Meeting Report:

Adenoviruses - from pathogens to therapeutics

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Abstract

The 10th International Adenovirus Meeting was held at Umea (June 13-17 2012), organized by G. Wadell, N. Arnberg and K. Isaksson. It gathered almost 140 scientists from Europe, North and South America, Asia and Australia. The meeting gave an overview of the state of the art of adenovirus research. Here, we highlight the advances in molecular and cell biology of adenoviruses, in particular virus structure and entry, replication, pathology and immunology, evolution and tropism of vectors. We conclude with an outlook into future developments in the field.

Introduction

The study of adenovirus biology has led to seminal discoveries in mRNA splicing (Nobel Prize to Sharp and Roberts in 1993, Sharp, 1994), the first cell-free DNA replication system (Stillman, 1983), and fundamental insight into antigen presentation to T cells (Burgert *et al.*, 1985). These and many other advances in adenovirus molecular and cellular biology and epidemiology laid a strong foundation for cancer research, immunology, and genetic engineering. Notably, adenoviruses (AdVs), whose members include >55 human types, are the most widely used vectors in clinical gene therapy fields. The types are classified into seven species, HAdV-A to G, mainly based on hemagglutination assays and genome sequences (Harrach *et al.*, 2011). Together with other mammalian adenoviruses, these HAdVs form the genus *Mastadenovirus*, distinct from the aviadenoviruses infecting birds only. Two other genera include AdVs from more diverse hosts. Atadenoviruses infect reptiles predominantly, but occur also in birds and ruminants, whereas the host origin of the genus *Siadenovirus* is unclear as yet. The only known piscine AdV alone represents the most recently approved sixth genus *Ichtadenovirus.*

Structure and entry

A major quest in public health has been to find out how viruses cause disease. To solve this complex issue investigators classically employ reductionist's approaches and break the problem down into small units with specific solutions. The ultimate aim of this line of approach is to determine the atomic structure of the virus. This knowledge is primordial because it links major processes of propagation: entry, assembly, egress and spreading.

The presentation from V. Reddy (The Scripps Research Institute, La Jolla, USA) gave new glimpses into the largest crystal structure of biomaterials solved so far, a human adenovirus type 5 from species HAdV-C (HAdV-5) containing the short fiber from HAdV-35 at 3.5 Å resolution (Reddy *et al.*, 2010). This structural analysis extended earlier cryo-EM studies (Fabry *et al.*, 2005, Liu *et al.*, 2010), and showed that the virus has a T=25 icosahedral symmetry, made up in large parts by the major

virus protein hexon. The new study assigned the 4 helical bundle on the capsid exterior to a segment of IIIa protein, which was previously assigned to the C-termini of IX by the cryo-EM studies. While both IIIa and IX stabilize the hexon shell on the capsid exterior, VIII and yet to be identified vertex proteins stabilize the hexon shell on the interior. The determination of the precise locations of the cementing proteins helps better understand how the virus is held together and how the double-stranded DNA genome is connected to the capsid.

Many of the connections of the major capsid proteins to the 'glue' proteins are broken in a stepwise manner during virus entry (Greber *et al.*, 1993). These connections determine if the viral DNA is deposited in the cell nucleus or degraded. A disassembly-defective HAdV-2 mutant, ts1 (temperature-sensitive 1), unable to deliver DNA into the nucleus is non-infectious (Imelli *et al.*, 2009). Ts1 lacks viral protease and contains immature non-processed 'glue' proteins. Remarkably, the outside structure of HAdV-2 and ts1 are identical as suggested by cryo-EM analyses at 10Å resolution (Silvestry *et al.*, 2009). The viruses interact with the same receptors, the coxsackievirus and adenovirus receptor (CAR) and alpha v integrins.

