Appointment with Department of Pathology in the Nicholls Lab) |
| ZHANG, Jingshu Tami | Post-Doctoral Fellow | |
| LI, Mingyuan | Technical Officer<br>Post-Doctoral Fellow | Start: 01-Jan-2017 |
| LAN, Yun | Technical Officer | |
| YAN, Sheng Selena | Research Associate | |
| GAYRAUD-MOREL Barbara | Honorary Research Associate | |
| BENET, Gabriel | Scientific Officer | (International Volunteer of the French Ministry of Foreiogn Affairs) |
| LI, Ping Hung | Research Technician | |
| SIU, Yu Lam Lewis | Research Technician | |
| TSE, Kong San Jane | Research Technician | |
| FAN, Lik Yan | Research Assistant | Until: Sept-2016 |
| IP, Ka Kay Kelvin | Research Assistant | Until: 31-Oct-2016 |
| LI, Shuting | Research Assistant | Until: 30-Aug-2016 |
| TI, Huihui | Research Assistant | Until: 08-Dec-2016 |
| WANG, Yizhuo | Research Assistant | |
| YIP, Tsz Fung | Research Assistant | |
| LI, Pui Yee Athena | Research Assistant<br>PhD Student | Until: 31-May-2017 |

| | | |
|---|---|---|
| MA, Nok Lam Fion | Research Assistant<br>MPhil Student | Until: 01-Sep-2016 |
| POMBO, Joao | Research Assistant<br>MPhil Student | Until: 31-Aug-2016 |
| AKHEE, Sabiha Jahan | PhD Student | |
| LEE, Hok Yeung Horace | PhD Student | |
| BULL, Máiréid | MPhil Student | |
| HERATH M Thusitha Kumara K | MPhil Student | Graduated: 28-Feb-2017 |
| SELIM Asisha Sami Mohammed Mohammed | MPhil Student | |
| WONG, Ho Him | MPhil Student | |
| MAK, Ganon | PhD Student (Part-time) | |
| DIA Ndongo | Trainee | from Institut Pasteur Dakar |
| CHAN Mei Lam | Student Intern (IVE) | |
| NG Hon Lam | Student Intern (IVE) | |
| YIP GARRICK | Student Intern (IVE) | |
| De SAINT GERMAN, Raphaelle | Student Intern | |
| GAUTHIER, Ninon | Student Intern | |
| LE QUANG Agathe | Student Intern | |
| LOMMEN Koen | Student Intern | |
| WICART Clotide | Student Intern | |
| LI, Suk Yin Anne | Administrative Assistant | |
| CHAN, King Chuen | Laboratory Attendant | Until: 01-Feb-2017 |
| CHEUNG, Wai Sze | Laboratory Attendant | |
| YIM, Yuk Chun | Laboratory Attendant | Until: 13-Mar-2017 |
| NG, Tsz WAI | Laboratory Attendant | |

## 5.2 Income & Expenses for the year ending 30 June 2016

## INCOME:

| | | | |
|---|---|---|---|
| Central Fund | | | $2,083,485.81 |
| Endownment Fund | c/f | $2,062,639.39 | |
| | | $3,330,496.27 | $5,393,135.66 |
| Grants | c/f | $2,327,361.64 | |
| | | $5,952,194.01 | $8,279,555,65 |
| Private Donation | c/f | $1,008,000.00 | |
| | | $300,000.00 | $1,308,000.00 |
| Teaching/Training | c/f | $184,634.77 | |
| | | $586,954.41 | $816,589.18 |

**TOTAL INCOME** **$17,880,766.30**

## EXPENSES:

| | |
|---|---|
| Staff Cost | $6,886,012.47 |
| Research | $2,322,931.72 |
| Conference/Meeting | $450,960.91 |
| Equipment/Maintenance | $223,198.10 |
| Administration | $108,667.60 |
| Teaching/Training | $885,635.11 |

**TOTAL EXPENSES** **$10,877,405.91**

## 5.3 Forecast for Income & Expenses for the year ending 30 June 2017

## INCOME:

| | | | |
|---|---|---|---|
| Central Fund | c/f | $(573,738.00)<br>$2,700,538.50 | $2,126,800.50 |
| Faculty in-kind | c/f | $-<br>$538,000.00 | $538,000.00 |
| Endownment Fund | c/f | $4,457,423.39<br>$956,452.00 | $5,413,875.39 |
| Grants | c/f | $2,118,895.09<br>$5,816,439.36 | $7,935,334.45 |
| Private Donation | c/f | $1,214,199.00 | $1,214,199.00 |
| Teaching/Training | c/f | $16,943.17<br>$525,136.85 | $542,080.02 |

