WELCOME LETTER

Dear Colleagues and friends,

Three years ago, impressed by events such as the emergence of the HIV and HCV epidemics or, more recently, the SARS episode, Christophe Mérieux asked us to initiate a series of workshops in les Pensières to address the risk that represents the fantastic evolutionary capacity of viruses that affect human health and economy. The first symposium, in January 2005, was focused on resistant variants of hepatitis B virus that may compromise our chances of successfully tackling this disease that affects more than 300 million people worldwide. Since then, the growing concern about the emergence of a deadly pandemic H5N1 virus comforted Christophe in his conviction that it was important to gather in les Pensières experts in different fields of virology to share their experiences and opinions on the best ways to fight emerging viruses.

Christophe Mérieux actively participated in the preparation of the programme of this second conference which bears his name and will highlight recent trends in virology.

We will try to draw general lessons from the most recent events in human and animal virology. Basic science sessions will help us understand how emerging viruses penetrate their host cells and replicate, how they develop strategies to interact, trigger or escape the immune defences of their host. Also, how their genetic plasticity allows them to infect new hosts, to become more virulent or resistant to treatments. Our hope is that the presentations made by experts of different viruses will help us to identify common virological or immunological mechanisms.

Two sessions will be devoted to direct applications of basic research in the fields of therapeutic targets and vaccination. It is obvious that the antiviral arsenal is not large enough. Many viral diseases still cannot be treated: respiratory or gastro-intestinal infections, hemorrhagic fevers, encephalitis. Of course, these diseases are generally acute but the example of Tamiflu shows that a drug may significantly reduce morbidity and, in the most severe forms, save lives. Recent successes in the treatment of chronic viral diseases and new and powerful technologies for drug design and screening bring hope, and some of them will be described. Vaccines against viruses have been used with success and may permit the eradication of many of them. However, strategies developed by some viruses make vaccine design very difficult. Presentations will deal with the specific problems of designing an efficient vaccine against an unpredictable pandemic influenza virus or against zoonotic viruses. Both therapeutic and preventive vaccine programs will be addressed in this session. The second example, after HBV, of a successful vaccine against virus-induced cancer involving HPV will also be described.

The changing epidemiology of viral infections, as well as the surveillance tools and the organizations that are necessary for this surveillance, will be the topic of a complete session. It is noteworthy that climate changes have a strong impact on vectors that carry dangerous viruses like dengue virus and hemorrhagic or encephalitis viruses. A large place will be made to speakers from India and far-east Asia, two areas which have to face serious emerging viral threats.

Finally, experts will share their experience in alert and response systems that play an essential role in the preparedness plans to fight and control epidemics and pandemics.

We would like to welcome all of you to the second Christophe Mérieux Conference and we are sure that the quality of the scientific committee, of speakers, chairmen and attendees as well as the great possibilities offered by les Pensières will open the way for a long series of workshops on emerging pathogens.

Guy Vernet - Fabien Zoulim
INTRODUCTION

In the recent years, the virologists community has been confronted to several events that had strong impact on human health worldwide. Several unknown viruses have been discovered. Some viruses have been transmitted from animals to humans. The genetic plasticity of viruses has created viral variants with dangerous pathobiological properties. Recent progresses in the development of antiviral treatments have been jeopardized by the emergence of resistant mutants. Human activities resulting in climatic and social changes have resulted in the emergence or re-emergence of viruses that affect human health, especially arboviruses.

The main objective of the Christophe Mérieux Conference will be to draw general lessons from recent events involving different emerging viruses and to improve the preparedness of the health community through a better surveillance of and response to viral spread and epidemics. Important topics in medical virology will be covered, including viral hepatitis, dengue, influenza, SARS, hemorrhagic fever, and emerging pathogens. All aspects, from basic science (taxonomy, species barrier crossing, pathogenesis, replication, immunology) to surveillance and early warning (including diagnostic and veterinary surveillance), as well as development of vaccines and treatment (including monitoring) will be covered.

This new conference will be the first of a series of workshops focused on this topic. This event will be held in the memory of Dr. Christophe Mérieux, MD, Vice Président Fondation Mérieux, who was deeply committed to the global fight against infectious diseases and has initiated this action.
Christophe Mérieux Conference  
Trends in Virology

SCIENTIFIC PROGRAM

Sunday, June 24

18.00 - 19.00: Introduction  
Alain Mérieux

Plenary Session  
Chair: G. Vernet, F. Zoulim
  • Emerging viral threats to human health in the last 25 years - Ab Osterhaus

19.45: Welcome Reception

Monday, June 25

8.30 - 11.00: Replication and virus entry  
Chair: S. Günther, T. Baumert
  • New insight into Hepatitis C virus entry and regulation of viral replication - Ralf Bartenschlager
  • Protease-mediated entry of SARS-CoV from cell surface; implication in pathogenesis - Fumihiro Taguchi
  • Replication of Lassa Virus - Stephan Günther
  • Structural studies of flavi- and alphavirus envelope proteins: insights for membrane fusion and entry - Félix Rey
  • Kaposi’s sarcoma associated herpesvirus (KSHV/HHV-8) entry, signaling and replication: implication in pathogenesis - Bala Chandran

Round Table  
11.00: Break

11.30 - 13.00: Drug design/screening and new antiviral treatments  
Chair: F. Penin, F. Hayden
  • Discovery of novel inhibitors of hepatitis C virus replication - Johan Neyts
  • Coronavirus proteases as drug targets - Rolf Hilgenfeld
  • The RNA methyltransferase and RNA-Dependent RNA Polymerase of Flavivirus Protein NS5 as drug targets - Bruno Canard

Round Table  
13.00: Lunch Break
SCIENTIFIC PROGRAM

14.30 - 16.15: Virus genome evolution: Mutants, resistance, virulence
Chair: J. Neyts, C. Trepo

• Antiviral drug resistance in Influenza viruses - Frederick G. Hayden
• Hepatitis B virus drug resistance - Fabien Zoulim
• HCV resistance to protease inhibitors - Tara Kieffer
• Zoonoses: modeling hotspots for infectious disease emergence - Peter Daszak

Round Table
16.15: Break

16.45 - 19.15: Surveillance, epidemiology
Chair: X. de Lamballerie, I. Lipkin

• Vector biology and arbovirus transmission is pretty generic - Barry Beaty
• Emerging viral diseases in South-East Asia and the Western Pacific - John Mackenzie
• Pathogen surveillance and discovery in acute and chronic disease - Ian Lipkin
• Molecular epidemiology of hepatitis viruses - Tetsuro Suzuki
• New molecular tools for epidemiology studies and diagnosis - Guy Vernet

Round Table
19.30: Poster Session
20.00: Gala Dinner

Tuesday, June 26

8.30 - 9.45: Immunology and pathogenesis
Chair: D. Lavillette, G. Leroux-Roels

• Innate immunity against flaviviruses - Christoph Seeger
• Exploited defense - How influenza virus hijacks cellular signalling responses - Stephan Ludwig
• Immune control and immunopathogenesis of viral infection - Daniel Pinschewer
10.15 - 13.00: Prophylactic and therapeutic vaccines
Chair: M-L. Michel, J-Y. Bonnefoy

• H5N1 vaccine studies - Geert Leroux-Roels
• Therapeutic vaccines for chronic HBV infection - Marie-Louise Michel
• Prophylactic and therapeutic HCV vaccines - Geneviève Inchauspé
• HPV vaccines - Samir Khleif
• Vaccination against flaviviruses - Christian Mandl
• Hantavirus vaccine development and large scale vaccination in China - Mifang Liang

14.30 - 16.00: Early warning and response
Chair: W-B. Xu, B. Lina

• Web-based infectious disease surveillance: the power of networks - Stephen Morse
• Surveillance and response to emerging diseases outbreak: a WHO perspective - Pierre Formenty
• Molecular epidemiology of Measles viruses in China from 1993-2006 - Wenbo Xu

16.30: Keynote lecture

• The evolutionary biology of emerging and re-emerging viruses - Edward C. Holmes

Conclusion
Guy Vernet, Fabien Zoulim
PLENARY SESSION
SUNDAY, JUNE 24
In the past century, pandemic outbreaks of influenza and AIDS have cost the lives of tens of millions of people. These events were all caused by multiple introductions of animal viruses – influenza A viruses and SIV of birds and non-human primates respectively – into the human population. Besides these introductions causing major pandemics in humans, a large number of other virus infections have spilled over from animal reservoirs to humans or other susceptible species, resulting in considerable morbidity and mortality as “virgin soil” epidemics. The most recent examples in humans are the introduction of SARS coronavirus and influenza A viruses (H5N1 and H7N7) from the animal world, which caused global concern about their potential to be at the origin of new pandemics. Over the last decades there seems to be a dramatic increase in the emergence or re-emergence of virus threats in humans and animals worldwide. A long list of exotic names like Ebola, Lassa, Rift-Valley, Crimea-Congo, Hendra, Nipah and West-Nile is the illustration of names of just some of the places associated with the origin of viruses that crossed the species boundary to humans, with dramatic consequences in the last ten years alone. Similarly, recent mass mortalities among wild aquatic and terrestrial mammals caused by previously known and newly discovered morbilliviruses, as well as outbreaks of hog cholera, foot-and-mouth disease and fowl plague among domestic animals, highlight this trend.

