Session 5: Molecular Virology and Gene Therapy

Lectures

L5.1

Conformational flexibility in the receptor-binding site of the hepatitis C virus glycoprotein E2

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The hepatitis C virus (HCV) glycoprotein E2 is the major target of neutralizing antibodies and therefore highly relevant for vaccine design. Its structure features a central immunoglobulin (Ig)-like β-sandwich that contributes to the binding site for the cellular receptor CD81. This presentation will focus on our recent studies that demonstrate that the Ig-like domain is present in two different conformations on infectious cell-culture derived HCV particles (HCVcc) and surrogate retrovirus based pseudoparticles (HCVpp). This conformational flexibility in one of the major neutralization epitopes characterized to date may contribute to the immune evasion of hepatitis C virus and - in this respect – shifts the current paradigm of antigenic sites targeted during chronic viral infections such as HCV. Moreover, such conformational plasticity of the HCV E2 receptor binding site has important implications for immunogen design.

L5.2

100 years from Spanish flu - can we control influenza today?

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The so-called "Spanish" flu of 1918/19, caused by an influenza A virus of the subtype H1N1, still ranges at the top of devastating human epidemics in recent history associated with rapid global spread and high mortality. Meanwhile three further influenza pandemics (1957/8: H2N2; 1968: H3N2; 2009: H1N1) have swept through the global human population. Human losses in the course of these epidemics have gradually declined, attributable probably in large parts to the wide availability of antibiotics and huge advances in intensive care compared to 1918. Deciphering the origins, modes of generation and spread of human pandemic influenza viruses and improving preventive and therapeutic measures have been a major focus of infectiological research ever since the Spanish flu. Influenza A viruses not only infect human being but instead expand to and evolve in a wide and intricate network of various avian and mammalian host species. In fact, metapopulations of aquatic wild birds have been identified a reservoir of all influenza A virus subtypes known to date. Accidental spill-over transmission from this reservoir into mammalian hosts may spark the emergence of new influenza A virus lineages that perpetuate in a new mammalian host species independently of the previous avian reservoirs. Contribution from avian origin influenza A virus has been documented for all pandemic viruses since 1918. Improved control of human influenza therefore must involve better understanding of influenza A virus ecology and epidemiology on a broad scale; this is a classical OneHealth issue. Recent research developments, perceived and true risks and advancements along these lines will be illustrated.

L5.3

Adenovirus vectors for genetic vaccination and oncolytic virotherapy

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Viral vectors are commonly used as vehicles delivering genetic material into cells. Adenovirus vectors (Ad) have been extensively used in gene therapy preclinical trials and over 500 clinical trials, representing over 20% of all gene therapy clinical trials worldwide. They are considered promising agents for both anti-cancer therapy and genetic vaccinations.

Genetic vaccines based on Ad vectors are potent inducers of broad immune responses. In preclinical studies on HCV vectored vaccine we observed both humoral responses and very high T-cell responses induced by Ad injection. Ad vectors have been tested in a number of clinical trials, including vaccinations against HIV, HCV, Ebola virus and malaria. Currently, simian adenoviruses are of great interest, as they can overcome host anti-Ad neutralizing immunity – the most significant limitation of Ad-based vaccines.

In recent years, adenovirus vectors have been evaluated as very promising anti-cancer agents. Selectively-replicating oncolytic adenoviruses can directly kill tumor cells without affecting healthy cells. In addition to tumor cytolysis, they create "pro-immune" environment that induces innate and adaptive anti-tumor immune responses. Thus, they hold capacity to promote vaccination against the whole tumor.

Posters

P5.1

BMV-based VLP – faster, cheaper, easier

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Recent studies indicate that plant viruses have a lot of advantageous features that predestinate them to be used as nanocontainers for efficient and site-specific drugs or diagnostic reagents delivery. Brome Mosaic Virus (BMV) is a model plant virus which has been commonly used in many types of research including trials of loading its capsid with different types of cargo. However the efficient production of BMV-based nanocontainers demands large amount of high purity virions. Usually BMV capsids are obtained through virus propagation in plants and their subsequent extraction and purification. Herein we describe an alternative and effective method of BMV-based VLPs (Virus-like particles) production, based on a bacterial expression system. The BMV coat protein is synthesized with a fusion protein partner which significantly improves its solubility. An additional benefit of VLPs obtained in bacterial culture is the absence of viral RNA, a part of the virus which excludes further medical applications. The method is fast and inexpensive and it can be easily scaled up. To improve VLPs formation we also propose modifications in capsid protein that should result in higher stability of the empty capsid due to stronger protein–protein interactions.

P5.2

The role of viral glycoprotein B and exosomes in the formation of antiviral immune response during alphaherpesvirus infection

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Alphaherpesviruses, members of the *Herpesviridae* family, belong to the most widespread human as well as farm and wild animal pathogens. Interestingly, during infection alphaherpesviruses can interfere with the host immune system and establish a latent infection.

