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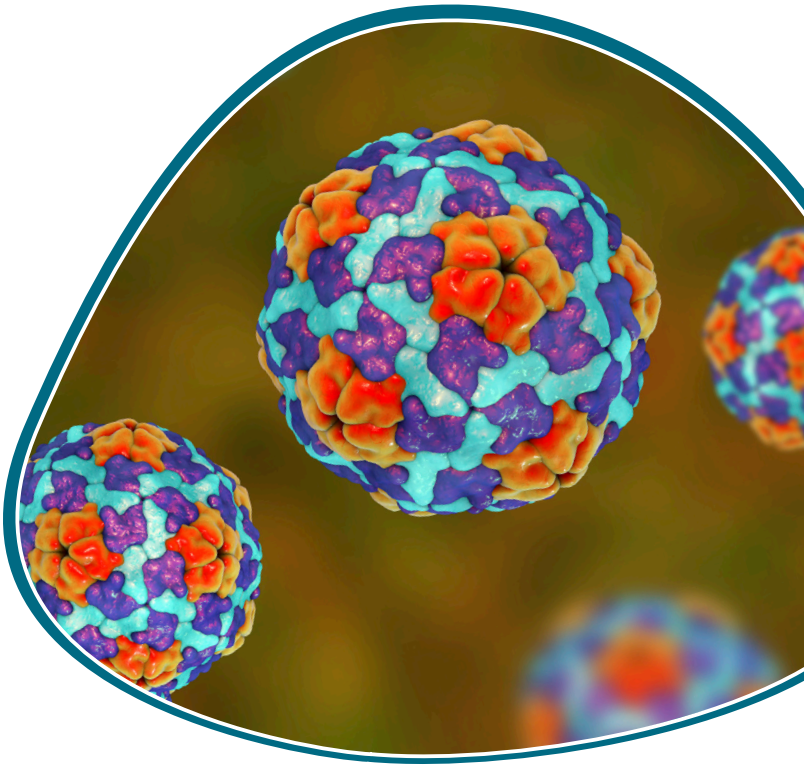
European Study Group on the Molecular Biology of Picornaviruses



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POSTER ABSTRACT BOOK

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Virus epidemiology and emergence

P01

Temporal and Spatial dynamics of Enteroviruses from Guatemalan sewage

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Abstract

Background: The Global Specialized Polio Laboratory at CDC collaborated with Guatemala's National Health Laboratory to monitor the emergence of vaccine-derived polioviruses and possible importation and transmission of wild polioviruses (WPVs) through environmental surveillance.

Methodology: Sewage samples from 6 sites in central Guatemala were collected monthly between April 2020 and September 2021, shipped to CDC, and concentrated. Murine fibroblast L-cells and Rhabdomyosarcoma cells were used for the isolation of polioviruses following a standard algorithm. Additionally, viruses were isolated on HLF cells, which were sensitive to non-polio-enteroviruses, which were detected by Illumina sequencing on a MiSeq platform, followed by sequence analysis and typing.

Results: Echoviruses 1, 11, 13, 25, 33; Coxsackievirus A13, A20, A24, Enterovirus (EV) C99, and oral poliovirus vaccine strains (OPV types 1 and 3) were identified through whole-genome sequencing. There was no difference in number or EV type when comparing dry or wet seasons over 18 months, except for EV-C99, which was present in samples collected during the wet season (May-October). More EV's were found from sites located in areas with smaller populations. Phylogenetic analysis of the unique EV genomes revealed virus lineages distinct from previously published sequences.

Conclusion: OPV's were detected from sewage samples from 5 out of 6 sites. The use of an additional cell line combined with next-generation sequencing revealed patterns of circulation of EVs in Guatemala. Spatial analysis showed that EV detection was inversely related to the catchment size of the population, and no clear seasonality was observed except for EV-C99.

P02

Parechovirus-A findings in Finland during years 2012-2019

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Abstract

The Parechovirus-A (PeV-A, formerly Human parechovirus, HPeV) species comprises 19 types. These are common around the globe, with PeV-A1 being the most common type. PeV-As infect mostly children and cause mild gastroenteritis and respiratory infections. However, especially type 3 can cause serious sepsis like disease and central nervous system infections, such as meningitis and encephalitis.

Helsinki University Hospital laboratory (HUSLAB) started PeV-A diagnostics in 2012 as a pilot study from serum and cerebrospinal fluid samples (CSF) followed by implementing the molecular method into daily diagnostics. During 2012 seven PeV-A caused sepsis-like disease cases were detected. After several low prevalence years, with only up to one case annually, an outbreak was detected in 2018 with nine hospitalized patients. The outbreak appeared in August and peaked in September/October. All PeV-A positive patients were under three months-old, and two of them needed treatment at the intensive care unit (ICU). PeV-A3 was typed from a 1-week-old patient treated in ICU, however, from the other PeV-A positive ICU patient no sample was available for typing. Typical symptoms were fever (>38°C), irritability, tachycardia, marmorizing skin and rash. In year 2019, only one PeV-A was detected from a 2.5-month-old girl from a CSF sample.

Most of the found PeV-As have been typed as PeV-A3, but interestingly the outbreak of year 2012 was caused by a rare PeV-A4 strain. Furthermore, one PeV-A5 was detected during the 2018 outbreak.

P03

Seroprevalence of enterovirus A71 prior to encephalitis outbreak ocurred in Spain during 2016: a pilot study

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Abstract

Background. Enterovirus A71 (EV-A71) is the major cause of hand, foot and mouth disease with neurological complications in the Asia-Pacific region while in Europe it is associated with neurological pathologies almost exclusively. In Spain, EV-A71 had circulated in low level until 2016, when an important outbreak causing severe encephalitis in children between 1-5 years occurred. We describe a pilot study of seroprevalence of EV-A71 to evaluate Spanish population susceptibility prior to the outbreak.

Methods. Seroprevalence of EV-A71 specific neutralizing antibodies was determined in age-stratified 86 serum samples collected in 2015 using micro-neutralization assay in RD cells and two EV-A71 strains (C12002 y C1v2016). Neutralizing titer and geometric mean titer (GMT) were also calculated. Antibody titers of > 1:16 were considered positive.

Results. The overall seroprevalence of both strains was 53%. It was significantly lower (33%, 7/21) in children between 1 and 5 years-old than in those between 6 and 14 years-old (66%, 12/18) ($p<0.05$). In adults, seroprevalence was over 55%. There were no differences in GMT between both strains in each age group. However, children between 1-5 years with antibodies against EV-A71 showed higher titers than the other groups of age (6-14, 15-39, 40-65, >65), with those > 65 years having the lowest titers.