G. Nemerow (The Scripps Research Institute, La Jolla, USA) presented atomic force microscopy (AFM) experiments showing that the 5-fold axis of Ad5/35F has lower tensile strength than the 2-fold or 3-fold axis of the virus. Together with W. Roos and colleagues at the University of Utrecht (The Netherlands) they showed that alpha defensin HD5 binding to the 5-fold axis stabilizes this region of the virus while binding of integrins to the same region loosens the 5-fold structure. These studies provide a link between the mechanical properties of the virus and the disassembly process. Whether the physical properties of HAdV-2 and ts1 are different will be interesting to see in the future, as it might give an indication on the impact of host factors, such as integrins, defensins or blood factors on virus stability. It will also be interesting to see, how the flexibility of ts1 contributes to the resistance of this virus to the cellular uncoating cues that trigger the stepwise disassembly process of the wild type virus.

M. Suomalainen (University of Zurich, Switzerland) presented the identification of cellular cues, which promote the uncoating of wild type HAdV-2 or 5. These cues are the drifting motions of CAR-bound virus on the cell surface, counteracted by the

stationary motions of the alpha v integrin co-receptors (Burckhardt *et al.*, 2011). The drifts are acto-myosin dependent. Together with integrins these motions trigger the shedding of the fibers and subsequently the exposure of the internal capsid protein VI, which appears to be the factor that disrupts endosomal membranes (Moyer *et al.*, 2011). C. San Martin (Spanish National Center of Biotechnology, Madrid, Spain) presented data from cell-free HAdV-2 uncoating experiments and suggested that a unique vertex structure is opened before the virion eventually falls apart. It can be speculated that the compaction of the viral core into the capsid and subsequent maturation steps of the core by the viral protease would lead to an increase of internal capsid pressure, which then might facilitates uncoating steps of the virus (Perez-Berna *et al.*, 2012). Whether such uncoating steps are modulated by posttranslational modifications of the HAdV-2/5 particle, for example phosphorylations as discussed by S. Lind (University of Uppsala, Sweden), remains to be demonstrated.

In addition to providing insight into stepwise disassembly during entry, a high-resolution structure may also determine the arrangement of the hyper-variable (HV) loops of hexon. These HV loops are recognized by neutralizing antibodies and blood factors and influence the residence time of the cell-free virus in an organism, for example in the blood system of the mouse (Waddington *et al.*, 2008). Work by A. Baker and colleagues (University of Glasgow, UK) showed that coagulation factor X (FX) is important for liver tropism of blood-exposed HAdV-5 in rodents. FX binds to the HAdV-5 hexon, not fiber, via an interaction between the FX Gla domain and HV loops of the hexon surface leading to heparansulfate-proteoglycan engagement. FX binding influences multiple HAdV types, but not canine type 2 (CAdV-2) (A. Bradshaw, University of Glasgow, UK). Baker suggested that liver gene transfer is mediated through a heparin-binding exo-site in the FX serine protease domain. A. Byrnes (Food and Drug Administration, Silver Spring, USA) showed that when the HV loops of hexon were genetically altered such that blood factors could no longer bind, these altered viruses were recognized by natural antibodies and the mouse complement system and subsequently degraded.

Receptors and trafficking

It is presently unknown if adenovirus types, which use different receptors than CAR use the same cues to expose their protein VI as HAdV-2/5, or other mechanisms. Towards answering this question, receptor identification is a crucial step. U. Greber (University of Zurich, Switzerland) presented evidence that HAdV-3/7 (species HAdV-B) use the membrane cofactor CD46 as an attachment receptor, provided that CD46 is abundantly present to allow avidity binding to the fiber proteins of the virus (Trinh *et al.*, 2012). A. Lieber (University of Washington, Seattle, USA) extended these studies and showed that HAdV-3 also uses human desmoglein 2 (DSG2) as a receptor (Wang *et al.*, 2011b). DSG2 is localized on the basal and lateral membrane domains of polarized epithelial cells and involved in cell adhesion. A recombinant, self-dimerizing knob of the HAdV-3 fiber bound to DSG2 and inhibited infection (Wang *et al.*, 2011a). Lieber also presented a transgenic mouse model expressing human DSG2, and suggested that the HAdV-3 fiber knob can be used to open the junctions between epithelial cells, and thereby enhance the therapeutic efficacy of adenovirus vectors in transducing epithelial cells, and possibly cancer targeting therapeutic antibodies (Wang *et al.*, 2012). Besides CAR, CD46 and DSG2, there may be additional receptors for particular HAdV types, such as glycoproteins or glycolipids carrying sialic acid residues (Nilsson *et al.*, 2011), as discussed by R. Storm (University of Umea, Sweden), or other soluble components that provide a link between the virus and the cells, as discussed by M. Nygren (University of Umea, Sweden). The biological relevance of these low affinity receptors remains to be determined. Efforts at the University of Umea are under way to develop sialic acid derivatives as anti-virals against adenoviruses causing epidemic keratoconjunctivitis, as pointed out by N. Arnberg (University of Umea, Sweden).

