Are viral proteins good targets for antiviral intervention?

In the session “Antivirals and Resistance” antiviral drug susceptibility in Europe during the last ten years was broadly discussed. On the one hand, the studies presented by Rong Hai and Emi Takashita on two different influenza virus subtypes illustrate the increasing resistance against NA inhibitors that we are currently facing worldwide. On the other hand, Cécile Helene Herbreuteau suggested an alternative antiviral strategy using polyclonal immunoglobulins to treat H5N1 infected patients, to make viral escape more difficult.

Antivirals buy time between pandemic onset and the availability of vaccines. Currently, two groups of drugs to treat influenza virus infections are available: M2 channel blockers and NA sialidase inhibitors. However, the usage of M2 inhibitors is no longer recommended due to high occurrence of drug resistance. Rong Hai from the Icahn School of Medicine at Mount Sinai in New York investigated the fitness of an H7N9 influenza A virus isolate bearing the NA-R292K resistance mutation. He could show that replication in primary human tracheobronchial epithelial cells, in a mouse model, and the transmissibility in the guinea pig model was not affected by this mutation. The presentation given by Emi Takashita from the Influenza Virus Research Center in Japan highlighted the viral resistance of pandemic H1N1 influenza A virus from 2009 (pH1N1) against four NA inhibitors (oseltamivir, peramivir, zanamivir and laninamivir), which are all commonly used in Japan. She found cross-resistance to oseltamivir and peramivir in a community cluster of a pH1N1 viruses isolated between November 2013 and February 2014. Sequence analyses show that this virus is closely related to viruses isolates from other regions of the country suggesting a clonal spread of a single mutant virus.

As the previous talks indicate more monitoring and screening for viral resistance is needed to better control and assess the effectiveness of antiviral drugs. Adam Meijer from the National Institute for Public Health and the Environment in the Netherlands presented the strategies of the WHO to monitor antiviral susceptibility in Europe since 2004. The collection of this data was mainly based on the activity of WHO National Influenza Centers and the ERLI-Net in Europe whose number of participating laboratories is still further increasing. Following the talks on increasing resistance of influenza viruses against antiviral inhibitors, Cécile Helene Herbreuteau from Fab’entech in France introduced the clinical efficiency of anti-H5N1 polyclonal immunoglobulins as an alternative antiviral intervention. Herbreuteau’s results including safety and pharmacokinetic data from phase I clinical trial and cross-reactivity data on 21 H5N1 strains demonstrate the neutralizing efficacy of polyclonal immunoglobulins. Although, this therapeutic approach is already used in some Asian countries and will soon be available in France, the long-term effects still need to be evaluated. In summary, during this session a nice overview on monitoring activities for antiviral susceptibility were given and the urgent need to identify new antiviral targets and to develop new antiviral strategies was demonstrated.
There will be a new pandemic and we are still unprepared

Will there ever be a new influenza pandemic? The simple answer is ‘yes’. Only the ‘when’ remains uncertain. And are we prepared? There the answer is a ‘no’ or a ‘not as good as we should be’. At this moment the revised WHO pandemic approach doesn’t really push preparedness forwards, since some mechanisms still remain unclear.

One thing is certain, seasonal flu preparedness leads to a better pandemic preparedness and vice versa. Clio Sellwood, who is pandemic FLU Lead NHS, UK, explained why it is important to build on already well imbedded systems. “Also in a pandemic, we need to deliver the vaccine in a way patients are familiar with. And lessons learned from the pandemic must inspire our seasonal flu approach.”

The H5N1 strain has already been proven lethal. Must we be concerned? Yes, we undoubtedly should be. H5N1 is a highly pathogenic virus. “The critical question though is why H5N1 didn’t cause a pandemic”, said Colin Russell from Cambridge University, UK. Only a handful of mutations are necessary to make H5N1 airborne transmissible in ferrets. Thirty percent of H5N1 variants that have been sequenced might even only require as few as three mutations to become airborne. In order to assess risks more precisely, we need improved surveillance in regions where mutations are already prevalent, Russell said.