**TOTAL INCOME** **$17,770,289.36**

## EXPENSES:

| | |
|---|---|
| Staff Cost | $7,550,600.98 |
| Research | $2,289,679.40 |
| Conference/Meeting | $236,466.64 |
| Equipment/Maintenance | $655,366.41 |
| Administration | $175,784.40 |
| Teaching/Training | $728,941.84 |

**TOTAL EXPENSES** **$11,636,839.67**

## 5.4 – 7th HKU-Pasteur Cell Biology Course 2016

# 7th HKU- PASTEUR CELL BIOLOGY COURSE

## 6 - 18 March 2016

### HKU-Pasteur Research Pole, Hong Kong

## Intracellular Traffic in Health and Disease

**New deadline for applications**

### Directors:

Roberto BRUZZONE (HKU-Pasteur Research Pole)
Philippe CHAVRIER (Institut Curie)
George TSAO (The University of Hong Kong)
Chiara ZURZOLO (Institut Pasteur)

### Lecturers:

Maria-Joao AMORIM (Portugal)
Roberto BRUZZONE (Hong Kong)
Philippe CHAVRIER (France)
Pier Paolo DI FIORE (Italy)
Linda KENNEY (Singapore)
Tom KIRCHHAUSEN (USA)
Jason MERCER (United Kingdom)
Jeanne SALJE (Thailand)
Sumana SANYAL (Hong Kong)
Giampietro SCHIAVO (United Kingdom)
Michael SHEETZ (Singapore)
Anne SPANG (Switzerland)
George TSAO (Hong Kong)
Cheng-han YU (Hong Kong)
Chiara ZURZOLO (France)

### Registration:

Candidates are invited to download course application forms at www.hkupasteur.hku.hk.

Please return completed form, including two letters of recommendation to hku-pasteur@hku.hk.

Registration fees (HKD 1,000), inculde accommodation (on sharing twin basis for overseas participants) and food (lunch, coffee breaks and two dinner) will be provided.

The course (MMPH6175) is included in the coursework curriculum for research postgraduate studies of the University of Hong Kong.

### New Deadline:

**December 18, 2015.**

### For more information:

Please contact Anne LI at +852 2831 5516 or hku-pasteur@hku.hk.

Check www.hkupasteur.hku.hk for programme updates.

International network Institut Pasteur
THE UNIVERSITY OF HONG KONG LI KA SHING FACULTY OF MEDICINE 香港大學李嘉誠醫學院
香港大學 - 巴斯德研究中心 HKU-Pasteur Research Pole
Liberté • Égalité • Fraternité RÉPUBLIQUE FRANÇAISE CONSULAT GÉNÉRAL DE FRANCE À HONG KONG ET À MACAO
Institut Pasteur

# 7th HKU- PASTEUR CELL BIOLOGY COURSE

## 6 - 18 March 2016

### HKU-Pasteur Research Pole, Hong Kong

## PUBLIC LECTURE

## "Cellular Transport Systems"

by

**Prof Anne SPANG**
*Biozentrum, University of Basel*
*Switzerland*

**DATE:** Monday, 7 March 2016
**TIME:** 08:30 - 11:00
**VENUE:** Seminar Room 2, Ground Floor
5 Sassoon Road, Pokfulam, HK

**ALL ARE WELCOME**

International network Institut Pasteur
THE UNIVERSITY OF HONG KONG LI KA SHING FACULTY OF MEDICINE
香港大學李嘉誠醫學院
香港大學・巴斯德研究中心
HKU-Pasteur Research Pole
Liberté • Égalité • Fraternité RÉPUBLIQUE FRANÇAISE CONSULAT GÉNÉRAL DE FRANCE À HONG KONG ET À MACAO
Institut Pasteur

# 7th HKU- PASTEUR CELL BIOLOGY COURSE

## 6 - 18 March 2016

## HKU-Pasteur Research Pole, Hong Kong

## PUBLIC LECTURE

## "Axonal transport in heath and disease"

by

**Prof Giampietro SCHIAVO**
*University College London*
*United Kingdom*

**DATE:** Tuesday, 8 March 2016
**TIME:** 09:00 - 11:30
**VENUE:** HRI-SR 2, Ground Floor
5 Sassoon Road, Pokfulam, HK

## ALL ARE WELCOME

International network Institut Pasteur
THE UNIVERSITY OF HONG KONG LI KA SHING FACULTY OF MEDICINE
香港大學李嘉誠醫學院
香港大學・巴斯德研究中心
HKU-Pasteur Research Pole
Liberté • Égalité • Fraternité RÉPUBLIQUE FRANÇAISE
CONSULAT GÉNÉRAL DE FRANCE À HONG KONG ET À MACAO
Institut Pasteur