Although improved detection and surveillance techniques, as well as increased media attention may have contributed to our perception of an increase in the incidence of outbreaks of virus infections, it is becoming more and more clear that major changes in our modern society increasingly create new opportunities for virus infections to emerge: a complex mix of changes in social environments, medical and agricultural technologies and ecosystems continues to create new niches for viruses to cross species boundaries and to rapidly adapt to new species. In combating this global threat, we should make optimal use of the new tools provided by the unprecedented advances made in the research areas of virology, molecular biology, immunology, epidemiology, genomics and bioinformatics. Serious investment in these areas in the future will not only be highly cost-effective but will also save many lives of humans and animals. In addition, better collaboration and coordination between all the stakeholders is urgently needed, to establish early warning systems and effective preparedness plans.
SESSION
REPLICATION AND VIRUS ENTRY
MONDAY, JUNE 25
The hepatitis C virus (HCV) is a hepatotropic virus that in 50 – 80% of infections establishes persistence. Although infections are associated with only mild and often no clinically overt symptoms, persistently infected people have a high risk to develop severe liver disease including liver cirrhosis and hepatocellular carcinoma. As a member of the flaviviruses, HCV is an enveloped virus with a single stranded RNA genome of positive polarity. The genome encodes a single polyprotein that is cleaved by cellular and viral proteases into 10 mature products that are required for virus particle formation and amplification of the RNA genome. To the most part, the mechanisms underlying individual steps of the viral replication cycle are not known. This is primarily due to the lack of adequate cell culture systems, which has slowed down HCV research for more than a decade since the first molecular cloning of the viral genome. However, with the advent of the HCV replicon system, HCV pseudoparticles and, most recently, the first system for the production of infectious HCV particles, it is now possible to study the complete replication cycle in a culture dish.

By using these systems several molecules contributing to or essential for HCV entry have been described. Most convincing evidence for an important role in infection has been provided for glycosaminoglycans that probably ‘trap’ HCV particles on the cell surface; scavenger receptor BI that presumably facilitates HCV uptake in a high-density lipoprotein-dependent manner; CD81, a tetraspanin molecule assumed to form oligomeric complexes with other cell surface proteins; claudin-1, a tight-junction protein recently shown to be essential for HCV infection. Thus, a complex interplay between HCV and these cell surface molecules appears to be required for productive entry.

HCV RNA replication occurs in the cytoplasm of the infected cell in a specialized virus-induced compartment designated the membranous web. It is composed of intracellular membranes, most likely derived from the endoplasmatic reticulum, viral proteins and most likely several cellular proteins that all together form membrane-associated replication complexes. Aspects that will be discussed are the biogenesis of the replication complex, its composition and the regulation of RNA translation/replication versus virus assembly.
Protease-mediated entry of SARS-CoV from cell surface; implication in pathogenesis

Fumihiro Taguchi
NIID - Tokyo
Japan

Severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) infection is inhibited by lysosomotropic agents and the infected cells undergo syncytia formation by the treatment of proteases that induce the cleavage of the spike (S) protein. From these observations, Bates et al hypothesized that SARS-CoV takes an endosomal pathway for infection and cleavage of the S protein in an endosome is critical for entry. To confirm their hypothesis, we forced the viruses attached on VeroE6 cells pretreated with lysosomotropic agent and treated those viruses with proteases that induce the S protein cleavage and its fusogenicity. SARS-CoV successfully infected cells under above condition, which indicates that SARS-CoV enters from cell surface, when cell-attached virions are treated with proteases. This observation is not in disagreement with the entry hypothesis proposed by Bates et al. Interestingly, viral infection in the presence of those proteases is much more efficient than the infection in their absence, indicating that the infection from cell surface is more efficient than that via endosomal pathway. Elastase that enhances SARS-CoV infection in cultured cells is a major protease produced in pneumonia, hinting that high replication of the virus in the lung could be attributed to the elastase produced in pneumonia. To explore this possibility, we infected mice with low-pathogenic bacterium Pasteurella that induces elastase in the lung and further with SARS-CoV. This resulted in a severe respiratory disease like human SARS with a 35-90 % of mortality when mouse-adapted SARS-CoV was used. Severe pneumonia was caused by enhanced infection of the virus in the lung, but not bacterial growth. Histopathological similarities were observed between severe pneumonia of mice and human SARS. So far, our findings could suggest that protease, most probably elastase, is an important factor to induce aggravated pneumonia like SARS in mice.
Lassa virus is an RNA virus and belongs to the family of Arenaviridae. It is the causative agent of Lassa fever, a hemorrhagic fever in humans. Molecular mechanisms of genome replication and gene expression are largely unknown. Using a minireplicon system, we characterised the putative 19-nucleotide long promoter at the RNA termini and the RNA-dependent RNA polymerase domain (~500 amino acids) predicted within the 2200-amino acid L protein.

For promoter analysis, each nucleotide of the 3' and 5' ends of the RNA genome analogue was changed into the three remaining residues by mutagenesis. Promoter activity was dependent on specific residues at both termini as well as specific base pairings predictably formed between the 3' and 5' termini, indicating that the functional promoter forms a 3'-5' duplex. Due to their high degree of sequence conservation among the virus family, the promoter ends were chosen as siRNA targets. The siRNA targeting the 5' end of NP mRNA led to reduction of minireplicon activity, while less reduction was seen with siRNAs targeting GPC/Z and L mRNA termini. NP-specific siRNA also inhibited replication of different Lassa virus strains and related arenaviruses in cell culture.

The RNA polymerase domain was characterised by mapping essential amino acids. Potentially relevant residues were identified using secondary structure prediction and data from crystal structures of known RNA polymerases. A novel method was established for generating L protein mutants in large scale by PCR. Three types of mutations were generated and functionally tested: conservative changes in the predicted catalytic sites (motifs pre-A to E); a charged-to-alanine scan throughout the domain, and insertions to search for loop regions (in total ~160 mutants). Northern blot analysis revealed one mutation that selectively impairs RNA transcription but not genome replication. Based on the data, a model of the RNA polymerase domain of Lassa virus is proposed.
Structural studies of flavi- and alphavirus envelope proteins: insights for membrane fusion and entry

Félix Rey
Institut Pasteur - Paris
France

For this meeting I will make an overview of the structural studies on viral envelope proteins involved in membrane fusion carried out in the last ten years or so, and the functional insights that have been extracted from these studies. I will focus in the intrinsic differences of the members of the two structural classes of viral membrane fusion proteins that these studies have identified - classes I and II - and the relations to budding of infectious particles on the one hand, and entry into target cells via membrane fusion on the other.

I will also describe the results of very recent structural studies, showing that the envelope glycoprotein of the vesicular stomatitis virus (VSV) does not belong to either of these two classes, but display features of both of them. In addition, this structure revealed a totally unanticipated homology with the herpes virus envelope glycoprotein gB.

The main emphasis of my talk will be on the structure of class II envelope proteins, from flavi- and alphaviruses, in the acid pH triggered fusogenic conformational change, and in special cooperative interactions among adjacent fusogenic trimers to carry out the membrane fusion reaction.
Herpesviruses encode for several envelope glycoproteins and interact with multiple host cell surface molecules during infection. However, the mechanism by which the herpesvirus-receptor interactions facilitate infection is not well understood. The γ-2 herpesvirus KSHV (HHV-8) is etiologically associated with Kaposi's sarcoma, primary effusion lymphoma (PEL), and multicentric Castleman's disease (MCD). KSHV infects B cells, endothelial cells, macrophages, keratinocytes and epithelial cells both in vivo and in vitro. In contrast to α and β- herpesviruses, KSHV in vitro infection of target cells does not lead to progeny virus formation, and instead, KSHV establishes latency. Infection of primary human dermal microvascular endothelial (HMVEC-d) cells is characterized by the sustained expression of latency-associated genes, transient expression of a limited number of lytic genes with anti-apoptotic and immunomodulatory functions, reprogramming of host cell genes and induction of several cytokines, growth and angiogenic factors. Establishment of latent infection by KSHV thus provides a good in vitro model for studying viral and host factors involved in the establishment and maintenance of latent infection.