Another central question has been how some viruses infect polarized cells, as these cells can be both gates and barriers to hosts. Paradoxically, many virus receptors are not present at the apical plasma membrane facing towards the outside. K. Excoffon (Wright State University, USA) using transfection and overexpression studies suggested that a low abundant splice variant of CAR is targeted to the apical domain of polarized canine MDCK cells, and this enhanced HAdV-5 infection from the apical side (Kolawole *et al.*, 2012). Only a fraction of CAR is apically expressed, but this small fraction can be reduced by overexpression of MAGI-1 (membrane-associated guanylate kinase with inverted orientation protein-1**)** and can be

enhanced by MAGI-1 down-regulation. It will be interesting to see if MAGI-1 is suppressed by cytokines, such as interleukin (IL)-8, which increase apical CAR levels in co-cultures of human epithelial cells and macrophages and boost apical infection with HAdV-2/5 (Lutschg *et al.*, 2011). It will also be interesting to analyze the trafficking routes that direct CAR to the apical plasma membrane domain in epithelial cells. S. Salinas (Institut de Génétique Moléculaire de Montpellier, France) discussed CAR trafficking in neuronal cells. Her results suggested that CAR engagement by CAdV-2 or its fibre knob induced virus trafficking from Rab5-positive to neutrally charged lumens of Rab7-positive vesicles in axons (Salinas *et al.*, 2009). This implies that virus-induced internalization of CAR can lead to CAR and virus cotransport. CAdV-2 appears to escape in the neuronal cell body while CAR is degraded.

Work from C. Wiethoff (Loyola University, Maywood, USA) suggested that the rupture of endosomes by incoming HAdV-5 in epithelial cells elicits danger signals (Barlan *et al.*, 2011). Wiethoff also suggested that the membrane lytic protein VI is required for the dissociation of the virus from broken endosomes, by virtue of its PPxY motive, and interactions with ubiquitin ligases. A mutant HAdV-5 lacking PPxY appeared to be cleared by autophagy. Wiethoff reported that the knock-down of the autophagy-related protein ATG5 fully restored the infectivity of HAdV-5 lacking the PPxY motive, suggesting that the PPxY motive had no additional functions for infection besides evasion of autophagy during cell entry. H. Wodrich (University of Bordeaux, France), however, suggested that the PPxY motive of the incoming protein VI was also required to release host-mediated repression of adenoviral early gene expression (Schreiner *et al.*, 2012). This was in some disagreement with the presentation of M. Suomalainen who reported that incoming protein VI was degraded within 2-3 h post infection, that is, before or at the onset of early viral gene expression. The latter was also in agreement with earlier reports (Greber *et al.*, 1993, Burckhardt *et al.*, 2011). Based on protein VI overexpression studies Wodrich et al. also suggested that protein VI displaces Daxx (death domain-associated protein) from PML-NBs, and that this was dependent on the PPxY motive, which facilitated interaction with Nedd4 ubiquitin ligases (Schreiner *et al.*, 2012). Surprisingly, the repression of Daxx by protein VI and activation of adenoviral early promoters could be mimicked by structural proteins from unrelated human viruses, such as human

cytomegalovirus (HCMV) or papillomavirus, and protein VI overexpression activated the early HCMV promoter. Wodrich suggested that capsid proteins contain trans-activating properties for viral gene expression. Whether these properties function during entry or replication, or if they work at high or low copy numbers of viral genomes remains to be determined. Regardless of the mechanism, further studies will be needed to explore how the trafficking of viral genomes relates to the way the genomes are detected by the cellular surveillance systems.