# 7th HKU- PASTEUR CELL BIOLOGY COURSE

## 6 - 18 March 2016

## HKU-Pasteur Research Pole, Hong Kong

## PUBLIC LECTURE

## "Mechanisms of virus entry"

by

**Dr Jason Mercer**
*University College London*
*United Kingdom*

**DATE:** Wednesday, 9 March 2016
**TIME:** 09:00 - 11:30
**VENUE:** HRI-SR7, 7th Floor
5 Sassoon Road, Pokfulam, HK

## ALL ARE WELCOME

International network Institut Pasteur
THE UNIVERSITY OF HONG KONG LI KA SHING FACULTY OF MEDICINE
香港大學李嘉誠醫學院
香港大學・巴斯德研究中心
HKU-Pasteur Research Pole
Liberté • Égalité • Fraternité RÉPUBLIQUE FRANÇAISE CONSULAT GÉNÉRAL DE FRANCE À HONG KONG ET À MACAO
Institut Pasteur

# 7th HKU- PASTEUR CELL BIOLOGY COURSE

## 6 - 18 March 2016

## HKU-Pasteur Research Pole, Hong Kong

## PUBLIC LECTURE

## "Viral usage of host recycling endosome"

by

**Prof Maria-Joao Amorim**

*Instituto Gulbenkian de Ciencia*

*Portugal*

**DATE:** Thursday, 10 March 2016

**TIME:** 09:30 - 11:30

**VENUE:** HRI-SR2, Ground Floor
5 Sassoon Road, Pokfulam, HK

## ALL ARE WELCOME

International network Institut Pasteur

THE UNIVERSITY OF HONG KONG LI KA SHING FACULTY OF MEDICINE 香港大學李嘉誠醫學院

香港大學・巴斯德研究中心 HKU-Pasteur Research Pole

Liberté • Égalité • Fraternité RÉPUBLIQUE FRANÇAISE CONSULAT GÉNÉRAL DE FRANCE À HONG KONG ET À MACAO

Institut Pasteur

# 7th HKU- PASTEUR CELL BIOLOGY COURSE

## 6 - 18 March 2016

HKU-Pasteur Research Pole, Hong Kong

## PUBLIC LECTURE

11:30 - 12:30

"An overview of Salmonella pathogenesis:
Imaging needle formation, regulation and effector secretion"

14:00 - 15:00

"How Salmonella coopts a pathogenicity island - encoded regulator to control ancestral genes to form biofilms"

by

Prof Linda KENNEY

*Mechanobiology Institute, NUS*

*Singapore*

**DATE:** Friday, 11 March 2016

**VENUE:** HRI - SR2, Ground Floor
5 Sassoon Road, Pokfulam, HK

## ALL ARE WELCOME

International network Institut Pasteur

THE UNIVERSITY OF HONG KONG
LI KA SHING FACULTY OF MEDICINE
香港大學李嘉誠醫學院

香港大學 · 巴斯德研究中心
HKU-Pasteur Research Pole

Liberté · Égalité · Fraternité
RÉPUBLIQUE FRANÇAISE
CONSULAT GÉNÉRAL DE FRANCE À HONG KONG ET À MACAO

Institut Pasteur

# 7th HKU- PASTEUR CELL BIOLOGY COURSE

## 6 - 18 March 2016

### HKU-Pasteur Research Pole, Hong Kong

## PUBLIC LECTURE

# "Mechanosensing Through Local Generation and Transduction of Forces"

by

**Prof Michael Sheetz**

*Mechanobiology Institute, NUS*

*Singapore*

**DATE:** Monday, 14 March 2016

**TIME:** 13:30 - 16:00

**VENUE:** HRI - SR7, 7th Floor

5 Sassoon Road, Pokfulam, HK

## ALL ARE WELCOME

International network Institut Pasteur

THE UNIVERSITY OF HONG KONG LI KA SHING FACULTY OF MEDICINE 香港大學李嘉誠醫學院

香港大學・巴斯德研究中心 HKU-Pasteur Research Pole

Liberté • Égalité • Fraternité RÉPUBLIQUE FRANÇAISE CONSULAT GÉNÉRAL DE FRANCE À HONG KONG ET À MACAO