Recent studies demonstrate these occurs with the participation of herpesviral glycoproteins, such as glycoprotein B (gB). gB is one of the most important herspesvirus proteins, mediating fusion during viral entry into the cell. There are also indications for the role of extracellular vesicles (EVs), such as exosomes, in the formation of antiviral immunity. Based on the evidence that human herpes simplex-1 (HSV-1)gB targets major histocompatibility class II processing pathway for immune evasion, this viral protein was chosen for construction of cell lines constitutively expressing HSV-1, bovine herpesvirus-1 and suid herpesvirus-1 gB.

Proposed immunomodulatory function of gB requires utilization of EVs biogenesis pathway. Analysis of EVs samples purified from stable cell lines and virus-infected cells, confirms that exosomal incorporation of gB is its conserved feature. Tested gB homologs differed, however, in their ability to interact with MHC II, reduce their surface levels and secrete the complex via EVs. Additionally, gB could also serve as a decoy for virus-neutralizing antibodies the exact mechanism of these phenomena remains to be elucidated.

P5.3

In search for crucial amino acid residues in the bovine herpesvirus-1-encoded UL49.5 involved in the inhibition of transporter associated with antigen presentation (TAP)

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Varicellovirus-encoded UL49.5 protein is a potent inhibitor of transporter associated with antigen processing (TAP), helping viruses to avoid recognition by cytotoxic T cells. Bovine herpesvirus 1 UL49.5 encodes a small, type I transmembrane protein acting as TAP inhibitor in two ways: causing conformational arrest within the transporter and inducing its degradation. The exact mechanism of UL49.5 activity remains elusive. Established 3D structure of UL49.5 protein was used to determine potentially active sites of the protein. We designed mutations within N-terminal UL49.5 residues: P48, 52PPQ54 and the 30RRE32 motif.

UL49.5 variants were expressed in human melanoma MJS cells transduced with retroviruses. Additionally, UL49.5PPQ/GGG and, as a control, previously published UL49.5RRE/AAA variant were introduced into BHV-1 genome. In constructed cell lines or infected cells we tested UL49.5 variants ability to block TAP function as well as cause TAP degradation. These observations were correlated with the 3D structure determination of the mutants by NMR in dodecylphosphocholine (DPC) micelles and molecular dynamics.

While UL49.5 RRE motif seemed redundant for MHC class I downregulation, 52PPQ54 mutations resulted in a massive loss in the protein function. What is more, in contrast to the 30RRE32/AAA mutation, which had moderate effect on the structure of the N-terminal domain, proline-hinge mutations affected structure of the whole protein.

P5.4

Fluorescent TAP as a model for virus-induced degradation of the antigenic peptide transporter

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The transporter associated with antigen processing (TAP) plays a pivotal role in MHC class I-restricted antigen presentation. This makes TAP an attractive target for viruses that aim to escape the immune system. During co-evolution with their hosts, herpesviruses have specialized in TAP inhibition via diverse mechanisms. Bovine herpesvirus-1 (BHV-1) encodes a UL49.5 protein, that is unique in its ability to target TAP for proteasomal degradation following a conformational arrest. To investigate the mechanism of TAP removal, a TAP-GFP fusion protein was instrumental, yet GFP-tagging may affect the susceptibility of TAP to UL49.5-induced degradation. Therefore, we constructed a series of TAP1 and TAP2 variants carrying either N- or C-terminal GFP with helical or unstructured linkers. Mel JuSo cells with CRISPR/Cas9 TAP1 or TAP2 knock outs were reconstituted with fluorescent TAP1 or TAP2 variants. Cellular distribution, functionality and susceptibility for BHV-1 infection-mediated degradation were assessed. Fluorescent TAP model was also used to re-evaluate TAP stability in the presence of other well-characterized viral TAP inhibitors-CPXV012 of cowpox virus, ICP47 of herpes simplex virus-1 or US6 of human cytomegalovirus. CPXV012, ICP47, or US6 were introduced into the cells using retroviruses and TAP-GFP levels were analyzed by flow cytometry. Only UL49.5 was able to reduce TAP levels, whereas CPXV012, US6, and especially ICP47, increased TAP-GFP fluorescence.

P5.5

Comparative proteomic analysis of the latex of virus infected and non-infected medicinal plant Greater Celandine (*Chelidonium majus* L.)

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Medicinal plant Greater Celandine (*Chelidonium majus* L.) belongs to *Papaveraceae* family. Its milky sap comprises latex-related exudate, which is rich in biologically active substances, like alkaloids, flavonoids, phenolic acids and defense-related proteins with antipathogenic activities. The aim of the study was to compare protein profiles of the latex of a healthy and virus-infected plant obtained using two-dimensional electrophoresis (2-DE) and mass spectrometry. Plants were inoculated with a suspension of potyvirus strains (PVY Ny/LW) and their latex was collected, from which proteins were isolated using TCA/acetone method. After analysis of 2-D profiles the differentiating proteins were selected and identified using tandem mass spectrometry (MS/MS). The results showed that the latex of infected plants was more abundant in two types of proteins identified as isoforms of major latex protein (MLP28) and heat shock protein. On the other hand the latex of non-infected plant showed lower abundance of phosphoglycerate kinase involved in basic metabolic reactions. The proteins with higher abundance after infection belong to pathogenesis-related (PR) protein family. These proteins are associated with plant defense response against viral infection, so they have possible antiviral properties, thus increasing the curing potential of *C. majus*.

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