Professor Peter Simmonds
Professor of Virology
Nuffield Department of Medicine, University of Oxford, United Kingdom

Topic:	**New insights into the recognition of viral RNA by mammalian cells – potential applications in vaccine design**
Date:	**15 August 2017 (Tuesday)**
Time:	**10:30 a.m. – 12:00 noon**
Venue:	**SR3, The Jockey Club Building for Interdisciplinary Research, 5 Sassoon Road**

Synopsis:
RNA viruses have evolved numerous strategies with which to evade interferon-coupled and other cellular defence pathways. Such mechanisms are ultimately dependent on the ability of the cell to recognise viral RNA as foreign through a battery of pattern recognition receptors (PRRs). These re frequently activated by viral RNA that is double-stranded or possesses non-physiological 5'phososporylated ends. In addition to the expression of immune evasion proteins, previous work has demonstrated that viruses may have evolved specific strategies to avoid detection through possessing RNA structured genomes to hide 5' ends and to modify their composition to more closely resemble cellular mRNAs. In particular, a wide range of vertebrate and plant RNA and small DNA viruses suppress genomic CpG and UpA dinucleotide frequencies, mimicking host mRNA composition.

We have explored the effects of compositional changes on virus replication in a variety of virus models, including the picornavirus, echovirus 7 (E7) and influenza A virus. Artificially increasing CpG/UpA dinucleotide frequencies greatly attenuated virus replication through a non-interferon-coupled recognition pathway. We have found that restriction occurred immediately after viral entry, with replication of E7 failing to initiate in the majority of infected cells. It was therefore not dependent on induction of a cellular interferon-coupled antiviral state. Sequences of CpG/UpA-high virus stocks showed no evidence of increased mutational errors that would render them replication defective, nor were these viral RNAs differentially sequestered in cytoplasmic stress granules or capable of inducing a systemic antiviral state. Restriction was not mediated through effects on translation efficiency since replicons with high CpG/UpA sequences inserted into a non-coding region were similarly replication defective. Host-cells thus appear to possess an intrinsic ability to resist replication of viruses with increased CpG/UpA frequencies independently of conventional cellular defence pathways and unrelated to changes in translational efficiency.

Although the mechanism(s) controlling the replication of high CpG/UpA viruses is only partly characterised, the greater visibility of such genomes to the cell can be exploited in vaccine design. Influenza A virus (IAV) with maximised frequencies of CpG or UpA dinucleotides in segment 5 both showed 10-fold attenuated replication in cell culture compared to unmodified virus. Attenuation was also manifested in more extreme form *in vivo*, with 10-100 fold reduced viral loads in lungs of mice infected with 200 PFU of CpG-high and UpA-high mutants. However, both induced powerful inflammatory cytokine and adaptive (T cell and neutralising antibody) responses disproportionate to their replication. CpG-high infected mice also showed markedly reduced clinical severity, minimal weight loss and reduced immmunopathology in lung, yet sterilising immunity to lethal dose WT challenge was achieved after low dose (20PFU) pre-immunisation with this mutant. Increasing CpG dinucleotide frequencies may represent a generic and potentially highly effective method for generating safe, highly immunoreactive vaccines.

A second means to exploit this phenomenon in vaccine design rests on the even more remarkable observation that elimination of CpG and/or UpA dinucleotides in E7 accelerates their replication to levels up to 50-fold greater than wild type virus. Such viruses appear to evade a level of physiological control of virus replication beyond that displayed by normally adapted viruses. In the future, it may be possible to engineer virus seed stocks for inactivated vaccines, such as poliovirus, by CpG/UpA elimination and greatly increase their production yield. In other biotechnology applications, low CpG/UpA reporter and selection genes show greatly enhanced expression and stability in cell culture and *in vivo*.