Institut Pasteur

# 7th HKU- PASTEUR CELL BIOLOGY COURSE

## 6 - 18 March 2016

### HKU-Pasteur Research Pole, Hong Kong

## PUBLIC LECTURE

## "Endocytosis Signaling and Diseases (mostly cancer)"

by

**Prof Pier Paolo DI FIORE**

*IFOM - Institute of Molecular Oncology*

*Italy*

**DATE:** Monday, 14 March 2016

**TIME:** 09:30 - 12:00

**VENUE:** HRI-SR7, 7th Floor
5 Sassoon Road, Pokfulam, HK

## ALL ARE WELCOME

International network Institut Pasteur

THE UNIVERSITY OF HONG KONG LI KA SHING FACULTY OF MEDICINE 香港大學李嘉誠醫學院

香港大學 · 巴斯德研究中心 HKU-Pasteur Research Pole

Liberté · Égalité · Fraternité RÉPUBLIQUE FRANÇAISE CONSULAT GÉNÉRAL DE FRANCE À HONG KONG ET À MACAO

Institut Pasteur

# 7th HKU- PASTEUR CELL BIOLOGY COURSE

## 6 - 18 March 2016

HKU-Pasteur Research Pole, Hong Kong

## PUBLIC LECTURE

9:30 - 12:00

"Endocytosis Signaling and Diseases (mostly cancer)"

by

Prof Pier Paolo DI FIORE

IFOM - Institute of Molecular Oncology, Italy

13:30 - 16:00

"Mechanosensing Through Local Generation and Transduction of Forces"

by

Prof Michael Sheetz

Mechanobiology Institute, NUS, Singapore

**DATE:** Monday, 14 March 2016

**VENUE:** HRI - SR7, 7th Floor
5 Sassoon Road, Pokfulam, HK

## ALL ARE WELCOME

International network Institut Pasteur

THE UNIVERSITY OF HONG KONG LI KA SHING FACULTY OF MEDICINE

香港大學李嘉誠醫學院

香港大學 · 巴斯德研究中心
HKU-Pasteur Research Pole

Liberté · Égalité · Fraternité
RÉPUBLIQUE FRANÇAISE
CONSULAT GÉNÉRAL DE FRANCE À HONG KONG ET À MACAO

Institut Pasteur

# 7th HKU- PASTEUR CELL BIOLOGY COURSE

## 6 - 18 March 2016

### HKU-Pasteur Research Pole, Hong Kong

## PUBLIC LECTURE

## "Cellular dynamics with high temporal and spatial resolution"

by

**Prof Tom Kirchhausen**

*Harvard Medical School*

*U.S.A.*

**DATE:** Wednesday, 16 March 2016

**TIME:** 09:30 - 12:30

**VENUE:** HRI - SR2, Ground Floor
5 Sassoon Road, Pokfulam, HK

## ALL ARE WELCOME

International network Institut Pasteur

THE UNIVERSITY OF HONG KONG LI KA SHING FACULTY OF MEDICINE 香港大學李嘉誠醫學院

香港大學 · 巴斯德研究中心 HKU-Pasteur Research Pole

Liberté · Égalité · Fraternité RÉPUBLIQUE FRANÇAISE CONSULAT GÉNÉRAL DE FRANCE À HONG KONG ET À MACAO

Institut Pasteur

## 5.5 13th HKU-Pasteur Virology Course 2016

# 13th HKU-PASTEUR VIROLOGY COURSE

## 10 - 22 July 2016

### HKU-Pasteur Research Pole, Hong Kong

# BATS and VIRUSES

**DIRECTORS**

**Roberto BRUZZONE** (HKU-PRP) | **Malik PEIRIS** (HKU-PRP & HKU) | **Noël TORDO** (Institut Pasteur)

**LECTURERS**

**Roberto BRUZZONE** (Hong Kong)
**Christian DROSTEN** (Germany)
**Anthony FOOKS** (United Kingdom)
**George Fu GAO** (PR China)
**Neil FUREY** (Cambodia)
**Emily GURLEY** (Bangladesh)
**Bart HAAGMANS** (The Netherlands)
**Mart LAMERS** (The Netherlands)
**Chris MOK** (Hong Kong)
**Malik PEIRIS** (Hong Kong)
**Leo POON** (Hong Kong)
**Tony SCHOUNTZ** (USA)
**Noël TORDO** (France)
**Jonathan TOWNER** (USA)
**Jun WANG** (PR China)
**Linfa WANG** (Singapore)
**Gudrun WIBBELT** (Germany)