Conclusions. This is the first report about seroprevalence of EV-A71 in Spain. The lower seroprevalence of EV-A71 in children under 5 years-old could explain the major susceptibility of this population to the EV-A71C1v strain that emerged during 2016 in the country causing more severe neurological infections in them.

P04

Enterovirus 71 detection in England (2012-2020).

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Abstract

Human enterovirus 71 (EV-71) is an emergent non-polio enterovirus (NPEV) of clinical importance associated with hand, foot, and mouth disease outbreaks and severe neurological complications including acute flaccid paralysis. Three genetic lineages A, B and C further subdivided in B1-B5 and C1-C5 have been described. In Europe, subgenogroups C1 and C2 predominate and recently C4 has been reported. Novel recombinant subgenogroups have been described in the Asia Pacific Region.

In England, the Enteric Virus Unit at UKHSA offers Enterovirus genotyping after an initial detection is performed at local Public Health or hospital laboratories.

This study reports a retrospective analysis of the EV-71 genogroups identified in referred samples from 2012 to 2020. Data was collected from local Laboratory Information System and Enterovirus Surveillance Database.

Over the 8-year period, a total of 11,204 samples were genotyped as NPEV with a 3.6% (n=403) confirmed as EV-71. Sequence data analysis shows a subgenogroup replacement from C1 in 2012-2015, to C2 in 2016-2020 period with a significant increase in detection in 2013 and 2019. In infants ≤5 years of age, EV-71 was detected in three different biological sites: CSF (cerebrospinal fluid), gastrointestinal and respiratory. Neurological clinical manifestations were detected with two-fold frequently in male children than female.

Sample submission for genotyping is voluntary in the UK and may be biased towards more severe cases limiting our capacity to assess community prevalence of NPEV. An extensive NPEV laboratory surveillance program is required to allow the monitoring of clinically important genotypes.

P05

Harmonising Non-polio Enterovirus (NPEV) sero-surveillance among the European Non-Polio Enterovirus Network (ENPEN) members

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Abstract

Sero-surveillance of non-polio enteroviruses (NPEV) can complement genotyping-based surveillance by providing information on the risk of upsurges. To assess the variability of Virus Neutralisation Assays (VNA), the standard for measuring neutralising antibodies (Ab) against NPEV, we performed a survey and VNA quality assessment among twelve members of the European Non-Polio Enterovirus Network (ENPEN).

We provided Intravenous Immunoglobulins (IVIG) and five echovirus (E) isolates (E1, E18, E30 G1, E30 G6 and E6) to perform VNA according to the laboratory’s own protocol. Data on nAb titres, TCID50 and protocol details were collected. Intra- and inter-laboratory variability were assessed by calculating coefficients of variation (CV). We checked linearity of the individual assays by measuring nAb titres against E6 in a IVIG dilution series (1:1, 1:4, 1:16, 1:64) and calculating correlation coefficients (Spearman’s rank).

Twelve laboratories from the Netherlands, Bulgaria, Croatia, Czech Republic, Finland, France, Germany, Portugal, Spain, Romania and United Kingdom participated in this study. VNA protocols varied (moreover cell lines, incubation lengths and nAb titre calculation methods). Wide ranges of nAb titres were observed between laboratories especially against E18 and E1 (1:4 to >1024). Intra-lab variability was minimal (CV<20%), whereas inter-lab CV values were 44%, 53%, 28%, 25% and 17% for E1, E18, E30G1, E30G6 and E6 respectively. There was a good correlation between IVIG dilution and nAb titre.

Harmonisation of VNA protocols is necessary to make results of NPEV sero-surveillance studies comparable. The inclusion of a reference sample and standardising cell line and incubation length will be further assessed.

P06

Detection of Enterovirus D68 clades B3 and D1 in wastewater samples from the UK between July 2021 and December 2021

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Abstract

Enterovirus D68 (EV-D68), a member of the *Picornaviridae* family, was first detected in the US in 1962. After a large outbreak in 2014, predominantly affecting children, more recent infections have been linked with severe neurological disease such as acute flaccid myelitis (AFM). However, active surveillance for EV-D68 is lacking, which makes full assessment of this association difficult. EV-D68 appears to present itself in a biennial cycle with occurrences of AFM coinciding with EV-D68 outbreaks in 2014, 2016 and 2018. However, in 2020, there was no apparent increase in EV-D68 detections or AFM cases. Here we describe an upsurge of EV-D68 detections in wastewater samples from the United Kingdom between July 2021 and December 2021 that appears to mirror an increase in recently reported clinical samples from Europe, a close association between EV-D68 historical prevalence in clinical and sewage samples that we have observed since 2014.

This increasing prevalence of EV-D68 and its association with AFM has resulted in this becoming a concern for public health. Vaccines are currently in development and as a result, we have recently generated an International standard for anti-EV-D68 serum. Establishing relevant reference reagents will aid the development and standardisation of safe and effective vaccines. These resources in partnership with the utilisation of environmental surveillance would, eventually, help in predicting and controlling future epidemics.

P07

Whole-genome sequence characterization of type 2 novel OPV-related recombinant isolates by deep sequencing

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Abstract

A novel type 2 oral polio vaccine (nOPV2) showing increased attenuation and enhanced genetic stability but similar antigenicity and immunogenicity properties to Sabin 2 is being used in several countries under World Health Organization (WHO) Emergency Use Listing (EUL). These properties were confirmed in small clinical trials and nOPV2 is thought to be an ideal vaccine to stop outbreaks due to type 2 circulating vaccine-derived polioviruses (cVDPV2s) without carrying a risk to cause disease and new polio outbreaks. Monitoring genetic stability and vaccine effectiveness of nOPV2 is essential to support the EUL and move through the different phases towards full licensure. Recombination events between polio and non-polio enteroviruses are very common during human to human transmission and could be a mechanism for nOPV2 isolates to quickly acquire new mutations that lead to reversion of its attenuation properties. For this reason, nOPV2 design includes genetic modifications to reduce the chance of recombination. Despite this, we did find in both clinical and environmental samples following immunisation campaigns, nOPV2 isolates showing recombinant structures containing heterotypic sequences from Sabin 1 or unidentified non-polio enteroviruses. Although none of the recombination events observed so far are expected to alter nOPV2 key properties, it is critical to develop methods for the sensitive detection and full characterization of recombinant viruses from surveillance activities. Deep sequencing methods to detect and characterize nOPV2 recombinant isolates alone or in virus mixtures using Illumina MiSeq and Oxford Nanopore MinoION technologies are described.