Transcriptional regulation and replication

An important mechanism of host interference with viral infection is transcriptional repression, as emphasized in the presentation of S. Schreiner (Heinrich Pette Institute, Hamburg, Germany). The Daxx protein located in promyelocytic leukemia nuclear bodies (PML-NBs) may be a negative regulator of adenovirus gene expression at low multiplicity of infection (Schreiner *et al.*, 2010). Schreiner et al. suggested that Daxx/ATRX (X-linked α-thalassemia mental retardation syndrome protein) and SWI/SNF (SWItch/Sucrose NonFermentable) chromatin remodelling complexes play a role in regulating viral gene expression. ATRX acts as the core ATPase subunit, while Daxx recruits histone deacetylase, and leads to transcriptional repression of viral promoters (Schreiner *et al.*, 2012). The early viral E1B-55K protein inhibits Daxx functions, and targets ATRX for proteasome degradation depending on the cullin E3-ubiquitin ligase, thereby counteracting host cell chromatin remodelling functions (Schreiner *et al.*, 2011). It is currently unknown if the impact of E1B-55K on the transcriptome profile is comparable to the large-scale epigenetic impact of the immediate early transcriptional regulator E1A (Ferrari *et al.*, 2008, Horwitz *et al.*, 2008), and how E1B-55K affects viral replication.

Adenoviral replication is a high fidelity process, in part due to the proof-reading features of the viral DNA polymerase. R. Hoeben et al. (Leiden University Medical Center, The Netherlands) presented an elegant series of experiments to make the viral DNA polymerase more error prone by some >50-fold, and therefore similar to some retroviruses. They introduced mutations in the nucleotide binding pocket and the exonuclease domain of the polymerase, and stably expressed the error-prone polymerase in a cell line. Polymerase-deleted viruses were amplified and screened

for features, such as improved growth. One virus with enhanced cytolytic activity contained mutations in the splice acceptor site preceding the adenovirus death protein, and induced greater plaque sizes, which was interpreted as increased cytolytic activity. A potential clinical application could be better oncolytic vectors.

Another important aspect of genome regulation is alternative splicing. G. Akusjarvi (University of Uppsala, Sweden) showed data to indicate that the late viral splice site regulatory protein L4-33K, which controls alternative splicing of the viral L1 transcript, is phosphorylated by DNA-dependent protein kinase (DNA-PK) and protein kinase A (PKA) (Tormanen Persson *et al.*, 2012). Phosphorylation by DNA-PK inhibits while phosphorylation by PKA enhances L1-IIIa alternative splicing. This toggle switch mechanism report suggested new roles for DNA-PK and PKA in alternative RNA splicing.

Assembly and egress

Two assembly models are currently debated in the field, a capsid intermediate model where a putative ATPase pumps the viral DNA into a preformed empty capsid shell, and a capsid-DNA co-assembly model driven by the self-assembly properties of capsid proteins. The work presented by W. Mangel (Brookhaven National Laboratory, New York, USA) favored the self-assembly model. He proposed that one-dimensional sliding of the viral protease along the AdV genome allows the protease to scan across the nascent capsid and thereby cleave the capsid and core proteins. Such a scanning process bypasses potentially inefficient 3-dimensional diffusion of the protease within the capsid, which is rich in DNA and protein. It will be important to determine how the protease slides along the viral chromatin, or if the protease preferentially slides on protein-free DNA.

After encapsidation, the newly assembled viruses exit the cell by a lytic process. Computational modeling and wet lab experiments were presented by A. Yakimovich (University of Zurich, Switzerland) to indicate that cell-cell transmission of replication-competent GFP expressing HAdV-2 occurred by passive transfer of infectious viruses which included advection and diffusive mass transfer (Yakimovich *et al.*, 2012). This implies that the contribution of lytic cell-free transmission to viral

infection spread between cells can be predicted by measurements or calculations of the virus diffusion constants in aqueous medium, and viral spreading can be computationally modeled.