**TOPIC**

Bats have been associated with a number of emerging zoonotic viral diseases. The course will explore the impact of viruses of bat origin with a holistic approach spanning virology, immunology, public health systems, sociology and anthropology. Various viral models and situations will be considered with the help of leading scientists in this area, including ecologists, field biologists, immunologists, virologists and clinicians. Practical workshops will address the dynamics of viral evolution and current experimental strategies for molecular surveillance and pathogenetic studies.

**REGISTRATION**

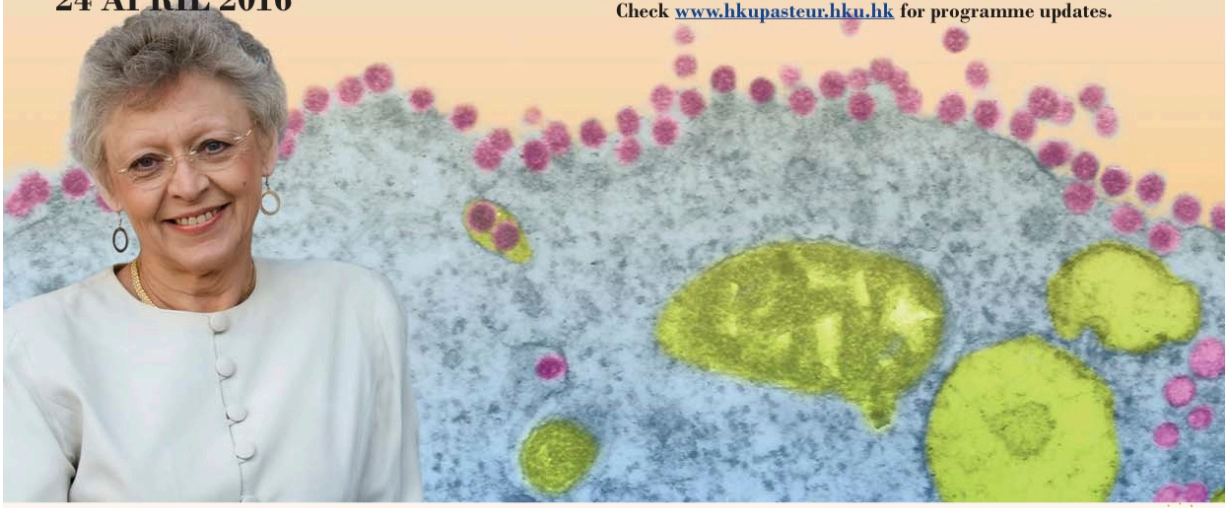
Candidates are invited to download course application form at www.hkupasteur.hku.hk
Please return the completed form, including two letters of recommendation to hku-pasteur@hku.hk.
Registration fees (HKD 1,000) include accommodation (on sharing twin basis for overseas participants) and food (lunch and coffee breaks).
The course (MMPH6171) is included in the coursework curriculum for research postgraduate studies of the University of Hong Kong.

**DEADLINE FOR APPLICATIONS**

**24 APRIL 2016**

**FOR MORE INFORMATION, PLEASE CONTACT**

Anne LI at +852 2831 5516 or hku-pasteur@hku.hk.
Check www.hkupasteur.hku.hk for programme updates.



Institut Pasteur | THE UNIVERSITY OF HONG KONG LI KA SHING FACULTY OF MEDICINE 香港大學李嘉誠醫學院 | 香港大學 · 巴斯德研究中心 HKU-Pasteur Research Pole | Liberté · Égalité · Fraternité RÉPUBLIQUE FRANÇAISE | international network Institut Pasteur

# HKU-PASTEUR VIROLOGY COURSE

FOR RESEARCH POSTGRADUATE STUDENTS

Institut Pasteur

香港大學 · 巴斯德研究中心
HKU-Pasteur Research Pole

THE UNIVERSITY OF HONG KONG
LI KA SHING FACULTY OF MEDICINE
香港大學李嘉誠醫學院

## 13th HKU – PASTEUR VIROLOGY COURSE

## OPEN LECTURES

| | |
|---|---|
| 09:00 – 11:00 | "Virus diversity and evolution in bats" Part 1<br>by Prof ChristianDrosten<br>University of Boon Medical Centre, Germany |
| 11:30 – 13:30 | "Virus diversity and evolution in bats" Part 2<br>by Prof Christian Drosten<br>University of Boon Medical Centre, Germany |
| 14:30 – 16:30 | "Immunology of bats and their viruses"<br>by Prof Tony Schountz<br>Colorado State University, USA |