And what about H7N9 and other looming flu threats? Sander Herfst, from the Department of Viroscience, Erasmus MC Rotterdam, The Netherlands, echoed the concluding remarks of Colin Russell. H7N9 already exhibits three of the properties that are needed for H5N1 to gain transmissibility between humans. There is currently little knowledge of the determinants of transmission of respiratory viruses, and further knowledge is needed to augment our understanding, he said. Herfst recommends the improvement of surveillance studies and epidemiological investigations by including simple virus phenotyping essays. Herfst also advocated a gain-of function approach in addition to other means of studying virus transmission.

How well are we prepared for the next pandemic? In the aftermath of the H1N1-pandemic, WHO revised its pandemic response strategy. Diane Gross, WHO Regional Office for Europe, Denmark, explained the how and why. The revision is inspired by demands for national flexibility. The new approach encourages this flexibility by basing national actions on risk assessment, resources and needs. But at the same time, some mechanisms remain unclear, as remarked by someone in the SPI track session. For example, vaccines will not be ready on time unless there is an effective mechanism informing companies to switch production from seasonal to pandemic vaccines. Obtaining clarity is urgent.

Do we have a problem? What is necessary to put the pandemic threat on the agenda again? Robert Dingwall from Dingwall Enterprises Ltd, UK, investigated the obstacles to influenza preparedness from a sociological point of view. “A pandemic is not just a public health problem”, Dingwall said. It presents a risk for the entire social, economic, and political system, and should be dealt with at the highest levels of government. The unknown nature of a pandemic destabilizes our trust in human contact in a way that is independent of the actual risk. A pandemic also creates a vacancy for moralization, Dingwall said.
Many roads lead to evolution

The session on Genetics and evolution of virus and host provided food for thought that perplexed even session chair Peter Palese (Mount Sinai School of Medicine, USA). From the evolution of highly pathogenic avian influenza viruses to antigenic drift, reassortment and semi-infectious particles, many roads lead to evolution, making our quest for predictions as tricky as it can get.

Jürgen Stech (Friedrich-Loeffler-Institut, Germany) presented two independent mechanisms for the evolution of highly pathogenic avian influenza viruses. He demonstrated that beyond the well-characterized polybasic cleavage site of the HA protein, the other influenza virus proteins can contribute to a high pathogenicity phenotype in chickens. Using an H5N1 strain, he showed that HA polybasic cleavage site and NA stalk deletion were a minimum set of virulence determinants leading to high pathogenicity. But interestingly, the combination of all but the NA gene of the studied highly pathogenic avian influenza virus and the NA gene of a low pathogenicity variant also maintained the reassortant’s high pathogenicity profile, despite lacking the NA stalk deletion.

Bjorn Koel (Erasmus MC, The Netherlands) presented the antigenic drift of influenza A (H1N1) and B viruses using the popular antigenic cartography method, and identified the substitutions responsible for their antigenic change. Punctuated antigenic drift characterized both influenza A (H1N1) and B viruses, with single substitutions located near the receptor-binding site responsible for the transition between antigenic clusters. Comparing his results to his previous study on the antigenic drift of influenza A H3N2 viruses, Koel observed slower antigenic and genetic evolution of influenza A H1N1, and even slower evolution of influenza B viruses.

Anice Lowen (Emory University, USA) reminded the audience that because of its segmented genome made of 8 genes, two parental influenza viruses can produce 256 different types of progeny upon co-infection, including 254 reassortants. A time course experiment in guinea pigs shed light on the intra-host evolution of genomic diversity as a function of the inoculation dose, completing her earlier study on the frequency of unbiased reassortment in vitro and in vivo. Later on in her presentation, Lowen explored the role that Defective Interfering Particles (DIPs) may play in favoring reassortment. DIPs are semi-infectious particles that cannot initiate productive infection unless co-infection with an infectious virus occurs, resulting in complementation of the defective genome. However, because of interference, co-infection was less frequent in cells infected with DIPs than in cells infected with infectious viruses, resulting in fewer infectious reassortants.