Pathology and immunology

Advances in this field were mainly reported on three levels, virus interactions with immune cells, viral genes controlling host immune responses, and genetic analyses of host factors in animal models. M. Bouvier (University of Illinois at Chicago, USA) presented a crystallographic analysis of the interaction of HAdV-2 E3 19K glycoprotein with the class I major histocompatibility complex (MHC) HLA-A2 for antigen presentation. Class I MHC molecules bind peptides generated upon degradation of cytosolic proteins by the proteasome or exogenous proteins by cross-presentation, and present these peptides to cytotoxic T cells at the cell surface. Bouvier discussed how HAdV-2 E3 19K blocks antigen presentation to T cells. T. Bru et al. (Institut de Génétique Moléculaire de Montpellier, France) addressed the question how adenovirus immune complexes affect the anti-viral response of dendritic cells (DC), in particular the maturation of immature DCs. It was found that shRNA mediated knock-down of nucleic acid pattern recognition receptors (PRRs) reduced DC maturation, while knock-down of the inflammasome pathway enhanced DC maturation. For example, knock-down of Toll-like receptor 7 (TLR7) or retinoid-inducible gene 1 protein (RIG1) reduced tissue necrosis factor (TNF) alpha secretion. This suggested that HAdV immune complexes differentially modulate DC maturation by interacting with innate sensors, and thereby link innate and pre-existing immunity to tweak T-cell immunity.

J. Radke (Loyola University and Hines VA, USA) reported interesting findings about apoptotic mimicry of AdV. A canonical function of apoptosis is to repress proinflammatory responses of macrophages and hence minimize overall inflammation. It is well known that HAdV infected cells develop severe cytopathic effects (CPE), which is more akin to necrosis than apoptosis. Nonetheless, the CPE corpses appeared to suppress proinflammatory cytokine production from macrophages, for example IL-6, which is downstream of nuclear factor kappa B (NFkB) activation. Radke suggested that clinical isolates of HAdV-14 (species

HAdV-B), which causes acute respiratory distress syndrome (ARDS) (Metzgar *et al.*, 2007), express less E1B-19K protein than prototype strains, and failed to repress NF-kB dependent transcription. Genetic knock out experiments further showed that the immune suppression depended on the viral early protein E1B-19K, which is a homologue of the anti-apoptotic Bcl-2 protein. E1B-19K hence appears to have immune suppressing effects on macrophages, and these effects may require macrophages CPE-corpses contacts. Further explorations of the cell biology of CPE corpses are likely to reveal more of the intricate details of AdV immune modulations. It will also be interesting to see if E1B-19K variations dictate the severity of ARDS. H.G. Burgert (University of Warwick, UK) described a novel secreted form of an E3 protein of 49 kDa from the keratoconjunctivitis-associated HAdV-19a (species HAdV-D), termed E3/49K. E3/49K appears to be unique to species HAdV-D, and binds to uninfected cells of the lymphoid lineage but not fibroblasts or epithelial cells. Functional studies suggested that E3/49K suppresses NK cell-mediated lysis of infected cells, and hence may contribute to HAdV-D pathogenicity.

A mouse genetics approach to uncover host genes involved in AdV pathology was described by K. Spindler (University of Michigan, Ann Arbor, USA). Animals susceptible to mouse adenovirus 1 (MAdV-1) develop encephalitis and lose the blood brain barrier (BBB) integrity due to direct virus-induced functional impairment of vascular endothelial cells (Gralinski *et al.*, 2009). Virus-induced loss of BBB integrity appears to be astrocyte-dependent and involves the matrix-metalloprotease MMP9. The MAdV-1 susceptibility locus *Msq1* on chromosome 15 was involved in BBB breakdown upon MAdV-1 infection. Interestingly, *Msq1* contains a number of *Ly6*-related genes that encode cell surface expressed proteins, some of which are interferon-induced. Spindler reported that the *Ly6* genes are also susceptibility factors for other viruses, including HIV, Marek's disease virus, and West Nile virus.