**Date:** Tuesday, 12th July 2016

**Venue:** Seminar Room 4, 4th Floor
HKJC Building for Interdisciplinary Research
5 Sassoon Road, Pokfulam, Hong Kong

# HKU-PASTEUR VIROLOGY COURSE

FOR RESEARCH POSTGRADUATE STUDENTS

Institut Pasteur

香港大學 · 巴斯德研究中心
HKU-Pasteur Research Pole

THE UNIVERSITY OF HONG KONG
LI KA SHING FACULTY OF MEDICINE
香港大學李嘉誠醫學院

## 13th HKU – PASTEUR VIROLOGY COURSE

## OPEN LECTURES

## "Henipaviruses: from discovery to vaccine development"

by

Prof Linfa WANG
Duke – NUS Graduate Medical School
Singapore

**Date:** **Thursday, 14th July 2016**

**Time:** **09:00 – 11:30**

**Venue:** **Seminar Room 4, 4th Floor**
**HKJC Building for Interdisciplinary Research**
**5 Sassoon Road, Pokfulam, Hong Kong**


## ALL ARE WELCOME

# HKU-PASTEUR VIROLOGY COURSE

FOR RESEARCH POSTGRADUATE STUDENTS

Institut Pasteur

香港大學 · 巴斯德研究中心
HKU-Pasteur Research Pole

THE UNIVERSITY OF HONG KONG
LI KA SHING FACULTY OF MEDICINE
香港大學李嘉誠醫學院

## 13th HKU – PASTEUR VIROLOGY COURSE

## OPEN LECTURES

| | |
|---|---|
| 09:00 – 11:00 | "MERS coronavirus: Ecology, epidemiology and transmission" by<br>Prof Malik Peiris<br>University of Hong Kong, HKSAR |
| 11:30 – 13:00 | "Virus diversity in bats: the case of astroviruses and coronaviruses" by<br>Dr Leo Poon<br>University of Hong Kong, HKSAR |
| 14:30 – 16:30 | "The biology of the emerging MERS-CoV" by<br>Dr Bart Haagmans<br>Erasmus Medical Center, The Netherlands |

**Date:** Monday, 18th July 2016

**Venue:** Seminar Room 4, 4th Floor
HKJC Building for Interdisciplinary Research
5 Sassoon Road, Pokfulam, Hong Kong

Françoise Barré-Sinoussi (b. 1947) co-recipient of the 2008 Nobel Prize in Medicine for the "discovery of human immunodeficiency virus"

SARS-CoV virions budding from infected cells (Departments of Pathology and Microbiology, HKU)

## ALL ARE WELCOME

# HKU-PASTEUR VIROLOGY COURSE

FOR RESEARCH POSTGRADUATE STUDENTS

Institut Pasteur

香港大學 - 巴斯德研究中心
HKU-Pasteur Research Pole

THE UNIVERSITY OF HONG KONG
LI KA SHING FACULTY OF MEDICINE
香港大學李嘉誠醫學院

## 13th HKU – PASTEUR VIROLOGY COURSE

**OPEN LECTURES**

*co-organized by*

*AoE on Control of Pandemic and Inter-Pandemic Influenza*

## Host jump: Flu, MERS-CoV, Ebola and Zika
## Part 1 & 2

by

Prof George Gao
Chinese Center for Disease Control and Prevention
PR China

**Date:** **Wednesday, 20th July 2016**

**Time:** **Part 1** **11:30 – 12:30**
**Part 2** **14:00 – 15:00**

**Venue:** **Mrs Chen Yang Foo Oi Telemedicine Centre**
**2nd Floor, Room A2-08, William M W Mong Block**
**Li Ka Shing Faculty of Medicine**

**ALL ARE WELCOME**

Area of Excellence
CONTROL OF PANDEMIC AND INTER-PANDEMIC INFLUENZA
FUNDED BY THE UNIVERSITY GRANTS COMMITTEE HONG KONG

SARS-CoV virions budding from infected cells (Departments of Pathology and Microbiology, HKU)
Françoise Barré-Sinoussi (b. 1947) co-recipient of the 2008 Nobel Prize in Medicine for the "discovery of human immunodeficiency virus"