The broad cellular tropism of KSHV is in part due to its interaction with the ubiquitous heparan sulfate (HS) proteoglycans. KSHV envelope glycoproteins gB and gpK8.1A interact with HS molecules. KSHV gB possesses the integrin interacting “RGD” motif, and infection of fibroblast or endothelial cells was neutralized by soluble α3β1, αVβ3 and αVβ5 integrins. KSHV or gB immunoprecipitated the α3β1, αV, β3 and β5 integrins from the cell surfaces. Virus binding and DNA internalization studies suggest that these integrins play roles in virus entry. KSHV also utilizes the cystine transporter protein xCT as the fusion-entry receptor in adherent cells, which is a part of the heterodimeric glycoprotein CD98 (4F2 antigen) complex associated with α3β1 integrin. Infection clusters these molecules suggesting that KSHV interacts with a family of functionally related proteins in the endothelial cells to mediate its entry and infection.
Within minutes after infection, KSHV enters the target cells by endocytosis, overlapping with the induction of pre-existing host cell signal pathways, such as FAK, Src, PI-3K, Rho-GTPases, Dia-2, Ezrin, PKC-ζ, ERK1/2 and NFκB. FAK, Src, PI-3K and RhoA play roles in virus entry, and RhoA-Dia2 play roles in microtubule acetylation and transport of capsid in the cytoplasm. Lipid rafts of endothelial cells play roles in KSHV infection and gene expression by modulating KSHV induced PI3-K, RhoA-GTPase, Dia-2 and NFκB molecules. Induction of ERK1/2 is essential for the initiation of viral gene expression, and sustained NFκB induction is essential for the initiation and maintenance of viral gene expression. The p38MAPkinase was activated only during the later time points and Akt was activated in a cyclic manner.

Early during infection of endothelial cells, KSHV also induced several pro-inflammatory cytokines and growth factors, including high levels of cyclooxygenase-2 (COX-2) and PGE2. Latent ORF73 gene expression and ORF73 promoter activity were significantly reduced by COX-2 inhibitors and relieved by exogenous supplementation with PGE2. Ongoing studies suggest that KSHV has evolved to utilize the inflammatory responses for the maintenance of latent gene expression.

Taken together, these studies suggest that KSHV interaction with host cell receptors is evolved, not to be a mere conduit for viral DNA entry, but is evolved to manipulate the signaling pathways and genes of the host cells to create an appropriate intracellular environment that is conducive for the establishment of a successful infection.
SESSION
DRUG DESIGN/SCREENING AND NEW ANTIVIRAL TREATMENTS
MONDAY, JUNE 25
Worldwide over 170 million people are chronically infected with the hepatitis C virus and hence at high risk to develop fatal liver disease. There is no vaccine available and the standard therapy [(pegylated) interferon alfa plus ribavirin] is only effective in 50 to 60% of patients and is associated with important side-effects. The discovery of novel antiviral strategies to selectively inhibit HCV replication has long been hindered by the lack of convenient cell culture models for the propagation of HCV. This hurdle has been overcome first with the establishment of the HCV replicon system in 1999 and, in 2005, with the development of robust HCV cell culture models. The viral serine protease and the viral RNA dependent RNA polymerase have shown to be excellent targets for selective anti-HCV therapy. Clinical studies with a limited number of HCV protease and polymerase inhibitors resulted in encouraging results. However, and not unexpected, preclinical evidence suggested, and clinical evidence confirms that the virus may become rapidly resistant to such inhibitors. Combination therapy of drugs with different mode of action and resistance profiles may thus be required. Alternative strategies, such as the use of non-immunosuppressive cyclophonylin binding compounds with potent anti-HCV activity, may prove important, in particular since such compounds may have a resistance profile that is very different from that of protease or polymerase inhibitors.
Coronaviruses have been in the spotlight since the SARS outbreak of the year 2003. These viruses feature the largest genome of all plus-stranded RNA viruses (27-31 kb of ssRNA). Open reading frame 1 covers 2/3 of the entire genome and codes for two polyproteins, pp1a and pp1ab, the latter of which arises through a ribosomal frameshift during translation. These polyproteins are processed by two or three viral proteases, yielding the non-structural proteins (Nsp1-16) required for virus replication. In most coronaviruses, these cleavage reactions are performed by three cysteine proteases, two of which are of the papain type (PL1\textsuperscript{pro} and PL2\textsuperscript{pro}) and one of the chymotrypsin type (M\textsuperscript{pro}). In SARS-CoV, there is only one PL\textsuperscript{pro}. The main protease of Transmissible Gastroenteritis Virus (TGEV) was the first protein of any coronavirus to have its three-dimensional structure determined [1]. This was followed by the structures of the homologous enzyme from human coronavirus 229E [2] and from the SARS coronavirus [3,4]. Soon it became clear that the main protease is an attractive target for the design of anticonvivial inhibitors. The latest inhibitors from our laboratory will be discussed, and a number of their complexes with the M\textsuperscript{pro} will be presented [2,5,6]. Some of our inhibitors also exhibit an interesting activity against coxsackievirus B3 and norovirus.

References:

The RNA methyltransferase and RNA-Dependent RNA Polymerase of Flavivirus Protein NS5 as drug targets

Bruno Canard
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France

Flaviviruses cause Dengue fever, yellow fever, Tick-borne West Nile and Japanese encephalitis. They are major diseases in many part of the world, with yet unmet medical needs.

The Flavivirus protein NS5 harbors two activities: a methyltransferase (MTase) and the RNA-dependent RNA polymerase (RdRp), unlike the Flaviviridae monofunctional RdRp gene of hepaciviruses and pestiviruses.

The MTase domain can be separated from the RdRp domain, and both exhibit enzymatic activity useful to carry structure function studies. The MTase domain has been crystallized in complex with several RNA-cap analogues and the methylation co-factor S-Adenosyl Homocysteine, providing a hint on the still elusive complete RNA capping pathway at work in (+)RNA viruses. The RdRp domain of dengue virus (DV) and West Nile virus (WNV) NS5 were purified with high yield relative to full-length NS5. Steady-state enzymatic parameters were determined on homopolymeric template poly(rC). The presence of the MTase domain did not affect the RdRp activity parameters. The structures of an active and an inactive WNV RdRp domain were determined to 3.0 Å and 2.35 Å resolution, respectively. They showed that the putative nuclear localization sequences (NLS), long thought to be the linker between the two domains of Flavivirus NS5, form an integral part of the RdRp domain, which shows a typical closed right-hand structure. It was captured in a putative pre-initiation state, where motif F adopts a different conformation and a position perpendicular to its canonical position as part of the NTP entry channel. Residue Trp800 is proposed to be the central part of the thumb-domain priming loop providing the platform for de novo initiation. Based on reverse genetics experiments, using our structure and the known structure of the DV MTase domain, a model of full-length NS5 is proposed.

With such detailed structural and functional models, both the MTase and the RdRp constitute antiviral targets of first choice against Flaviviral diseases.
SESSION
VIRUS GENOME EVOLUTION: MUTANTS, RESISTANCE, VIRULENCE
MONDAY, JUNE 25
Antiviral drug resistance in Influenza viruses

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Switzerland

Single nucleotide changes and associated amino acid changes can lead to high-level antiviral resistance to M2 inhibitors (amantadine, rimantadine) or to neuraminidase inhibitors (oseltamivir, zanamivir) (NAIs). M2 inhibitor resistance emerges frequently with treatment, confers cross-resistance to the class, and is associated with retention of pathogenicity and transmissibility. At present M2 inhibitors are not a reliable intervention due to frequent antiviral resistance in seasonal (H3N2 > H1N1) viruses and often A/H5N1 viruses, such that their use needs to be guided by knowledge of local susceptibility patterns. In contrast to M2 inhibitors, primary resistance is very rare to NAIs, and this class is inhibitory for M2 inhibitor-resistant variants. NAI cross-resistance patterns depend on NA type/subtype, specific mutation, and drug. Many NAI resistant variants show reduced infectivity and virulence in animal models but some are replication competent and transmissible. Recent community surveys have found evidence for low-level transmission of oseltamivir-resistant influenza A and B viruses. Oseltamivir-resistant N1 variants due to a H274Y mutation emerge during therapy and may be associated with virologic and probably clinical failure in H5N1-infected patients, as well as immunocompromised hosts. Viruses with this mutation are less fit in preclinical assays and retain susceptibility to zanamivir. Another mutation N294S that confers reduced oseltamivir susceptibility has also been recognized in several H5N1 and H3N2-infected patients.