New adenovirus based vaccines

The strong potential of adenoviruses to elicit CD8+ and CD4+ T cell responses in animal models has spurred major efforts to develop adenovirus vectors for vaccinations in humans and animals. Because E1-deleted vectors from HAdV-2/5 are capable of inducing strong global immune responses, it has become the *de facto*

gold standard. While revisiting this notion, several presentations suggested that AdV types with low pre-existing immunity in humans (e.g. types isolated from animals), preferentially and efficiently infect DCs. For example, HAdV-19a, or surprisingly the less immunogenic helper-dependent vectors may thus be better inducers of protective immunity. It is a priority to clarify the underlying mechanisms of AdV immunogenicity. Presentations from A. Hill, M. Dicks (Oxford University, UK) and S. Colloca (Okairos, Rome, Italy), M. Barry (Mayo Clinics, Rochester, USA), as well as P. Holst (University of Copenhagen, Denmark), F. Kreppel (University of Ulm, Germany), and H.G. Burgert (University of Warwick, UK) outlined exciting data with human and chimpanzee adenovirus (ChAdV) vectors (ChAds) expressing antigens from malaria, human immune deficiency virus or hepatitis B or C viruses either alone or in combination with a heterologous booster regime with MVA (modified vaccinia Ankara) strain or a different adenoviral vector using the same antigen. Anti-malaria phase IIa vaccination trials against liver- and blood-stage antigens of the parasite indicate that CD8+ T cells have been elicited against the parasite by both species C and species E ChAd vectors (Colloca *et al.*, 2012, Sheehy *et al.*, 2012). J. Richardson (INRA, Maisons-Alfort, France) reported that antigens encoded by HAdV-5 vaccine vectors were preferentially presented to CD8+ T lymphocytes by the CD8alpha+ DCs (Suleman *et al.*, 2011). With a better, yet still incomplete understanding of how T- and B-cell responses are generated and sustained, the vaccine field is now moving forward to harness the power of innate responses.

New isolates, evolution and diversity

Adenoviruses have been known for nearly 60 years (Rowe *et al.*, 1953), yet new family members continue to be identified in humans and animal hosts. This is possible in part because partial or full genome sequences can be obtained without having to isolate the virus in tissue culture. A direct sequencing approach is different from the classical serological typing tests, such as cross neutralization studies, and there have been debates in favor of or against using the direct sequencing or serology for identifications of new adenoviruses (Seto *et al.*, 2011, Aoki *et al.*, 2011). Arguments in favor of direct sequencing include a rapid identification and information release, albeit at certain risks, such as to be defined quality criteria and information how many nucleotide differences define a new virus type. Two participants, J. de

Jong (Erasmus University, Rotterdam, The Netherlands) and D. Seto (George Mason University, Manassas, USA) presented opposing views on this issue. De Jong mentioned that sequence information is important for clinical virology and research but he believed that the new molecular type designation system is not relevant in clinical virology or research. de Jong argued that the HAdV types 52, 53, 54, 55 and 56 identified by sequencing were either not typeable by classical serology, or crossreacted with sera from previously recognized HAdVs, such as type 8, 11, 15, 22 or 29. He proposed that claims for new types should not be approved without establishing the corresponding serotype. Seto on the other hand argued for the necessity of considering the entire genome sequence as an excellent marker for evolutionary events and a basis for typing, and including the hexon and fiber sequences corresponding to the epitopes. To this end, Seto et al. completed the full genome sequencing of every prototype HAdV strain, and have established a typing algorithm, which aims to include the sequences supporting serological features. Regardless of the method used to identify new virus types, members of both camps agreed that it is of utmost importance that the available types are adequately preserved and made available to the research community at impeccable quality. In addition, J.R. Brister (NCBI NLM NIH, Bethesda, USA) argued in favor of an expert group that would take the responsibility for defining guidelines and demarcation criteria.