# HKU-PASTEUR VIROLOGY COURSE

FOR RESEARCH POSTGRADUATE STUDENTS

Institut Pasteur

香港大學 · 巴斯德研究中心
HKU-Pasteur Research Pole

THE UNIVERSITY OF HONG KONG
LI KA SHING FACULTY OF MEDICINE
香港大學李嘉誠醫學院

## 13th HKU – PASTEUR VIROLOGY COURSE

## OPEN LECTURES

## "Filovirus Replication in Bats"

By

Prof Jonathan Towner
Centers for Disease Control & Prevention
USA

**Date:** Wednesday, 20th July 2016

**Time:** 09:00 – 11:00

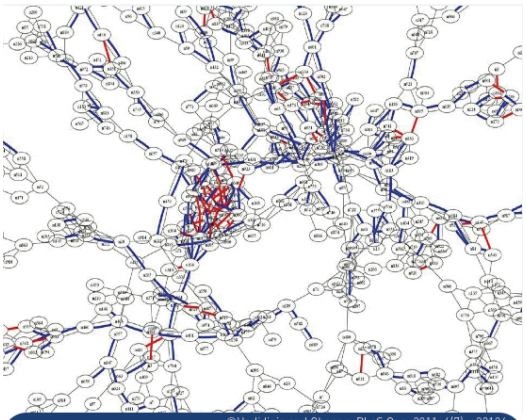
**Venue:** Mrs Chen Yang Foo Oi Telemedicine Centre
2nd Floor, Room A2-08, William M W Mong Block
Li Ka Shing Faculty of Medicine

## 5.6 6th Epidemiology Workshop

International Workshop

# INTRODUCTION TO MODELING OF INFECTIOUS DISEASES

**October 30-November 4, 2016** Ho Chi Minh City, Vietnam



©Hadidjojo and Cheong, PLoS One. 2011; 6(7): e22124.

**NO REGISTRATION FEES - FREE**

**ACCOMMODATION WILL BE PROVIDED**

**DEADLINE FOR APPLICATIONS: SEPTEMBER 5, 2016**

## Course's Objectives

The course will introduce professionals working on infectious diseases to mathematical modeling of infectious diseases, an exciting and expanding new area of research that is being applied to inform public health policies.
It will place emphasis on developing a conceptual understanding of the basic methods and on learning their practical application.
The methods will be illustrated by "hands-on" experience of setting up models in spreadsheets as well as other specialist modeling packages, small group work, and seminars in which recent applications of infectious disease modeling to public health problems will be discussed.

## Faculty

**B. COWLING, T. LAM & J.T. WU** The University of Hong Kong, **S. CAUCHEMEZ** Institut Pasteur, **M. LIPSITCH** Harvard TH Chan School of Public Health, **R. BRUZZONE** HKU-Pasteur Research Pole, **M. CHOISY** Oxford University Clinical Research Unit & IRD, **M. JIT** London School of Hygiene & Tropical Medicine, **C. HOANG CUOC, H. VU THI QUE & Q. PHAM DUY** Pasteur Institute - Ho Chi Minh City.

## Participants' Profile

The course is specifically designed for health personnel, mainly but not exclusively from countries in South East Asia, including medical and health professionals, policy makers, veterinary scientists, medical statisticians and infectious disease researchers interested in expanding their knowledge of the techniques available for analyzing and interpreting epidemiological data on infectious diseases and for predicting the impact of control programs. Specialist mathematical training is not a prerequisite.
**Taught in English. Limited to 24 participants.**

## Applications

Candidates are invited to download application forms at www.hkupasteur.hku.hk. Please return the completed form, including 1-2 letters of recommendation, to hku-pasteur@hku.hk. The course teaching committee will review applications and select participants.

香港大學・巴斯德研究中心 HKU-Pasteur Research Pole
Pasteur Institute Ho Chi Minh City Training Center
international network Institut Pasteur
MIDAS
Center for Communicable Disease Dynamics
Liberté • Égalité • Fraternité République Française

## 5.7 Infectious Disease Outbreak Investigation

# Infectious Disease Outbreak Investigation
# 4-15 April 2016, Paris France

**Course organizers:** Maria Van Kerkhove
Malik Peiris

Institut Pasteur

centre d'enseignement

This course will cover methods and processes used to detect, investigate, interpret and report on infectious disease outbreaks and include wide ranging topics from preparing to respond to an emerging threat, implementing multi-disciplinary field investigations, analyzing and interpreting results from field studies, to communicating results to different partners.