Continued monitoring of resistance patterns in both human and animal influenza viruses is essential. Due to increasing resistance among both human and avian strains of influenza, the adamantanes are unreliable in seasonal and have uncertain role in pandemic influenza. The uncertain efficacy of current NAIs in severe human influenza or H5N1 disease, occurrence of oseltamivir resistance, and limitations imposed by zanamivir inhalation indicate the need for parenterally administered agents and alternative antivirals. New agents will provide opportunities for studying drug combinations, coping with the problem of antiviral resistance to current agents, and expanding the therapeutic repertoire for influenza management in the setting of a pandemic event.
Despite the development of new antivirals, the development of HBV resistance to antiviral drugs is becoming a major clinical concern. Indeed, most patients who have been exposed to lamivudine are now infected with HBV drug resistant strains and are receiving second line therapy with adefovir dipivoxil or entecavir. The latter drugs can be prescribed as a first line treatment as well as telbivudine. Other nucleoside analogs are in development such as tenofovir and clevudine. However, the development of resistance is inevitable with any nucleoside analog prescribed in monotherapy.

The selection of drug resistant mutants during antiviral therapy depends mainly on the liver space available for the spread of the mutant and the viral fitness of these polymerase gene mutants. Other important factors are the pharmacodynamic properties of the antiviral compounds and the host immune response against the HBV infected cells.

The characterization of the phenotype of these clinical isolates is therefore critical to gain more insight in their replication capacities and fitness and to determine their cross-resistance profile to tailor therapy to the virological situation. Clonal analysis of the viral quasi-species during therapy and the analysis of the in vitro replication capacity of the main variants in presence or not of antiviral pressure allowed to define models for the selection of the main drug resistant mutants and to provide recommendations for treatment adaptation.

More effort is needed to develop experimental models to define in more detail their fitness and identify new antiviral target to combat and prevent the development of drug resistant mutants as well as the progression of liver disease towards cirrhosis and hepatocellular carcinoma.

References:

Over 170 million people are infected with hepatitis C virus (HCV) worldwide and the current treatment with pegylated interferon (Peg-IFN) and ribavirin (RBV) has limited efficacy and causes significant side effects. Novel, specifically-targeted antiviral therapies for HCV (STAT-Cs) have the potential to revolutionize the treatment of HCV infected patients. STAT-Cs include protease inhibitors, which block the NS3/4A protease-dependent cleavage of the polyprotein, and polymerase inhibitors, which block viral replication. Because HCV replicates at high levels and uses an error-prone RNA polymerase, mutations conferring resistance to virally-targeted inhibitors are generated many times every day. The presence of numerous variants provides a source for the selection of drug resistant virus in patients treated with STAT-Cs. Therefore, it is extremely important to understand how HCV resistant variants could be selected during therapy and what can be done to avoid their appearance. A sensitive analysis for the initial detection and characterization of novel resistant variants is important in the development of all STAT-Cs.

The emergence of drug resistance to any STAT-C that is potent enough to significantly suppress wild-type virus is expected, and limits the use of these drugs in monotherapy. However, as was discovered for HIV, combinations of HCV drugs could potentially limit the risk of viral resistance. Indeed, it was shown that the combination of a protease inhibitor, telaprevir (VX-950), and peg-IFN can suppress both wild-type and resistant variants in two phase Ib clinical trials. Although HIV is probably the best studied model of viral drug resistance, and there are important lessons to be learned from the development and use of HAART, there are also significant differences from HCV that impact treatment strategies and outcome. HIV is a chronic infection requiring life-long treatment to suppress viral replication due to a small pool of latently infected CD4+ resting memory T cells. This latent reservoir also acts as an archive for any resistant virus selected in a patient, limiting treatment possibilities. In contrast, HCV does not integrate into the host cell genome and some infected patients are able to achieve a sustained viral response (SVR) with the current treatment. Therefore, drug resistance may be a problem that is overcome in HCV treatment with the development of clinical strategies involving STAT-Cs in combination with other drugs (Peg-IFN or other STAT-Cs).
Zoonoses: modeling hotspots for infectious disease emergence

Peter Daszak
Consortium for Conservation Medicine
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One of the great challenges of emerging infectious diseases is the frequency with which new pathogens cross the species barrier from wildlife to humans and the seemingly random nature of this process. This is exacerbated by the vast reservoir of animal pathogens from which new emerging zoonoses could originate (the “Zoonotic Pool” of Stephen Morse[1]). In this talk, I will dissect the process of zoonotic disease emergence into a series of analyzable and ultimately predictable steps:

1) The interactions among anthropogenic environmental changes on interspecies transmission of animal pathogens; 2) repeated ‘spill-over’ of animal pathogens into the human population driven by anthropogenic factors affecting contact rates; and 3) the globalized spread of localized outbreaks. Our group has specialized in using a combination of fieldwork, molecular ecology and predictive modeling to analyze these processes for a number of emerging pathogens: SARS CoV, Nipah virus, West Nile virus and H5N1 avian influenza virus. I will highlight these case studies, then present a longterm research strategy to identify the global distribution of “emerging disease hotspots” from which the next new zoonosis is most likely to emerge. This approach has value in targeting surveillance and control measures, and may ultimately provide an approach to predicting the emergence of the next new zoonotic EID.

SESSION
SURVEILLANCE, EPIDEMIOLOGY
MONDAY, JUNE 25
Vector biology and arbovirus transmission is pretty generic

Barry Beaty
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USA

The molecular bases of arbovirus transmission and persistent infections in vectors are poorly understood. Indeed, the vector portion of an arbovirus cycle is still frequently depicted as a black box. Modern molecular and genetic approaches have revealed unexpected determinants of productive arbovirus infection of mosquitoes. We have demonstrated that RNAi constitutes a robust innate immune response in mosquitoes to arbovirus infections. Perturbation of the RNAi response of by silencing Argonaute 2, a component of the RNAi silencing complex, changes Anopheles gambiae from an O’nyong nyong virus resistant to a permissive phenotype. We have also demonstrated that the RNAi response has also modulates vector competence for dengue and LaCrosse viruses, and thus is likely a critical component of vector competence for all arbovirus families. Genetic studies have revealed that several of the components of the mosquito RNAi response map to major QTLs that condition vector competence. We have now exploited the RNAi response to generate transgenic mosquitoes refractory to dengue virus infection. The RNAi response provides new opportunities and targets for control of dengue and dengue vectors. Studies of dengue in Mexico suggest that vector competence is epidemiologically significant and should be included in risk assessment and management schema.
The South-East Asian and Western Pacific regions have provided a number of examples of emerging viral diseases over the past decade and a half, the majority of which have been either vector-borne diseases or zoonoses. Two of the major vector-borne viral diseases are Japanese encephalitis virus (JEV) and Chikungunya virus (CHIKV). JEV has spread westwards into Pakistan and eastwards through the Indonesian archipelago into Papua New Guinea and islands of the Torres Strait of northern Australia. It now threatens to spread into mainland Australia and to other Pacific island territories. CHIKV has re-appeared in various parts of the south-east Asia, following a period of intense activity in islands of the south-west Indian Ocean. Outbreaks have been reported from India, Malaysia, and Indonesia. Other arboviruses, such as Sepik, Me Tri, New Mapoon and Chandipura, remain as enigmatic causes of occasional human disease. Several novel zoonotic viruses have emerged from apparent reservoirs in fruit bat or flying fox populations belonging to the genus *Pteropus* in the suborder *Megachiroptera*. These include Hendra and Nipah viruses, the first two members of a significant new genus, *Henipaviruses*, in the family *Paramyxoviridae*, as well as Menangle and Tioman viruses, two new members of the genus *Rubulaviruses* in the family *Paramyxoviridae*, and a rabies-related virus, Australian bat lyssavirus. Hendra virus first appeared in north-eastern Australia in 1994 as the cause of a fatal respiratory disease in racehorses and humans and a cause of fatal encephalitis in humans. Five years later, Nipah virus was found to be the cause of an outbreak of severe respiratory and/or neurological disease with high mortality in pigs and humans in Malaysia.
The sequence of transmission of Hendra and Nipah viruses was from pteropid bats to a livestock species (horses for Hendra and pigs for Nipah), and then from the livestock species to humans, but no evidence was found for human-to-human transmission. Subsequent outbreaks of Nipah virus disease have occurred in Bangladesh and India with circumstantial evidence to suggest that human-to-human transmission may have occurred for the first time, and that transmission may have not required an intermediate livestock host. Menangle virus has been associated with porcine stillbirths and malformations and with an influenza-like disease in humans, but Tioman virus has not been linked to any human or livestock disease. Australian bat lyssavirus (ABLV) has also been found in pteropid bats and in at least one species of small insectivorous bat in the suborder Microchiroptera; ABLV is closely related to classical rabies, being classified as antigenic type 1, but has not been associated with terrestrial rabies except for two fatal human infections. Of non-zoonotic emerging viruses, the most important regionally have been enterovirus 71 and HIV. The latter is beyond the scope of this brief presentation. Enterovirus 71 has caused several large outbreaks of hand, foot and mouth disease, with some cases of severe neurological diseases. Of most concern, however, has been the emergence of a syndrome of rapidly fatal neurogenic pulmonary oedema and haemorrhage. Most recently, a novel polyoma-like virus has emerged as a cause of respiratory disease.
The roles of heredity and environment as disease determinants have been debated for millennia. The emphasis on one or the other has largely been driven by technology. Despite the periodic appearance of new pathogens, we have likely collected much of the “low hanging fruit” (microbes readily associated with diseases). The challenge now is to understand those disorders that reflect the interaction of environmental factors (microbes, toxins, other stressors) with susceptibility genes.