P08

Long-term replication of poliovirus in immunodeficient patients and potential anti-viral treatments

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Abstract

With the significant decline in poliomyelitis cases due to wild poliovirus in recent years, cases related to the use of live-attenuated oral polio vaccine assume greater importance. Poliovirus strains in the oral vaccine are known to quickly revert to neurovirulent phenotype following replication in humans after immunisation. These strains can transmit from person to person leading to poliomyelitis outbreaks and can replicate for long periods of time in immunodeficient individuals leading to paralysis or chronic infection, with currently no effective treatment available to stop excretion from these patients. We describe the properties of poliovirus strains from these patients focusing on their genetic properties and evolution patterns including co-circulation of different genetic lineages for long periods of time in the same patient and frequent recombination events between them.

We also tested the potential of different anti-viral compounds to inhibit virus replication showing that Pocapavir and Remdesivir inhibit virus growth in cell culture assays of a type 2 vaccine-derived poliovirus strain obtained from an immunodeficient patient after 30 years of continuous infection. Interestingly, following administration of Remdesivir to this patient to treat SARS-CoV-2 infection, neither SARS-CoV-2 nor poliovirus has been detected for the 6 months following treatment. However, although every single stool sample taken from this patient during the previous 23 years since the infection was first detected has been positive for type 2 poliovirus, no samples were available for testing for several months preceding antiviral treatment making the possible link between Remdesivir treatment and interruption of poliovirus excretion not fully conclusive.

P09

Molecular dissection of type 3 oral polio vaccines by deep sequencing

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Abstract

Oral polio vaccine (OPV) has been the preferred vaccine throughout the World Health Organization (WHO) Global Poliovirus Eradication Initiative. However, Sabin poliovirus strains in OPV are genetically unstable and have been shown to lose their attenuating mutations and revert to a neurovirulent phenotype during passage in-vivo and in-vitro. In this study we evaluated the suitability of Next generation sequencing (NGS) analysis to identify key poliovirus sequence signatures, which can then be used to help monitoring the genetic stability and consistency of OPV during vaccine production.

Whole-genome SNP profiles of Sabin type 3 poliovirus vaccine strains were found to be consistent between vaccine seeds and lots from the same manufacturer, but differences were observed between vaccine products from manufacturers using different seeds and manufactures using different cell substrates to grow the seed viruses during vaccine production. These differences allowed us to identify the vaccine origin of type 3 poliovirus isolates from vaccinees from which whole-genome sequences are available in GenBank.

NGS has the potential to monitor genetic signatures throughout the poliovirus genome and would be beneficial for the quality assessment of vaccine seeds and production bulks used for manufacturing. Furthermore, NGS is an ideal test for the molecular characterization of novel OPV (nOPV) strains, that incorporate several genetically engineered mutations in their genome and for which the current molecular lot release test MAPREC, relying on single mutation quantification, would be meaningless. Further validation studies are ongoing with a view to replace OPV vaccine safety tests using animals with whole-genome NGS analysis.

P10

Tracking non-polio enteroviruses (NPEV) in urban wastewater during the SARS-CoV-2 pandemic in Lombardy (Italy)

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Abstract

Aim: This study aimed at monitoring the circulation of non-polio enteroviruses (NPEVs) in urban wastewater samples (WWS) in Lombardy (northern-Italy) during the COVID-19 pandemic.

Methods: 167 composite 24-hour WWSs were collected once a week from 03/24/2020 to 10/27/2021 at the inlet of 3 wastewater-treatment-plants (catchment of 1,500,000 inhabitants) in Milan. After concentration of WWSs by PEG-8000, RNA was extracted by commercial method (MinElute-Kit, QIAGEN) according to a modified protocol. NPEVs were detected by a one-step real-time RT-PCR (5'NTC region). Molecular characterization of NPEVs was performed by sequence analysis of a fragment of the VP1/VP3 gene (nt. 2602–2965).

Results: NPEV-RNA was identified in 87.4% (146/167) of WWSs. Viral load ranged between 8.8E+6 and 3.8E8 copies/L. The viral load in WSSs collected from 06/16/2021 to 10/27/2021 was significant higher compared to that from 03/24/2020 to 06/09/2021 (2.6E+7 vs 7.5E+7 copies/L, p=0.00014). The highest viral load was reported on 09/09/2021 (2.9E+8 copies/L). Unfortunately, molecular characterization of NPEVs was inconclusive in all positive-WWSs as the sequences revealed mixed electropherogram peaks, suggesting the co-presence of several genotypes.

Conclusion: This surveillance can monitor the NPEV circulation and its extent in the community. Although the identification of viral genotypes was not feasible, the significant increase of NPEV load in WWSs overlapped the surge of NPEV circulation in our area. It will be pivotal to implement innovative molecular approaches and pipelines (i.e. NGS) to deeply tract NPEV circulation and to uncover the introduction of viruses that may pose a threat to human health.

P11

Molecular identification of enterovirus (EV) and human parechovirus (HPeV) in Influenza-like illness (ILI) cases in Lombardy (Northern Italy) in 2018-2019 and 2021-2022 winter season

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Abstract

Background

In the framework of Influenza-like illness (ILI) virological surveillance activities, the molecular detection of enterovirus (EV) and human parechovirus (HPeV) was carried out in Lombardy region (Northern Italy) in 2018-2019 and 2021-2022 winter season, to compare the frequencies of EV/HPeV detection between these two seasons.

Methods

Nasal-pharyngeal swabs (NPS) collected from ILIs were tested for EV and HPeV with a specific real-time RT-multiplex-PCR assay. EV/HPeV-positive samples were molecularly characterized through sequence analysis. For this study, were considered NPSs collected from week-46 to week-10 of the following year.

Results

438 and 694 NPS were collected in 2018-2019 and 2021-2022, respectively. Sex distribution and mean age were similar between these seasons (males: 51.5% vs 54.0%, P=0.41) (28.2 years [IQR: 40.5] vs 23 years [IQR: 41]). EV was detected in 3.4% of ILI samples in 2018-2019 (from week-46 to week-6, peaking in week-51) and in 4.5% (P=0.36) of NPS in 2021-2022 (from week-46 to week-9, peaking in week-48). 12.5% and 22.6% (P<0.0001) of EV-positive samples were EV-D68 in 2018-2019 and 2021-2022, respectively.

HPeV was detected in one (0.2%) ILI case in 2018-2019 (in week-47) and in 1.7% (P=0.01) in 2021-2022 circulating from week-46 to week-6 and peaking in week-47. All HPeVs sequenced in 2021-2022 season were HPeV1.

Conclusions

These results confirm the re-start of circulation of respiratory viruses in the 2021-2022 winter season; whereas EV was detected at similar frequency in the pre-pandemic and pandemic season, EV-D68 and HPeV detections significantly increased in the current season.