Lowen argued that other semi-infectious particles might favor reassortment, argument taken up by Chris Brooke (NIAID, USA) during his talk on incomplete gene segment packaging. He showed that influenza virus populations consist primarily of semi-infectious particles failing to express one or more gene products, and requiring complementation to propagate. The production of semi-particles varies widely across influenza strains yet may contribute to evolution. Using an example based on the serendipitous evolution of a PR8 variant with a single substitution in the NP gene that increased replication levels and contact transmissibility in guinea-pigs, he showed that the packaging and expression of NA was selectively decreased due to intersegment interactions. This resulted in an increase in the production of semi-infectious particles favoring co-infection, reassortment and complementation of semi-infectious particles for effective propagation.
‘Vaccibodies’ as a novel vaccine platform

Though many speakers had speculated on the subject of novel influenza vaccines, Tuesday was the first day of the meeting that included sessions focused directly on developing influenza vaccines. In the first of two consecutive afternoon sessions, the subject ‘novel vaccine platforms’ was extensively discussed.

Novel influenza vaccine platforms

With two out of four speakers presenting research on Modified Vaccinia Virus Ankara (MVA) vaccines, novel vector-based vaccines very much dominated the session. However, to balance out the session, an interesting vaccination study in mice was also presented. This presentation showed the use of a novel DNA structure (‘vaccibodies’) that could be specifically targeted to professional antigen presenting cells.

MVA-based vaccines

The first speaker of the session, Thomas Friedrich, showed that vaccination with MVA vaccines expressing influenza HA or NP induced a very potent homologous humoral immune response in rhesus macaques. Heterologous antibodies and influenza-specific cellular immune responses could not be detected, however the macaques in this study were partially protected from challenge with a heterologous virus. This heterosubtypic immunity was attributed to antibody-dependent cellular cytotoxicity (ADCC). The next speaker, Rory de Vries, showed that an MVA-H5 vaccine was capable of inducing potent humoral and cellular responses – at least in preliminary experiments – in a randomized clinical trial, both against homologous and heterologous H5 viruses. The two MVA talks were followed by an interesting discussion chaired by Ab Osterhaus and co-chaired by Natalie Pica, focusing on the contrasting observations from these two studies.

‘Vaccibodies’

Gunnveig Grødeland switched gears completely to describe another type of novel influenza vaccine, the so-called ‘vaccibodies’, a novel type of vaccine that could be administered as DNA or protein. The ‘vaccibodies’ consist of multiple subunits (see figure). These include a targeting domain, which directs the molecule specifically to different types of antigen presenting cells and an antigenic domain, which contains the vaccine antigen. Both subunits are coupled together via a dimerization unit.

Using HA and NP as antigenic units, Grodeland showed that these ‘vaccibodies’ could be tailored to induce a particular type of adaptive immune response, by specifically targeting different surface molecules on antigen presenting cells. Interestingly, this novel vaccine platform was capable of inducing protective immune responses in mice, demonstrating proof-of-concept.

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The Heinrich Pette Institute (HPI), located in Hamburg/Germany, is dedicated to basic research of the biology of most-relevant human pathogenic viruses and the pathogenesis underlying the respective virus-induced diseases. The institute’s long-established mission is to provide new technologies and solutions to improve therapeutic procedures for established and emerging viral diseases. These include AIDS, Influenza and Hepatitis as well as certain types of cancer linked to infections with Herpes-, Polyoma- and other DNA-Viruses.

The institute has created several advanced technology platforms that facilitate comprehensive investigation of the infection processes, including a High-Throughput Sequencing Facility, BSL2/BSL3 Small Animal Models and advanced Microscopy and Image Analysis. Research projects at the HPI comprise multi-disciplinary approaches and are closely linked with research groups in the Hamburg metropolitan region as well as other national and international collaboration partners.

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