The objectives of our research program are to investigate the role of infectious agents in the pathogenesis of acute and chronic diseases; dissect the mechanisms by which microbes and host responses result in damage and dysfunction; and find strategies to reduce the burden of morbidity and mortality due to infectious diseases. I will discuss a staged strategy for diagnosis of infection and pathogen discovery, and future perspectives based on global surveillance networks and prospective birth cohorts.
Viral hepatitis exists throughout the world and is a major global public health problem. Here an update on the molecular epidemiology of infections of hepatitis B virus (HBV) and hepatitis E virus (HEV) will be reviewed. Worldwide two billion people have serologic evidence of past or present HBV infection, and 360 million are chronically infected. Approximately 15-40% of infected patients will develop cirrhosis, liver failure or hepatocellular carcinoma. HBV is estimated to be responsible for 500,000-700,000 deaths each year. HBV is transmitted by percutaneous or mucosal exposure to infected blood or other body fluids. HBV transmission has been observed with various forms of human contact: perinatal/mother-to-child, sexual, needle-sharing, and occupational/health-care-related. The prevalence of HBV infection varies markedly in different geographic areas of the world as well as in different population subgroups. DNA sequencing has allowed replacement of the initial serotypic classification of HBV strains by a more systematic genotype system that currently consists of 8 members (genotypes A-H). Recent studies suggest that certain genotypes and some of mutational hot spots in the HBV genomes may correlate with the patient’s immunological and/or disease status.

HEV infection is a major cause of epidemic and acute sporadic hepatitis in many areas of Asia, Africa, and Mexico. HEV is transmitted primarily through the fecal-oral route in contaminated drinking water. In countries where HEV is endemic, the virus is associated with about a half of sporadic acute hepatitis cases. Hepatitis E is self-limited but sometimes has severe complications and a high case-fatality rate, particularly in pregnant women. There are 4 known HEV genotypes (G1-G4). The majority of HEV infections in developing countries in Asia and Africa are caused by G1, one epidemic of G2 infection has been described in Mexico, and infection with G3 and G4 has been reported in several industrialized countries. It has been known that sporadic cases of HEV infection in non-endemic countries are usually associated with travel to areas where the virus is endemic. However, evidence is accumulating that hepatitis E also occurs among individuals in industrialized countries that have no history of travel to endemic countries and that hepatitis E is a zoonotic food-borne disease.
Molecular diagnostics have dramatically changed clinical microbiology, especially the identification and characterization of viruses which can not easily be done using phenotypic techniques. In the recent years, the introduction of real-time detection technologies has improved performances and reliability of nucleic acid testing (NAT). Automation and integration, particularly of DNA and RNA purification has brought simplicity and practicability to clinical virologists. New technologies will allow further breakthroughs such as the simultaneous detection of all pathogens responsible for a given syndrome, automated and high-throughput genotyping of viruses and decentralization of NAT to primary health-care centres and even to patient’s bed side. Such technologies have already been described and some of them are commercially available.

The simultaneous detection of viruses following multiplex amplification can be done using microarrays or particles. Microarrays have also been used to identify clinically relevant mutations and to determine genotypes or subtypes of HIV, HBV and influenza A virus. The throughput and practicability of sequencing, which is the gold standard for genotyping, has also been significantly improved, making it more accessible to virology laboratories. However, NAT still requires highly skilled technicians and relatively complex infrastructures. Several companies have initiated programs for the development of fully integrated NAT systems in which all reagents are ready-to-use in a single sealed cartridge and all steps from specimen preparation to result analysis are automated. Such platforms will open avenues to decentralized molecular diagnosis. The next step would be to design real fields tests, on the model of immunochromatographic assays or lab-on-a-card tests. Such research projects are ongoing but will not result in marketed products before years.
SESSION
IMMUNOLOGY AND PATHOGENESIS
TUESDAY, JUNE 26
Innate immunity against flaviviruses

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Flaviviruses have acquired the remarkable ability to infect and propagate in almost any animal species. For example, West Nile virus (WNV) exists in an enzootic cycle between insects and birds and is transmitted by mosquitoes to mammals including humans. More than five decades ago, Isaacs and colleagues observed that alpha interferon (IFN) could inhibit the production of WNV when administered prior to infection, but was much less effective once an infection has been established (1). Subsequent studies by several laboratories confirmed these results.

With the help of subgenomic WNV replicons, we demonstrated that WNV inhibits the activation of IFN-induced genes. Experiments aimed at understanding the mechanism underlying this inhibition revealed that, in response to IFN, WNV could impair phosphorylation of the Janus kinases JAK1 and Tyk2 (2). Phosphorylation of the kinases is required to activate a signal transduction cascade, resulting in establishment of an antiviral state in the cell. Similar observations with other flaviviruses including Japanese encephalitis virus, dengue virus and Langat virus, a tick borne encephalitis virus, have been reported.

These findings raised questions about the mechanism(s) responsible for the inhibition of JAK1 and Tyk2 phosphorylation. What viral proteins play a role in this process? Using replication competent subgenomic WNV isolates, we identified a mutant with two amino acid changes at the N-terminus of NS4B that replicated like the wildtype, but was sensitive to IFN treatment. Interestingly, infectious WNV genomes with the same NS4B mutations behaved like the wildtype. While our results demonstrated that NS4B carried a determinant for inhibiting IFN signaling, they also invoked a role for structural genes in abrogating the IFN response pathway.
Results from other laboratories based on ectopic expression of individual NS proteins yielded different results. In one study, all WNV NS proteins save for NS1 and NS5 (polymerase) could inhibit the IFN response (3). Another report identified NS4B of Dengue and WNV as the major determinant for IFN resistance (4). Finally, experiments with Japanese encephalitis virus suggested that NS5 is the protein responsible for this function (5). Thus, the mechanism by which flaviviruses divert the innate immune response might be much more complex than previously anticipated.

References:

Exploited defense - How influenza virus hijacks cellular signalling responses

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Cell fate decisions in response to extracellular agents, including pathogenic invaders are commonly mediated by intracellular signaling cascades that transduce signals into stimulus specific actions, e.g. changes in gene expression patterns, alterations in the metabolic state of the cell or induction of programmed cell death (apoptosis). Many of these signaling pathways are also activated in response to influenza virus infections. With regard to the function of these responses the overall picture that has emerged suggests that most of the signaling events are initiated as a cellular response to defend the invading pathogen. While at one hand influenza viruses have evolved strategies to keep these antiviral responses in a tolerable limit there is accumulating evidence that the virus also has acquired the capability to exploit some of the remaining activities to ensure efficient replication. To illustrate this concept, some examples will be discussed with regard to influenza virus induced NF-kappaB and PI3K signaling and how the pathogens take advantage of these activities within the infected cell to support propagation. Furthermore, first approaches to exploit these viral dependencies on cellular factors towards development of novel antiviral strategies will also be presented.

Selected recent publications:

Ludwig (2007) Future Virology 2, 91-100
Ehrhardt et al. (2007) J. Virol. 81, 3058-3067
Ehrhardt et al. (2006) Cell Microbiol. 8,1336-1348
Immune control and immunopathogenesis of viral infection

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Background:
Immune control of viral infection, particularly in vaccinated individuals, relies to a substantial extent on neutralizing antibodies (nAb). Accordingly, persistence-prone viruses have developed strategies to evade antibody neutralization, and a better molecular understanding of the underlying mechanisms will be essential for refining future vaccine strategies.