P12

Population level serologic reactivity to Enterovirus D68, enterovirus A71 and CVA6 in England

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Abstract

Non-polio enteroviruses cause hand, foot and mouth disease (HFMD) and neurological infections such as meningitis, encephalitis and acute flaccid myelitis. As the majority of infections occur without clinical symptoms and hence remain undiagnosed, seroprevalance data can be used to determine degrees of population exposure. We have analysed neutralising antibody frequencies obtained for three enterovirus types of concern (EV-D68, EV-A71 and CVA6) in serum samples collected in England in 2006, 2011 and 2017 [1,2]. Of 1507 samples tested, 1416 were seropositive for EV-D68 (94%, geometric mean titre, GMT=207 [95%CI 193-223]), 1129 for EV-A71 (75%, GMT=53 [95%CI 49-59]) and 1211 for CVA6 (80%, GMT=70 [95%CI 64-76]); 921 samples had antibodies against all three viruses (61%). A higher seroprevalence of EV-D68 was observed in young children up to 10 years and EV-A71 up to 20 years in 2011 and 2017 compared to 2006. Although no change in seroprevalence was observed for CVA6, higher GMTs were recorded in children in 2011 and 2017 than in 2006.

Overall, the data shows substantial population exposure and immunity to the three enteroviruses. Infection pressure seems to be increasing in those under 20 years of age based on seroprevalence and GMT for all three viruses. This in turn might explain the declining antibody titres demonstrated in older adults (or another way around!). Changes in EV-D68 seroprevalence and infection dynamics have been recently related to changes in virus transmissibility [3] but could they be a consequence of changing poliovirus vaccination programme. This needs to be investigated further.

P13

Complete Genome Analyses of Foot-and-Mouth Disease Viruses Belonging to Serotypes O, A and SAT 2 in East, West and North Africa

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Abstract

Foot-and-mouth disease is generally considered to be endemic in Egypt, but only to occur sporadically in other North African countries. FMD has always been endemic in East and West Africa, with the two regions having their own FMD virus (FMDV) pools (named Pool 4 and Pool 5, respectively). To examine the origin, evolution and interaction of FMD viruses in these three areas, we have sequenced the near-complete genomes of 66 O/EA-3, 35 A/AFRICA/G-IV and 39 SAT2/VII viruses from East, West and North Africa, and compared them with previously published sequences. Phylogenetic analyses of different genome regions revealed that, while the outer capsid-coding regions (VP2, VP3 and VP1) show serotype-specific phylogenies, the non-structural coding and untranslated regions do not, suggesting the possibility of extensive recombination. Recombination analyses further identified at least 25 events of both inter- and intra-typic recombination outside the outer capsid-coding region. Phylogenetic analyses indicate that both FMDV types O and A have spread from East Africa to West Africa and Egypt, independently. Spread of these two serotypes into the Maghreb countries occurred from West Africa in 2017 and 2018. FMDV serotype SAT2 also spread in Egypt on at least three occasions possibly from East Africa twice and West Africa once. The building of new trans-Saharan highways along with changes in the political situation in the region have probably led to increased legal and illegal trade between North Africa and both East and West African countries leading to the expansion of FMDV lineages from their traditional pools.

P14

Circulation of rhinoviruses in Denmark before and during the SARS-CoV-2 pandemic

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Abstract

Background: Systematic typing of rhinovirus positive samples was initiated at Statens Serum Institut (SSI) in 2014 as a response to the global emergence of enterovirus D68. In this study we describe the seasonality and circulating genotypes of rhinovirus in a five year period preceding, and overlapping with, the SARS-CoV-2 pandemic.

Methods: A total of 1074 rhinovirus positive samples were collected from different sources; routine respiratory diagnostics at SSI (n=162), influenza sentinel surveillance (n=224), enterovirus surveillance (n=51), rhinovirus surveillance (n=635), and SARS-CoV-2 sentinel (n=2). A fragment of the VP4 and VP2 genes was sequenced and typed by BLAST analysis and phylogenetic analysis.

Results: Rhinovirus was detected all year round for all five years, 167 samples were from 2017, 211 from 2018, 229 from 2019, 139 from 2020, and 328 from 2021. Fewer samples were positive during the summer months, especially in 2020. Sample numbers were highest from October to March. In 2021 the peak was seen between August and December, and was markedly higher than the previous years. Of successfully typed samples 210 were species A types, 31 species B, and 136 species C. 677 samples could either not be genotyped or where typed as enterovirus. 125 different genotypes were identified across the three species.

Conclusion: Rhinovirus infections occur all year round, with a peak in the late autumn and winter. Rhinovirus positive samples decreased in number during the first lockdown phase in Denmark in 2020, but detections were constant. Species A genotypes are most common, followed by species C.

Translation and replication

P15

Real time visualization of viral RNA during enterovirus infection

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Abstract

Enteroviruses are a group of prevalent pathogenic viruses that pose a significant health and socio-economic threat. To develop novel therapeutic options for enteroviral disease it is of great importance to have a good understanding of the early phases of infection, which are increasingly recognized as critical determinants of disease outcome. However, most current technologies fall short of the requirements to study these dynamic processes at the single cell level. Previously our lab has developed VIRIM, which enables the real-time visualization of translation dynamics of positive-sense RNA viruses. Expanding on our imaging toolbox, we employed the BiRhoBAST system to visualize viral RNA molecules during live infection. BiRhoBAST is an aptamer that was evolved to have high affinity towards the cell-permeable bivalent chemical dye tetramethylrhodamine-2 (TMR2). In solution TMR2 is quenched, but upon binding to BiRhoBAST it fluoresces in the orange spectrum. We successfully engineered Coxsackievirus B3 to have a cassette of 4 BiRhoBAST aptamers at the 3’-prime side of its genome. This allowed us, upon infection with this virus of cells in the presence of TMR2, to visualize viral RNA as bright spots. The combination of this powerful tool with VIRIM and other advanced imaging technologies will allow for the dissection of many aspects of the (early) virus life cycle. Moreover, these tools are not limited to enteroviruses, but can potentially be employed for a wide range of other RNA viruses.

P16

Expression analysis of the novel upstream ORF in enteroviruses.

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Abstract

The genus *Enterovirus* consists of a large group of positive-sense RNA viruses, with over 100 serotypes known to be human pathogens. Disease phenotype in humans ranges from sub-clinical to acute flaccid paralysis, myocarditis, and meningitis. Until recently, it was believed that enteroviruses encode all their proteins in a single long open reading frame (ORF), termed the polyprotein ORF (ppORF). However, it is now known that in many enteroviruses a small upstream ORF (uORF) encodes an additional protein termed UP (Upstream Protein). The mechanism by which ribosomes are recruited to the uORF initiation codon is unknown and thus the regulation of UP expression is undetermined. We address these questions using reporter systems and ribosome profiling, amongst other techniques. Building on previously published work, we investigate the relationship between the structure of the domain VI stem loop of the enterovirus internal ribosome entry site and translation efficiency of the uORF and ppORF. Through the profiling of initiating ribosomes in cells infected with different enteroviruses, we characterise the viral and cellular profiles of initiation events during different stages of virus infection. Our findings provide new insights into enterovirus gene expression.