This one-week course will include a combination of lectures and real-world practicals provided by experts at Institut Pasteur Paris, the Institut Pasteur International Network, the World Health Organization and other international agencies. Topics to be covered include:

- Field epidemiology (study designs and outcomes expected)
- Planning for field investigations (development of protocols, assembling a team, developing standard operating procedures, logistics planning, hiring teams, if necessary, ethical approvals)
- Partnerships in field investigations
- Questionnaire development and data collection
- Biological sample collection and laboratory testing
- Implementation of outbreak investigations
- Data entry and statistical analysis – from basic to advanced
- The use of mathematical modeling during outbreaks
- Report/presentation preparation for local partners
- Manuscript writing for peer-reviewed journals

**Participants must have a post graduate degree in epidemiology, microbiology, medicine, public health or a related discipline and some experience in field work of infectious diseases.**

The committee of the course will evaluate applications.

Only people from the Institut Pasteur Network are eligible to the course and the course is free of charge.

## 5.8 List of Public Lectures organized by HKU-PRP

19/12/2016

"Developmental Options and Functional Plasticity of Human Innate Lymphoid Cells" by Prof James DI-SANTO, Institut Pasteur, France

10/11/2016

"Investigating development and function of myeloid cells using chemical and genetic screens"by Dr Lee Kim Swee, Biomedx Innovation Centre, Germany

20/07/2016

"Filovirus Replication in Bats" by Dr Jonathan Towner, Centers for Disease Control and Prevention, USA

20/07/2016

"Host jump: Flu, MERS-CoV, Ebola and Zika" by Professor George Gao, Chinese Center for Disease Control and Prevention, PR China

18/07/2016

"MERS coronavirus: Ecology, epidemiology and transmission" by Professor Malik Peiris, The University of Hong Kong, Hong Kong

18/07/2016

"Virus diversity in bats: the case of astroviruses and coronaviruses" by Prof Leo Poon, The University of Hong Kong, Hong Kong

18/07/2016

"The biology of the emerging MERS-CoV" by Dr Bart Haagmans, Erasmus Medical Center, The Netherlands

14/07/2016

"Henipaviruses: from discovery to vaccine development" by Professor Linfa Wang, Duke-NUS Graduate Medical School, Singapore

12/07/2016

"Immunology of bats and their viruses" by Prof Tony Schountz, Colorado State University, USA

12/07/2016

"Virus diversity and evolution in bats - Part 1" by Professor Christian Drosten, University of Bonn Medical Centre, Germany

12/07/2016

"Virus diversity and evolution in bats - Part 2" by Professor Christian Drosten, University of Bonn Medical Centre, Germany

07/07/2016

"The multiple faces of activation-induced cytidine deaminase: the DNA mutating enzyme that drives antibody diversity, autoimmunity, and B-cell lymphoma" by Dr Tineke Cantaert, Institut Pasteur in Cambodia, Phnom Penh

16/03/2016

"Cellular dynamics with high temporal and spatial resolution" by Prof Tom Kirchhausen, Harvard Medical School, USA

14/03/2016

"Endocytosis Signaling and Diseases (mostly cancer)" by Prof Pier Paolo Di Fiore, IFOM – Institute of Molecular Oncology, Italy

14/03/2016

"Mechanosensing Through Local Generation and Transduction of Forces" by Prof Michael Sheetz, Mechanobiology Institute, NUS, Singapore

11/03/2016

"An overview of Salmonella pathogenesis: imaging needle formation, regulation and effector secretion" will be given by Prof Linda Kenney, Mechanobiology Institute, NUS, Singapore

11/03/2016

"How Salmonella coopts a pathogenicity island-encoded regulator to control ancestral genes to form biofilms" will be given by Prof Linda Kenney, Mechanobiology Institute, NUS, Singapore

10/03/2016

"Viral usage of host recycling endosome" by Prof Maria-Joao Amorim, Instituto Gulbenkian de Ciência, Portugal

09/03/2016

"Mechanisms of virus entry" by Dr Jason Mercer, University College London, United Kingdom

08/03/2016

"Axonal transport in heath and disease" by Prof Giampietro Schiavo, University College London, United Kingdom

07/03/2016

"Cellular Transport Systems" by Prof Anne Spang, Biozentrum, University of Basel, Switzerland