T cells may also be harnessed for immune protection. Yet, T cell reactions are often intimately linked with immunopathology and "autoimmunity". For many entities in the latter category of diseases, epidemiological observations are suggestive for a viral pathogenesis: Autoimmune diseases are often precipitated by viral infections. Moreover, in genetically susceptible individuals, early childhood infections seem to predispose them to multiple sclerosis (MS) or type 1 diabetes years or even decades before clinical onset. The underlying mechanisms and possible links between the two observations remain, however, unresolved.

Experimental approach and model system:
The prototypic arenavirus lymphocytic choriomeningitis virus (LCMV) and also the closely related high-risk pathogen Lassa fever virus are notorious at inducing very poor and vastly delayed neutralizing antibody responses. Moreover, LCMV has served as a primary workhorse for studying virus-host relationship and immunopathology in persistent infection. We have developed reverse genetic techniques to manipulate the infectious LCMV genome, and have exploited them I) for the investigation of viral determinants of neutralizing antibody evasion II) for delineating a novel concept of virus-induced "autoimmunity".
Immune control and immunopathogenesis of viral infection

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Results:

I) Swapping the glycoproteins (GPs) of LCMV (inefficient nAb inducer) and vesicular stomatitis virus (VSV, potent nAb inducer) transferred the only target of nAb's from either virus to the other. We analyzed the nAb response to each of the two recombinant and parent viruses in infected mice and found that nAb kinetics were solely determined by the viral surface GP and not by the virus backbone. Moreover, the slowly and poorly nAb-triggering LCMV virion was a potent immunogenic matrix for the more antigenic VSV-GP. These findings indicate that the viral GP determines nAb kinetics largely independently of the specific viral infection context. They further suggest that structural features of viral GPs or coevolutionary adaptation of the virus's GP to the host's naive B cell repertoire, or both, may critically limit nAb kinetics and improvement of vaccine efficacy.

II) We observed that the innate immune system of neonatal mice was sufficient to eliminate an attenuated LCM virus from most tissues except for the CNS, where the virus persisted in neurons ("predisposing virus"). Virus-specific cytotoxic T cells (CTLs) were neither deleted nor sufficiently primed to cause disease, but they were efficiently triggered in adulthood upon wild type LCMV infection ("precipitating virus"). This defined sequence of viral infections caused severe CNS inflammation that was histomorphologically reminiscent of Rasmussen encephalitis, a fatal human autoimmune disease. Yet disease in mice was mediated by antiviral CTLs targeting an epitope shared by the precipitating virus and the predisposing virus persisting in neurons (déjà vu). Thus the concept of viral "déjà vu" demonstrates how two related but independently encountered viral infections can cause organ-specific immune disease without molecular mimicry of self and without breaking self tolerance.
SESSION
PROPHYLACTIC AND THERAPEUTIC VACCINES
TUESDAY, JUNE 26
H5N1 influenza viruses are widely considered to be a likely source of the next influenza pandemic. Influenza vaccines are expected to form the main prophylactic measure against pandemic influenza and a vast number of doses will be needed to meet the global demand. Conventional seasonal influenza vaccines against circulating inter-pandemic strains are not expected to protect against H5N1; thus safe and effective H5N1 vaccines are urgently needed.

The principal strategy to develop such vaccines uses reverse genetics to generate attenuated strains which express H5 surface antigen but the yield of vaccine antigen from these strains is typically less than half of that achieved with inter-pandemic strains. Furthermore, avian H5 haemagglutinin appears to be an inherently poor immunogen in humans and antigen doses above the 15µg present in seasonal flu vaccines are required to induce protective antibody levels. Also, because the population is largely immunologically naïve to H5 haemagglutinin, the one dose schedule routinely employed for seasonal influenza vaccines is unlikely to be sufficient. Clinical studies with split-virion or killed whole-virion vaccines based on H5N1, as well as other vaccines based on non-H5N1 avian strains, all indicate that two immunizations appear necessary to elicit the level of immunity required by licensure criteria in individuals who are immunologically naïve to these strains. These factors exacerbate what is already a critical situation with respect to the global manufacturing capacity to supply sufficient influenza antigen for meeting pandemic demand. Therefore antigen sparing is considered to be critical for pandemic vaccine development. Numerous strategies are being explored to reach this goal.

An overview will be given of the different approaches taken by major vaccine manufacturers and detailed results will be presented of the results generated at our Center with GlaxoSmithKline Biologicals’ H5N1 candidate vaccine adjuvanted with a novel proprietary oil-in-water based adjuvant system. In this phase I study 400 adults received, 21 days apart, 2 doses of a A/Vietnam/1194/2004 (H5N1) vaccine, adjuvanted or not, at 4 dose levels (3.8µg, 7.5µg, 15µg and 30µg).
This vaccine showed a good safety profile and induced a high level of functional antibodies (haemagglutination inhibition, neutralization) directed against the H5N1 A/Vietnam/1194/2004 (clade 1) vaccine strain as well as against a drift H5N1 A/Indonesia/05/2005 strain (clade 2) which could potentially cause a pandemic. At the lowest antigenic dose (3.8µg), immune responses for the adjuvanted vaccine against the recombinant homologous vaccine strain (A/Vietnam/1194/2004) met or exceeded all FDA and EU licensure criteria. The cell-mediated immunity (CMI) of the subjects who received 2 doses of either the 3.8µg or the 7.5µg H5N1 vaccine was examined. For this, PBMC obtained before vaccination, at day 21 and day 42, were restimulated in vitro using either the H5N1 split antigen (A/Vietnam/1194/2004, vaccine strain) or a pool of 87 peptides corresponding to the amino acid sequence of H5 haemagglutinin which is conserved between the clade 1 A/Vietnam/1194/2004 vaccine strain and the clade 2 A/Indonesia/05/2005 strain. Influenza-specific T-cells were enumerated by flow cytometry following conventional immuno-fluorescence labeling of intracellular cytokine production. Even when a low dose of antigen (3.8µg) is used, the adjuvanted H5N1 candidate vaccine induces significantly better levels of T- and B-cell memory responses than the unadjuvanted vaccine. Additionally, induction of strong cross-reactive T-cell responses against a drift strain has been demonstrated.

Adjuvantation confers a significant antigen sparing that could increase pandemic influenza vaccine production capacity. In addition, the cross-clade neutralization antibody and T cell responses observed, indicate that the vaccine could effectively prime individuals to respond to either infection or re-vaccination with a related emergent pandemic strain.
Despite effective prophylactic vaccines against hepatitis B virus (HBV) exist since more than 20 years, more than 2.5 billion people worldwide have been exposed to HBV and around 370 million people are chronically infected with HBV. Chronic infection in more than two thirds of infected patients result in chronic liver disease, which may lead to cirrhosis, exposing to non carcinomatous complications and hepatocellular carcinoma. Currently available anti-viral therapies fail to allow a complete control of viral replication in most patients. Because adaptive immune responses are associated with spontaneous resolution of acute HBV infection, therapeutic enhancement of immune responses has been proposed as alternative or supplementary therapy for chronic HBV infection. This include HBV envelope- and nucleocapsid-based vaccines, new formulations for recombinant vaccines and DNA-based vaccines, which are currently evaluated in clinical trials. However, efforts have been hampered by poor proliferation and effector function of HBV-specific T cells. As the immunological defects are proportional to the level of HBV replication, inhibiting virus replication through antiviral treatment before therapeutic vaccination is a new strategy currently under investigation.


Prophylactic and therapeutic HCV vaccines

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Chronic hepatitis C virus (HCV) infection represents an increasing medical and economical burden to societies. In striking contrast to treatment of HBV and HIV infections, the result of therapeutic intervention in HCV carriers is that an overall 40-50% of treated patients successfully resolve infection. Yet, the need for more efficient therapies, including immunotherapies, better tolerated and less expensive, is of obvious worldwide interest as is, in a great number of countries, the development of a preventive vaccine. This talk will outline some major advances reached by the HCV vaccine community in the last 3-4 years.