P17

Non-polio Enteroviruses C replicate in both human gut and airway organoids

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Abstract

Enteroviruses (EVs) are globally widespread causing a variety of clinical symptoms. Despite the great interest in some EVs like Poliovirus, EV-A71, and EV-D68, many are still missing a proper characterization. Non-polio EVs-C, highly prevalent in Africa among children, can recombine with Poliovirus but little is known about their pathogenicity due to their poor cultivability.

We used five cell lines (BGM, Caco2, Gabi, LLCMK2, MCF7) for culturing six hard-to-grow EVs-C (Coxsackievirus A-1 (CVA-1), CVA-13, CVA-20, CVA-24, EV-C99, EV-C116). Viruses able to grow were further cultured on Human Airway Epithelium (HAE) and Human Intestinal Epithelium (HIE) mimicking EV primary replication sites. Adult HAE from a pool of donors (Epithelix Sàrl), in-house fetal HAE, adult and fetal HIE from 3 donors each were infected from apical and basolateral sides. Viral replication was measured by RT-qPCR.

We successfully cultured CVA-13, CVA-20, and EV-C99 on Caco2. All three viruses showed high replication on adult HAE and lower replication on fetal HAE upon apical infection. They were also able to efficiently infect fetal HIE, however, CVA-20 showed less replication. Cell tropism will be determined by immunostaining.

HAE from a pool of adult donors is a suitable culture system for some EV-C which also replicate in fetal HAE and HIE. This indicates that airway and gut may both serve as primary entry and replication sites for non-polio EV-C. We did not find differences in viral replication efficacy between fetal and adult tissue that could explain the preferential infection of children by EV-C but more studies are needed.

P18

Co-folding of Poliovirus P3 Polyprotein Precursors

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Abstract

The picornaviral open reading frame encodes a single polyprotein that is divided into P1, P2, and P3 regions. The P3 proteins are involved in genome replication though the actions of two active proteases ($3C^{pro}$ and $3CD^{pro}$) and the viral RNA polymerase ($3D^{pol}$). The structures of several individual P3 proteins are known, but not in the context of larger precursor proteins wherein they may co-fold and have functionally relevant interactions. In particular, 3A is composed of a soluble region that contains a pair of short helices based on NMR studies and a hydrophobic membrane association domain. However, most of 3A is disordered and poised for interactions with other viral and host proteins, as has been shown for kobuviral 3A interacting with the host ACBD3 protein GOLD domain. In this work we examined the structure of the individual and precursor P3 proteins using CD spectroscopy and crystallography. The data show that 3AB plays a role in stabilizing the structure of the 3Cpro in the context of 3ABC and this stabilization requires the complete 3AB sequence as an *in cis* interaction. 3ABC co-folding may play an important functional role to limit 3A interactions with host factors prior to the major processing step that yields 3AB and $3CD^{pro}$. In addition, we have solved a new monomeric crystal form of poliovirus $3C^{pro}$ in which the active site contains the cleavage site from an adjacent molecule. Using this crystal form, we have solved the 1.2–2.2Å resolution structures of multiple cleavage site sequences bound to $3C^{pro}$.

P19

The use of novel tools to uncover the ultrastructure of the FMDV replication complex by correlative light-electron microscopy

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Abstract

Foot-and-mouth disease virus (FMDV) is a veterinary pathogen of major global concern due to the economic impact of sanctions imposed during outbreaks of foot-and-mouth disease. FMDV reorganises cellular membranes to form replication complexes (RCs) which support viral replication. The structure of FMDV RCs remains poorly characterised, in part because SAPO4 classification of FMDV requires the use of specialist containment facilities. However, the use of a replicon system allows replication to be studied without high containment and without the complexity of other parts of the viral lifecycle. To study FMDV RCs we have developed modified replicons, incorporating tags for use in room temperature correlative light electron microscopy (CLEM). Osmium resistant fluorescent protein, mEosEM maintains its fluorescence after osmium tetroxide staining, which greatly increases sample contrast for EM. We have generated an FMDV replicon containing mEosEM and shown that the fluorescent signal can be detected by live cell and confocal imaging. This will allow us to identify cells where active viral replication is taking place. On the other hand, metallothionein tags bind gold nanoparticles to form detectable dense clusters providing a specific signal for imaging by electron microscopy. We have generated a replicon with an MT tag fused to one of the three 3B proteins, which locates within the RCs, and demonstrated that this is still replication-competent. Metallothionein tags will allow us to locate 3B within RCs, unravelling its molecular arrangement during viral replication. These tools are allowing us to elucidate the ultrastructure of FMDV RCs, enhancing our understanding of FMDV replication.

P20

DYNAMICS OF *IN VITRO* SIMULTANEOUS REPLICATION OF TWO SEROTYPES OF FOOT-AND-MOUTH DISEASE VIRUS

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Abstract

Foot-and-mouth disease (FMD) is one of the most infectious viral diseases of livestock. Its causative agent is present as seven serotypes that may co-circulate in some endemic areas, making virus isolation tricky and misleading.

This work aimed at gaining insight into the dynamics of simultaneous *in vitro* replication of two FMDV serotypes.

BHK-21, IBRS-2, and LFBK$_{\alpha\nu\beta6}$ cell lines were infected both simultaneously and separately, with two FMDV serotypes (O and A) at infecting concentration 10^3 TCID$_{50}$/ml each. Samples were collected at specific time points and quantified by topotype-specific qRT-PCRs. Two experiments were carried out using different topotypes of each serotype.

Both serotypes reached the highest concentration (~10^9 genomic copies) at 48 hpi when cultured in a single infection on BHK-21. However, from 24 hpi the serotype O in co-infection was quantified 1-2 log$_{10}$ lower than the same serotype in the single infection, while serotype A was not inhibited by the presence of the serotype O.

In IBRS-2 cells, serotype O was weakly inhibited by co-infection with serotype A (1 log$_{10}$ difference at 48hpi). Viral growth on IBRS-2 and LFBK$_{\alpha\nu\beta6}$ cell lines reached ~10^8 genomic copies for both serotypes in the single infection. Interestingly, in co-infection on LFBK$_{\alpha\nu\beta6}$ serotype A replicated less than in the single infections, but with moderate quantification differences (lower than 1 log$_{10}$, still under investigation).