SCHOOL OF PUBLIC HEALTH
THE UNIVERSITY OF HONG KONG
香 港 大 學 公 共 衞 生 學 院

Professor Peter Simmonds
Professor of Virology
Nuffield Department of Medicine, University of Oxford, United Kingdom

Topic:	**How to Succeed in Getting Research Grants?**
Date:	**15 August 2017 (Tuesday)**
Time:	**2:30 p.m. – 3:30 p.m.**
Venue:	**SR3, The Jockey Club Building for Interdisciplinary Research, 5 Sassoon Road**

Synopsis:

An ability to successfully obtaining grant funding is a key attribute both aspiring young scientist entering a research career and for more established scientists to maintain their research groups. While there is a huge variety in the type of funding available, from junior fellowships to long term programme grants, the success of applications is fundamentally dependent on a demonstration that the applicants are effective scientists and possess the ability to translate funding into solid scientific achievements. In preparing a grant application, there are some important factors to consider and these are all relevant to the success of the grant:

- It is vital to ensure that the grant scheme is appropriate for the application. Factors to consider are whether the funding is at the right seniority level for the applicant and whether the area of proposed research lies within the remit of the funding area.

- The applicant needs to impress the award panel and reviewers that he/she possesses the ability to contribute effectively in the research area. Key elements in an applicant's record that will be scrutinised include: i) publication record of the applicant; ii) does the application build on knowledge and ability gained in previous research? iii) does the applicant have broader experience in standard techniques and research methodologies appropriate for the planned research programme.

- The applicant must be completed to the highest standard. Key attributes that are assessed include:
    - Does the applicant display a firm understanding of the subject area of the application? Close attention must be put to the background review and formulation of research objectives
    - Is the planned research feasible and does it represent an effective and novel way to progress in the area? Application must have specific goals that the reviewers will consider to be achievable within the funding term.
    - Is the application appropriately resourced? Calculation of costs must demonstrate that the planned research in budgeted appropriately, not too much, not too little.
    - Despite the often numerous sections that need to be filled, the key element of a grant application for the reviewers is the description of the research proposal and the resources needed.

As a final comment, I should mention Persistence. Grant schemes often have relatively low acceptance rates and there is always an element of chance in being successful. Applicants should not be put off by rejection if they believe that they have the ability and track record to ultimately succeed.

Bio-sketch:

Professor Peter Simmonds' main current research programme is in the investigation of the evolutionary and functional basis for the pervasive suppression of CpG and UpA dinucleotides in RNA viruses. Modelling evidence he obtained for selection against them in cytoplasmically-expressed RNA sequences demanded functional investigations. The discovery that viruses with high frequencies of CpG/UpA are recognised and suppressed by as yet uncharacterised intrinsic defence mechanisms suddenly opens exciting, new areas of enquiry into innate immunity in mammalian cells. Conversely, accelerated replication of viruses with suppressed frequencies confronts us with a seeming evolutionary paradox which the team will investigate through experimental investigations of relationships between disease processes and transmission dynamics. Enhanced expression of genes with low CpG and UpA frequencies can be exploited as a generic technology in vaccine and vector design.

Other ongoing and recent research has been focused on the characterisation of RNA secondary structure in viruses, and its effect on virus evolution. Discovery of large scale RNA structure in many families of positive-stranded RNA viruses followed the development of large-scale bioinformatic methods to quantify and characterise RNA secondary structure formation in viral genomes. The association of genome-scale organised RNA structure (GORS) with host persistence, and the finding that their genomes are structured in a fundamentally different way from those causing acute infections has been the subject of ongoing investigations of the nature of virus interactions with host cell defences modulated by double-stranded RNA, the physical structure of the predicted RNA structures and the influence of RNA structure as a sequence constraint on the evolution of persistent viruses. This area of research has also initiated a series of functional studies of the role of RNA secondary structure elements in the replication of HCV (Professor David Evans, University of Warwick), caliciviruses (Dr Ian Goodfellow, Imperial College, London) and hepatitis A virus (Professor S. Lemon, University of Texas Medical Branch).

Patrick Manson Building (North Wing), 7 Sassoon Road, Pokfulam, Hong Kong
Tel: 3917 9928 Fax: 2855 9528 Email: kmylau@hku.hk