Data collected in the chimpanzee model unambiguously show that a number of vaccine formulations can control acute infection following experimental challenge. In most cases however, control of viremia appears transient and is often followed by resurgence of low level infection leading to rampant chronicity. Induced T cell responses appear to be key players when they are sustained and broad. Viremia control has been achieved by vaccine formulations solely based on induction of T-cell immunity. Yet, novel assays have also shown that neutralizing antibodies may in some instances contribute to viral clearance. Lessons from other chronic infectious models (HBV) have taught us that a strong pre-existing T cell immunity is associated with increased chances to eradicate viral infection following therapeutic intervention. They have also told us that decrease of viremia prior to therapeutic vaccination (LCMV) provides for a more successful impact of a therapeutic vaccine. Both antiviral agents and vaccine candidates are now available to start validating these concepts in the treatment or prevention of HCV infection.

References:
Youn, J.W. et al. (2005) Sustained E2 antibody response correlates with reduced peak viremia after hepatitis C virus infection in the chimpanzee. Hepatology 42(6), 1429-1436.
Despite the availability of an effective screening test, cervical cancer is still one of the deadliest malignancies worldwide. Epidemiological studies demonstrate that The human papilloma virus (HPV), which is a small, double stranded, DNA virus, is the causative agent of almost all cervical cancers and few of the other types of cancers. Therefore, prevention of HPV infection is crucial for the elimination of this deadly disease. HPV has more than 100 subtypes, the most common of which that cause the development of malignancies are HPV 16 and 18. The recent development of prophylactic HPV vaccine against these two subtypes and its availability on the market formed a major advancement in the control of cervical cancer. However, even if all women were able to have access to the vaccine and that the vaccine is proven to have a long term efficacy, it is likely that cervical cancer will not be eradicated in decades. Therefore, it is of crucial importance that efforts also be placed to develop therapeutic approaches for cervical cancer including the development of therapeutic vaccines. The HPV early gene products provide attractive targets for such therapies.

Cancer vaccines are administered either with a prophylactic or therapeutic intent. The prophylactic vaccines are vaccines that target the Viron and intend to prevent HPV viral infection. The immunologic basis of such a vaccine is humoral antibody-driven. A major body of work has been published on Virus-Like Particle (VLP) vaccines and the FDA has already approved a prophylactic VLP based vaccine at the end of 2006. Prophylactic vaccines will be covered in a different presentation in this book. On the other hand, the therapeutic vaccines are vaccines that intend to treat established lesions, whether pre-cancerous or invasive. These vaccines are designed to target intracellular proteins produced by the virus. As such, they will be directed against E6 and E7 after the integration stage and E2 before integration of HPV into the cellular genome. Accordingly, vaccines directed against the E6 and E7 will target high grade CIN lesions and invasive disease and those directed against E2 will target low and some high grade lesions. Based on that, therapeutic vaccines can be used with the intention of: 1) prevention of the development of precancerous into cancerous lesions and 2) treatment: which would be directed to treat established invasive carcinoma or prevent its recurrence.

We will be discussing the different strategy of HPV vaccines and the state-of-the-art of both the prophylactic and therapeutic vaccines.
Flaviviruses are a group of arthropod-transmitted pathogens of major and ever-increasing, medical importance. Among the most relevant human pathogens are yellow fever virus (YFV), Japanese encephalitis virus (JEV), West Nile virus (WNV), the dengue viruses (DV), and tick-borne encephalitis virus (TBEV). In the absence of any available specific antiviral drugs, disease prevention by vaccination represents the most relevant and effective measurement to combat flavivirus diseases.

In the past, both live attenuated vaccines (against YFV and JEV in China) and inactivated whole virus vaccines (JEV and TBEV) have proven very successful to reduce disease burden. Nevertheless, new vaccination strategies are in big demand and are being developed to meet the many challenges imposed by flavivirus infections. The quick invasion of North America by West Nile virus illustrates the potential of these pathogens to establish themselves in new geographical regions and to infect a multitude of host organisms. The geographical expansion of endemic areas, socioeconomic changes in, and the rising travel activities to such areas steadily increases the number of people at risk of infection. A particular problem for vaccine development is imposed by the difficult immunopathogenesis that is seen with successive dengue virus infections, which has hampered all attempts to develop dengue vaccines. New concepts include non-replicating vaccines such as subviral particles or DNA vaccines, as well as live vaccines which can be produced with various genetically engineered attenuating mutations or also by chimerization. New cell culture produced vaccines against JEV will very soon replace the previously used mouse brain-derived vaccines. While it is very likely that such a “classical” inactivated whole virus vaccine will be efficient and a very satisfactory tool to prevent Japanese encephalitis, new concepts are apparently needed to meet the challenges imposed by other flaviviruses, in particular dengue viruses.

We have explored the possibility of using genetically engineered flavivirus mutants with specific deletions in the capsid protein gene as a new vaccine approach. This method, which so far has been tested for TBEV and WNV allows generating highly attenuated and immunogenic vaccine strains which exhibit a higher attenuation index than observed for any other attenuating principle.

Mutants with very large deletions can be constructed and represent subviral particle-producing replicons, which can be applied as self-replicating RNA vaccines. This approach combines features of classical live and inactivated vaccines and may thus help to overcome the particular challenges imposed by dengue viruses.
Hantavirus (HV), which belongs to Genus *Hantavirus*, Family Bunyaviridae, is single-stranded, negative and segmented RNA virus. There are three segments, named S, M and L, which encode nucleoprotein (NP), glycoprotein G1 and G2, and RNA-dependent polymerase, respectively. Recent data indicated that there are about 30 serotypes or genotypes circulating in nature, and there should be more to be found as the studies putting forward.

Hantavirus infection is becoming a global serious problem in the field of public health with its wide epidemic and the severity of the diseases. It is most serious in China with more than 90% of the worldwide new HFRS cases occurred per year and with the emerging of new foci and some outbreaks. Since 1970, there have been more than 1.8 million HFRS cases worldwide, more than 95% of the cases happened in China, with average 3-5% mortality. There are mainly two types of hantavirus, Hantaan virus (HTN) and Seoul virus(SEO). During these years, Puu-mala virus has also been found in northeast China, though its distribution and pathogenesis has not been identified yet.

The HFRS emergency has promoted the vaccine research and development in the country, and the efforts have primarily focused on inactivated virus vaccines. Since 1996, there are multiple forms of hantavirus vaccines have been developed, licensed, commercial produced and progressed into large-scale vaccination. These vaccines included earlier version of rodent brain derived HTN vaccine, golden hamster kidney cells (GHKC) derived monovalent inactivated SEO virus vaccine, Mongolian gerbil kidney cells (MGKC) HTN vaccines and later version of GHKC or MGKC produced bivalent HTN and SEO vaccines, as well as the most recent version of purified GHKC or MGKC or Vero cell derived bivalent hantavirus vaccines. Other of hantavirus vaccines such us DNA vaccine, virus like particle (VLP) vaccines and so on are still at laboratory level. The vaccines developed and commercialized in China appear to be effective against two most important hantaviruses in Asia, HTNV and SEOV, with and minor side effects. More than about 20 millions peoples were vaccinated during last 10 years , the protective efficacy of each vaccine was determined at beginning after large number of human trial. Four years after the primary vaccination, average preventative rates were more than 90% . So far there is no evidence of antibody-dependent enhancement of infection or diseases. Details will be described in presentation at the conference.
Current concerns about the emergence and spread of infectious diseases have renewed the focus on the critical importance of global early warning and rapid response. However, while considerable progress has been made, many critical gaps remain, including major disconnects between human and veterinary disease reporting. In an attempt to address the fragmentation of disease surveillance systems and the lack of global capacity, ProMED (the Program for Monitoring Emerging Diseases) was founded in 1993 by a group of concerned scientists. In 1994, in order to improve communications among the 60 ProMED Steering Committee members from around the world, members were connected by e-mail (at that time still novel). This rapidly evolved into a prototype outbreak reporting system, ProMED-mail (http://www.promedmail.org). ProMED-mail (PMM), available free of charge and open to all, now has 37,000 subscribers in over 150 countries. Because many important emerging infections are zoonotic, PMM includes both human and animal diseases (as well as plant diseases). The team of moderators/editors, who are subject matter experts, evaluate and edit the outbreak reports sent in by clinicians and public health and laboratory scientists, or identified in the press, and often add additional context. The moderators may also use their own e-mail lists and personal networks for follow-up.

Recently, a number of other systems have begun utilizing the potential of the information and communications “revolutions” to improve surveillance and response. During the SARS outbreak in 2003, the WHO and collaborating scientists made very effective use of e-mail and video teleconferences. The establishment of PMM in 1994 inspired the development of several other systems, including CDC’s “Epi-X” and an e-mail listserv by the Infectious Diseases Society of America.
When PMM started, virtually no press content was available electronically. In the late 1990’s, when news reports became available on the Web, the Canadian government started an innovative limited access system, GPHIN (the Global Public Health Intelligence Network), that electronically searches the Web globally to identify news reports of possible disease outbreaks and other events of concern. In recent years, an increasing amount of useful content on PMM, GPHIN, and other systems is gleaned from press reports and other open sources. The growth of the web, and improved methods for searching, have made such strategies feasible. One promising recent initiative at WHO involves the new International Health Regulations [IHR(2005)], which will require each nation to have a real-time event monitoring system, and strengthened surveillance capabilities.