These preliminary results corroborate previous observations that different serotypes present in field samples can be isolated depending on the cell line used. Further studies are needed to confirm these findings.

P21

Assembled Foot-and-mouth disease virus capsids accumulate in close proximity to sites of replication

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Abstract

Foot-and-mouth disease virus (FMDV), like other positive stranded RNA viruses, induces membrane rearrangements within infected cells, which provide a platform for recruitment of replication proteins, enabling viral replication. Newly produced genomic RNA and capsid proteins assemble into new virions.

We have used confocal microscopy to investigate the relationship between sites of replication and assembled capsids. We used an antibody specific for viral protein 3A as a marker of sites of replication. To distinguish between assembled and unassembled capsid material, we used two antibodies, one which recognises only assembled capsids, the other which recognises capsid protein VP1 regardless of assembly status. Our data shows that assembled capsids are located close to, but not colocalised with, sites of replication. In cells co-labelled for assembled capsid and VP1, accumulations of capsid colocalise with bright puncta of VP1 labelling, but not with the bulk of VP1 signal. Inhibiting VP4 myristoylation or the chaperone HSP90 did not affect virus replication as judged by accumulation of 3A, but greatly reduced the signal for assembled virions within cells, confirming that these processes are required for assembly in situ.

Our data indicate that sites where assembled capsids accumulate are located close to, but distinct from, sites of replication within infected cells, and that even at later timepoints, the assembled capsid proteins represent only a proportion of the total capsid protein within the cell. To our knowledge this is the first time the localisation of assembled picornavirus capsids within cells has been investigated by light microscopy

Virus entry and structure

P22

Enteroviruses reach cytoplasm by endosome disruption

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Abstract

Enteroviruses, a large group of non-enveloped picornaviruses, are human pathogens causing a range of diseases, from the common cold to poliomyelitis. To initiate infection, enteroviruses enter cells by receptor-mediated endocytosis. However, the details of enterovirus genome delivery are not well understood.

In this project, we used cryo-electron tomography of thin parts of cells grown on electron microscopy grids to characterize the interactions of the cell and enterovirus particles *in situ*. The reconstructed areas of cells provide information about the endosome membrane remodeling and disruption, followed by virus release into the cytoplasm. We demonstrate that the endosome disruption is mediated by overactivation of a cellular mechanism by showing that endocytosis of very-low-density lipoprotein, the natural substrate of rhinovirus 2 receptor, also results in endosome disruption. The described mechanism of human rhinovirus 2 cell entry is supported by data collected on other enteroviruses.

Our results give overview of the cellular mechanisms that enteroviruses utilize to enter cell hosts. The enterovirus cell entry and the release of the viruses from endosomes are potential targets for antiviral therapeutics.

P23

Does the Pocket Factor Symmetrize the Rhinovirus RNA-Protein Interface?

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Abstract

Determining the binding site of the antiviral pyrazolopyrimidine OBR-5-340 in RV-B5 by cryo-EM, the 3D-structure of the OBR-5-340 resistant RV-A89, in the presence of OBR-5-340 in 10 % DMSO, had been obtained at 2.9 Å and used as a control. RV-A89 was devoid of OBR-5-340 and the hydrophobic pocket lacked a natural pocket factor; the side chain of a methionine partially occupied the pocket (1). The 3D-structure of RV-A89 in plain buffer was also determined (2.1 Å). Here, the hydrophobic pocket contained a myristate suggesting that the pocket factor had been eluted by the DMSO in the previous experiment.

Thee-D classification of computationally extracted single protomers of RV-A89 lacking the pocket factor revealed conformational differences of the amino acid residues in the vicinity of the RNA genome most probably resulting from interactions with different sequences and folds of the asymmetric RNA (2). Such differences were not detected in the 3D-structure of the myristate-containing RV-A89. This suggests that loss of the pocket factor might allow subtle structural adaptations of the viral capsid to the dissimilar conformations of the RNA at the interface with the viral protomers. This might facilitate and direct RNA exit. Similar differences were not found when comparing the 3D-structures of RV-B5 with an empty pocket and RV-B5 with the pocket containing OBR-5-340. The significance of this finding is thus currently unclear.

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P24

Needle in a haystack: virions of coxsackievirus A6 are required for infection

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Abstract

Coxsackievirus A6 (CV-A6) displaced enterovirus A71 and CV-A16 as the predominant causative agent of hand, foot, and mouth disease worldwide. No vaccine against CV-A6 is available, and vaccine development against CV-A16 and EV-A71 has proven challenging and trivalent vaccine candidates fail to cross-protect. Virions of CV-A6 were not identified in previous structural studies, and it was speculated that the virus is unique among enteroviruses in using altered particles with expanded capsids to infect cells. In contrast, the virions of other enteroviruses are required for infection. Here we used a very large cryo-electron microscopy (cryo-EM) dataset to determine the structures of the CV-A6 virion, altered particle, and empty capsid. We show that the CV-A6 virion has features characteristic of virions of other enteroviruses, including a compact capsid, VP4 attached to the inner capsid surface, and fatty acid-like molecules occupying the hydrophobic pockets in VP1 subunits. Furthermore, using a fluorescence-based infection assay, we found that in a purified sample of CV-A6, the ratio of infectious units to virions is 1 to 500. Therefore, it is likely that virions of CV-A6 initiate infection, like those of other enteroviruses. Our results provide evidence that future vaccines against CV-A6 should target its virions instead of the antigenically distinct altered particles. Moreover, the structure of the virion provides the basis for the rational development of capsid-binding antiviral inhibitors that block the genome release of CV-A6.

P25

Low-density lipoprotein receptor (LDLR) is an entry factor for naked hepatovirus

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Abstract

Mechanisms by which naked (nHAV) and quasi-enveloped (eHAV) hepatovirus virions enter cells to initiate infection have yet to be fully elucidated. TIM1, suggested previously suggested to be a receptor, binds to phosphatidylserine on the surface of eHAV and promotes eHAV endocytosis. However, TIM1 is not required for infection by either virion type and its depletion has no impact on nHAV entry. By contrast, gangliosides (particularly GD1a) are essential for entry of both eHAV and nHAV, binding capsid within late endosomes following endocytosis and degradation of the quasi-envelope (Das *et al.*, Nat Microbiol, 2020). Factors mediating endocytosis of nHAV are unknown. Thus, we were intrigued to identify LDLR, a receptor for some rhinoviruses, in a genome-wide CRISPR screen for HAV host factors (Das *et al.*, Nat Microbiol, 2020). Hepatoma cells with targeted LDLR knockout were less susceptible to nHAV infection, but demonstrated increased susceptibility to eHAV infection and no difference from control cells in amplifcation of transfected subgenomic replicon RNA. Soluble recombinant protein representing the LDLR ectodomain (sLDLR) bound nHAV in ELISA assays, and exerted a dose-dependent inhibition of nHAV, but not eHAV, infection. sLDLR binding to the capsid was greatest at pH 4.5-5.0, and strongly inhibited by the neutralizing anti-HAV mAb K24F2, but not R10. Incubation with sLDLR did not alter the thermostability of nHAV, suggesting that it does not initiate uncoating. These results suggest a role for LDLR early in the cellular entry of nHAV prior to interactions of the capsid with GD1a in late endosomes.