Two colleagues from the groups of K. Aoki and H. Watanabe (Hokkaido University, Sapporo, Japan) illustrated the usefulness of genome sequencing. S. Yamane showed that full genome sequencing was instrumental to detect a contaminant virus in the HAdV-8 stock of the American Type Culture Collection. G. Gonzalez presented a comprehensive comparative genome analysis of 26 viruses from HAdV-D. The aim was to define conserved sequences that can facilitate homologous recombination and characterize the frequently recombined regions. This may help distinguish between new types and recombination variants. Comparisons of full genome sequences have also been applied in the characterisation and identification of adenoviruses of veterinary importance, such as poultry AdVs, as presented by A. Marek (University of Veterinary Medicine, Vienna, Austria). Marek spoke about her recent experience in fowl adenovirus genome analyses using Illumina high-throughput sequencing. In addition, several new adenovirus species were

discovered by PCR and sequencing in a number of wild living, exotic animals in the past three years. M. Benkő (Institute for Veterinary Medical Research, CAR, Hungarian Academy of Sciences, Budapest) suggested that some of these viruses might represent a new genus. The size and contents of the early regions at the ends of the linear viral genome show great diversity among AdVs classified into different genera. This may indicate the existence of an additional alternative replication strategy compared to the canonical HAdVs and mastadenoviruses.

G. Gonzalez presented a poster showing an alternative method for molecular typing, based on the genetic diversity of the loop-1 region of hexon from 51 established HAdV serotypes and including analyses of the recombinant new types.
Additional posters from the M. Benko and B. Harrach groups (Institute for Veterinary Medical Research, CAR, Hungarian Academy of Sciences, Budapest) presented genome analyses of an unusual bovine AdV (E. Kovács), the detection of novel adenoviruses in representatives of European bat species (M. Vidovszky) and different captive reptiles (J. Pénzes). G. Kaján presented sequences from the fowl adenovirus types presently circulating in Hungary. Finally, P. Steer (The University of Melbourne, Victoria) presented a comprehensive study about the pathogenicity of fowl adenoviruses isolated from outbreaks of inclusion body hepatitis in chickens, a disease having a significant economic impact on the Australian poultry industry.

Future developments

The meeting gave insight into a wealth of different adenovirus types, and how they navigate cells in culture and the host. A major challenge is still to translate the basic biology to serve human health. This will require the development of model systems, for example differentiated cells, organ cultures, and small animal models, which can replicate human or animal AdVs, as well as nonhuman primates and clinical studies. Additional approaches will be developed to better characterize virus-host interactions, for example ‘omics approaches to correlate features from transcriptomes, proteomes, metabolomes or lipidomes with infection phenotypes. All these developments will further promote the use of adenoviruses in cancer and cardiovascular therapies, and vaccine development. Personalized medicine may become a cornerstone for translational approaches and lead to better virotherapies, as emphasized by D. Curiel

(University of Washington, St. Louis, USA). Vector developments will continue to generate new tools tailored for innovative treatments. This may lead to further enhancements of the immunogenicity of adenovirus vectors, for example by increasing T-cell numbers (Dicks *et al.*, 2012).

Finally, there is a growing need for anti-adenovirus drugs, in particular for immune-suppressed patients receiving hematopoetic stem cell and solid organ transplants. Conspicuously, the titers of HAdV (especially HAdV-2) have been observed to rise in such patients. The available polymerase inhibitor Cidofovir is insufficient to control these viral bursts, as pointed out by A. Heim (University of Hannover, Germany). Drugs designed to inhibit AdV splicing (Bayo-Puxen, Institut de Génétique Moléculaire de Montpellier) or replication (Oberg *et al.*, 2012) were also described. Whether these compounds make their way into the clinics remains to be seen, however. In summary, we anticipate that the field continues to provide deep mechanistic insights into basic infection biology, and thereby increase rational applications of vectors in gene delivery and vaccinations, and new anti-viral strategies.

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