These efforts have all helped to demonstrate the power of networks and the feasibility of implementing real-time reporting systems at low cost. All these efforts are helping to build the networked and inter-operable surveillance systems that will be needed to deal with the threats in our increasingly globalized and unpredictable world.

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Surveillance and response to emerging diseases outbreak: a WHO perspective.

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Emerging Infectious Diseases (EID) outbreaks often cause serious problems for public health services because of their epidemic potential, the often high case-fatality ratio and difficulties in their treatment and prevention. Outbreaks of EID tend to occur in remote rural areas with limited or non-existent medical and or public health services but have the potential to spread in large metropolitan areas (e.g. SARS, dengue fever/dengue hemorrhagic fever, West Nile) challenging cities capacities in controlling large scale epidemics. Lack of timely laboratory diagnosis and functional epidemiological surveillance, poor infection control practices at health-care facilities, inadequate communication with affected population and weak vector control programs often result in prolonged outbreaks, population suffering and disease spread. 75% of EID being of animal origin, we are threaten not only by pathogens spreading across countries and regions but also by diseases crossing borders between human, domestic animal and wildlife. To ensure Global Health Security, WHO need to develop a system to monitor the pathogens in the 3 worlds. At WHO, the new IHR2005 and the Global Alert and Response Network (GOARN) provides a framework for rapid and efficient interventions during EID outbreaks of international importance.

Forecasting systems based on satellite images, weather/climate forecasting data (e.g. Rift Valley Fever, Hantavirus, CCHF) and/or animal disease surveillance and vector monitoring data (Ebola, Yellow Fever) should be more systematically used to enable the early detection of cases and to allow authorities to implement measures averting impending epidemics. Forecasting of EID outbreaks, as well as risk assessment of possibilities of diffusion to new areas, should be consider as essential tools to enable implementation of effective and timely control measures.

International timely detection and verification of EID outbreak events, such as SARS, Ebola, Nipah, Avian flu, whether they are natural, accidental or deliberately, is based on a systematic gathering of epidemic intelligence and a rapid verification of these events. Surveillance and early detection of EID rely on competent national and regional surveillance systems and on close collaboration and cooperation with wildlife and domestic animal health authorities.
Surveillance and response to emerging diseases outbreak: a WHO perspective.

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A multisectoral and multidisciplinary approach for EID outbreak control have been develop by WHO to ensure that appropriate control measures are taken by affected population and countries but also that progresses in our understanding of new diseases are made in a timely manner to benefit global preparedness:

• **The behavior key.** Response to EID outbreaks requires not only medical expertise but also adhesion to control measures by the concerned populations. The acceptance of control measures, essential element of response operations, remain the fruit of an intense social mobilization based on a technique called COMBI which focuses on influencing behavior change at both individual and community levels. Taking into account the local socio-anthropological background also seems necessary.

• **Biotechnologies for field response.** Pathognomonic signs and symptoms are atypical in infectious diseases, notably for emerging infectious disease, and particularly in early stages when interventional strategies are most likely to be effective. Timely differential diagnosis is often confounded by the absence of tools that allow rapid, efficient testing for a wide range of potential pathogens. Recent developments of the advance BIO-technology fit the need for diagnostic tools that includes probes for viruses, bacteria, fungi, protozoa, helminth and ectoparasites. Panmicrobial array and others biotechnologies should be rapidly field test to improve our capacity for emerging infectious diseases surveillance and outbreak response.

• **More science and Better Care.** Considering the general lack of information on these new diseases, there is a pressing need to improved clinical data collection in order to establish the effectiveness of some treatment measures and to progress in our knowledge on pathogenesis. Post Mortem, that is underutilized, should be performed as appropriate to ameliorate the understanding of the pathogenesis and pathology of emerging diseases. The absence of effective therapies or vaccines for most of EID severely limits monitoring activities. The possible availability in the near future of postexposure vaccines and new “treatments” could change the situation and improve the perception of the medical profession in areas of the world where modern medicine is often lacking.
Christophe Mérieux Conference
Trends in Virology

LECTURE

Molecular epidemiology of Measles viruses in China from 1993-2006

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Measles is an acute respiratory infectious disease that severely threatens children’s health and life, and it is the third viral disease that will be eliminated after the eradication of smallpox and poliomyelitis in the world. This project initiated the molecular epidemiology studies on measles viruses in China during 1993-1994, and a novel wild-type measles viruses clade H, clustered into 2 novel genotypes H1 and H2, was FIRST reported in the world by this project. The H1 and H2 genotypes were adopted by World Health Organization (WHO) for the standard nomenclature of wide-type measles viruses, and were standardized to be used in the molecular epidemiology studies of measles viruses in the world.

Molecular epidemiology of measles viruses between 1993 and 2006 showed the characterization of genetic variation and the geographic distribution of the measles viruses in different years, and revealed that genotype H1 was the predominant indigenous measles viruses genotype in China mainland. Based on the genetic characterization of nucleoprotein (N) gene and Hemagglutinin (HA) gene of Measles viruses, genotype H1 could be further clustered into 3 sub-genotypes, H1a, H1b and H1c. The pattern of circulation and epidemic of H1a measles viruses became more predominant in the recent years, while H1b viruses became the minority, and H1c viruses was no longer circulating in China since 1995.

450 nucleotides at the C-terminal of the N gene, which was the most variable region in H1 genotype measles viruses, were targeted for molecular epidemiology studies of measles viruses in China, and based on this, the protocol for genetic characterization of wild-type measles viruses was standardized.

Through the 14-years molecular epidemiological studies, 3-level laboratory network (1 National Measles Laboratory, 31 provincial Measles Laboratories and 331 prefecture Measles Laboratories) was established, and the protocols for specimen collection, cell culture, viral isolation and genotyping were standardized in the laboratory network. The protocol for molecular epidemiology studies on measles viruses was standardized and expanded to be used in provincial measles laboratory network in China. The genetic variation of genotype and sub-genotype of wild-type measles viruses circulating in each province was identified, and scientific strategy was provided to further accelerating control and elimination of wild-type measles viruses in each province.
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This project is the only one that systematically developed and standardized molecular epidemiology studies on measles viruses in China, and it is also the only one that performed consecutive molecular epidemiology surveillance of measles viruses in countries with endemic measles transmission in the world. In this project, resources of measles isolates and measles gene bank with native Chinese characterization were collected; a number of laboratory data of molecular epidemiology of measles viruses was accumulated. These data provided a technique platform for scientific, quick and effective distinguishing indigenous viruses from imported wild-type measles viruses during measles elimination stage, and it could provided scientific technique guarantee for eliminating wild-type measles viruses in China in 2012, and also it could provided important measles viruses resources, gene resources and laboratory network resources for measles control, prevention and elimination, screening candidate of vaccine strains and establishing the strategy of the regional elimination program in the world.

The protocol for molecular epidemiology of measles viruses and the resources in the laboratory network established in this project played important roles in other viral diseases surveillance systems, and drove the establishment of other viral diseases laboratory network and the studies on molecular epidemiology.
The evolutionary biology of emerging and re-emerging viruses

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Despite the public health burden due to emerging viruses, little is known about the evolutionary processes that allow viruses to jump species barriers and establish productive infections in new hosts. Understanding the evolutionary basis to virus emergence, and whether generalities exist in this process, is therefore a key research goal in the study of infectious disease. Herein, I discuss the evolutionary biology of viral emergence, set within the conceptual framework of fitness landscapes provided by population genetics, and the possible reasons why some viruses may be more likely to emerge than others.

I will explore this problem both theoretically, and by considering a number of illustrative case studies, namely dengue virus, West Nile virus and influenza virus, each of which provides a different window on the process of emergence.

Alarmingly, there is still a lack of definitive data on many key aspects of viral emergence, particularly whether this process routinely requires adaptation to the new host species during the early stages of infection, or is in part a chance process involving the transmission of a viral strain with the necessary genetic and phenotypic characteristics. I will conclude by showing how systematic studies of genetic and phenotypic diversity within individual hosts, a critical aspect of viral fitness, are particularly notable for their absence.
Christophe Mérieux Conference
Trends in Virology

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