P26

From uncoating to assembly, an RNA tale.

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Abstract

Rhinovirus (RV) is an important upper respiratory tract pathogen. After binding to susceptible cells, RV enters cells via endocytosis and releases its genetic material using a not fully understood mechanism. Aiming to investigate how the endosomal main ions could interfere with the genome release, we exposed purified RV-A2 to low pH in Na^+ or K^+ containing buffers and quantified the native particle conversion into subviral A and B particles. By using different negative stains, we identified a distinct subviral particle stable for an hour at low pH in the presence of a physiological concentration of K^+. Using single-particle cryoEM, we reconstructed this intermediate and compared it with deposited RV-A2 native molecular model revealing changes centred at the fivefold axis (outer and inner faces). This new subviral E0 particle does not present loosening of the genome packing, loss of the pocket factor, loss of VP4, and formation of larger openings at the VP2 dimer interface, characteristic of E1 and A subviral particles. Afterwards, we evaluated the morphology of the RV-A2 ex-virion RNA using rotary shadowing, searching for hints of the Na^+/K^+ effect on the viral particle. The genome presents round and compact when kept in the presence of physiological concentrations of K^+, explaining the stability of the E0 particle. Differently, exposure to physiological concentrations of Na^+ resulted in the appearance of dissimilar levels of RNA unwinding, separated into 3 classes. Finally, we propose the exposition to Na^+ during the initial stages of endocytosis triggers RNA rearranging and permitting the uncoating.

P27

Heparan sulfate-mediated entry of enterovirus A71 via pH-independent endocytic pathway

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Abstract

Enterovirus A71 (EV-A71) is the enterovirus that has received the most attention after poliovirus as it causes major hand, foot and mouth disease together with severe neurological complications and deaths across many countries. To date, its pathogenesis remains poorly understood. We have previously characterized a new mutation that emerged in the EV-A71 capsid (VP1-L97R) during a disseminated infection in an immunocompromised patient. This mutation is always associated with a second mutation (VP1-E167G) after propagation in cell lines. We demonstrated that together, the two mutations confer high affinity for heparan sulfate (HS), an EV-A71 attachment receptor, thereby modifying viral tissue tropism. To better characterize wild-type (HS-independent) and HS-binding variants, we tested their sensitivity to different inhibitors. We highlighted that hydroxychloroquine (HCQ), a weak base known to elevate pH of acidic intracellular organelles, specifically inhibits the HS-independent variant. We further showed that HCQ impacts virus uncoating, a process in which endosomal acidification is required. Resistance to HCQ treatment suggests that the HS-binding variant does not use endosomal acidification as uncoating cue. We thus investigated the respective sensitivity of the two variants to low pH and analysed the interplay between HS and SCARB2, the EV-A71 uncoating receptor. Our results shed some light on the different entry mechanisms used by EV-A71 HS-dependent and independent variants and how HS-binding may modulate capsid stability and viral entry.

Evolution and diversity

P28

Recombination events and RGD/heparan sulphate receptor-binding sites in coxsackievirus A9

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Abstract

Coxsackievirus A9 (CVA9) is an enterically transmitted human picornavirus in Enterovirus B species. Atypically to other members in this species, it uses αV integrins in receptor-dependent infectious entry. In addition, heparan sulphate has also been suggested to mediate CVA9 infection. Fifty-four (54) novel CVA9 samples were investigated by partial sequencing of the VP1 and 3Dpol genes, as well as including the corresponding areas from GenBank sequences. VP1 gene was analyzed for receptor binding sites including the integrin-binding RGD motif and the putative heparan sulfate (HS) site. Furthermore, phylogenetic analyses were combined with clinical data in a further attempt to analyze whether sequence evolution reflects CVA9 pathogenicity in the phylogenies. Analysis of the 559-nucleotide-long VP1 sequences identified six clades. Although most of the strains within each clade showed geographical clustering, the grouping pattern of the isolates in the analysis of the VP1 gene was strikingly different from grouping of 3Dpol, which suggested that recombination events may have occurred in the region encoding the nonstructural proteins. Inclusion of clinical data did not provide any evidence of symptom based phylogenetic clustering of CVA9 isolates. Amino acid sequence analysis of the VP1 polypeptide demonstrated that the RGD motif was fully conserved among the isolates while the putative HS binding site was found only in one isolate. These data suggest that integrin binding is essential for virus tropism, but do not explain the symptom repertoire.

P29

Exclusion of human rhinovirus type A16 by A1a in cell culture coinfections

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Abstract

Background

Rhinoviruses (RV) are a common cause for human upper respiratory infections. They circulate in communities worldwide at high frequency. Coinfections of an individual with different RV-types can alter disease progression and severity, while the superinfection of a cell with two or more RVs can lead to recombination and drive evolution.

Aim

We used the A1a and A16 types as a model in cell culture to explore exclusion mechanisms in coinfections. A1a uses the low density lipoprotein receptor (LDLR), and A16 the intercellular cell adhesion molecule 1 (ICAM1).

Methods

Viral genomes were visualized by type-specific branched DNA-fluorescence *in situ* hybridization. The results show that an infection by A1a reduced the number of A16-infected cells compared to single infections, but not vice versa. To determine the genetic element(s) of A1a leading to A16 exclusion, we manufactured recombinant A1a / A16 RV, and tested them in coinfection settings.

Results

We found that the recombinant RV harbouring the A1a P1 region (VP4, VP2, VP3, VP1 capsid encoding region) alone was excluded by A1a, akin to wild type A16, indicating that virus entry through the ICAM1 or LDLR pathway was not subject to exclusion. In contrast, the recombinant harbouring the 5’UTR and P1 of A1a was no longer excluded by A1a, which indicates that the 5’UTR of A1a is important in the exclusion of A16.

Conclusion

We provide initial genetic insights into superinfection exclusion of RVs, which is a basis for RV recombination.

P30

Global Diversity and Evolution of Encephalomyocarditis Virus

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Abstract

The near-complete genomes of 170 encephalomyocarditis (EMC) viruses were determined using Illumina-based Next Generation Sequencing. They originated from 17 countries: Australia (71), Belgium (6), Brazil (2), Canada (6), Cyprus (8), France (1), Germany (2), Greece (22), India (2), Italy (17), Netherlands (1), New Zealand (2), Panama (1), Puerto Rico (2), South Africa (3), United Kingdom (3) and the USA (21) and were isolated from hosts principally belonging to species within the family Suidae (75%) and the orders Rodentia (12.5%) and Primates (3%), with a selection from other animals including African elephants, tree kangaroos and a mosquito. Samples from which virus isolates were made had originally been collected between 1940 and 2000. The new sequences were compared to 54 published genome sequences. Phylogenetically, EMC viruses have been classified into two genotypes, EMCV-1 (most isolates) and EMCV-2 (only three examples have been found, one from a mouse in Germany; the second from and an agouti in Singapore; and the third from a greater bandicoot rat in Thailand). Our analyses suggest that a third genotype, which we have named EMCV-3, was isolated from orangutans in Singapore in 2002. Forty-four sequences (12 new and 32 published) were almost identical to the ATCC VR-129B prototype strain (which was purportedly isolated from a chimpanzee in Florida in 1944). Since many of these published sequences were isolated post-2000, it suggests the possibility of many instances of laboratory contamination.

Virus-host interactions and immune response

P31

Modulation of nucleotide metabolism by picornaviruses

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Abstract

Viruses actively reprogram the metabolism of the host (e.g. glucose, glutamine, fatty acid and nucleotide metabolism) to ensure the availability of sufficient building blocks for virus replication. We are beginning to understand how picornaviruses - a large family of small, non-enveloped positive-strand RNA viruses - actively modulate host cell processes to ensure efficient replication and to evade innate immune responses, but relatively little is known about modulation of host-metabolism by these viruses. Here, we studied the modulation of host-metabolism by the picornaviruses CVB3 and EMCV using both steady-state as well as 13C-glucose tracing metabolomics. We demonstrate that both CVB3 and EMCV increase the levels of pyrimidine and purine metabolites mainly through the degradation of nucleic acids and nucleotides and the recycling of nucleotides. Inhibition of this recycling by compounds restricts CVB3 replication dose-dependently. Finally, by integrating our metabolomics data with a previously acquired phosphoproteomics dataset, we identify several phosphorylations on enzymes directly involved in nucleotide metabolism, e.g. TK1 S13 and CAD S1859, that significantly change during CVB3 infection.

P32

The encephalomyocarditis virus frameshift protein 2B* temporally regulates lytic virus release.

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Abstract

Background

The genome of encephalomyocarditis virus (EMCV) encodes a novel protein 2B* of unknown function[1]. 2B* is translated following programmed ribosomal frameshifting (PRF) which is temporally regulated, increasing in efficiency from 0% early in infection to 70-80% at 6-8 hours post-infection (hpi)[2]. 2B* has no homology to other known proteins.

Methods

Premature termination codons were inserted into the 2B* open reading frame (ORF) to create a 2B* knock-out virus. Viral growth curves, cytotoxicity assays, immunofluorescent imaging and electron microscopy experiments were performed.

Results

While total viral titre was similar between wild-type (WT) and 2B* knock-out (KO) viruses, virus release was delayed in cells infected with the latter. Cytotoxicity assays confirmed that the bulk of virus release coincides with the time of lytic cell death. This occurred at 8-10 hpi in WT infection, but not till 16-24 hpi in 2B* KO infection.

Conclusion

Our data suggest that the timed production of 2B* enables temporal regulation of lytic virus release. We are currently testing two hypotheses: (i) that 2B* induces lytic viral release indirectly by preventing apoptosis or another cell death pathway, and (ii) that it does so directly via a cell signalling pathway.

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P33

Cytolytic properties and genome analysis of Rigvir® oncolytic virotherapy virus and other echovirus 7 isolates

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Abstract

Rigvir® is a cell-adapted, oncolytic virotherapy enterovirus, which derives from an echovirus 7 (E7) isolate. While Rigvir® is claimed to be oncolytic, causing a cytolytic infection in several cancer cell lines, there is little molecular evidence about its effectiveness when comparing against clinical echovirus strains. Phylogenetic analysis of the full-length data suggested Rigvir® being most distant from other E7 isolates used in the study. Rigvir® contained 9 unique mutations in the viral capsid proteins VP1-VP4, with 6 of the mutations exhibiting cytoplasmic exposure. One mutation, E/Q/N162G, was located in region that forms the DAF-E7 contact interface. Rigvir® and 5 other isolates were also subjected to cell infectivity assays performed on 8 different cell lines. The used cell lines contained both cancer and non-cancer cell lines to observe Rigvir®'s claimed properties of being both oncolytic and oncotropic. Infectivity assays showed Rigvir® having no discernable difference in the viruses' oncolytic effect when compared to the Wallace prototype or the four other E7 isolates. Rigvir® was also seen infecting non-cancer cell lines, bringing its claimed effect of being a safe oncotropic virotherapy under question. We conclude that Rigvir®'s claim of being an effective treatment against multiple different cancers is not warranted under the evidence presented here. Bioinformatic analyses do not reveal a clear mechanism that could elucidate Rigvir®'s function at a molecular level, and cell infectivity tests do not show a discernable difference in either oncolytic or oncotropic effect between Rigvir® and other clinical E7 isolates used in the study.

P34

Proteolytic activities of enterovirus 2A do not depend on its interaction with SETD3

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Abstract

Enterovirus $2A^{pro}$ is a protease that proteolytically processes the viral polyprotein and cleaves several host factors to antagonize host responses during enteroviral infection. Recently, a host protein SETD3 was identified to interact with $2A^{pro}$ and to be essential for virus replication. The role of SETD3 and its interaction with $2A^{pro}$ remain unclear. In this study, we tested the possibility that SETD3 participates in some of the functions of $2A^{pro}$. For this, we introduced the CVB3 $2A^{pro}$ in an EMCV mutant containing an inactivated Leader protein (EMCV-L^{zn}) that is unable to shut-down host mRNA translation, to trigger nucleocytoplasmic transport disorder (NCTD), and to suppress stress granule (SG) formation and type I IFN induction. We show that the resulting virus containing active $2A^{pro}$ (EMCV-$2A^{pro}$) efficiently cleaves eIF4G to shut off host mRNA translation and nucleoporins to trigger NCTD, and actively suppresses SG formation and IFN gene transcription both in wt HeLa cells as well in HeLa SETD3 knock-out ($SETD3^{KO}$) cells, arguing against a role of SETD3 in these $2A^{pro}$-mediated functions. Surprisingly, using a virus containing an inactivated $2A^{pro}$ mutant (EMCV-$2A^{m}$), we observed that the catalytic activity of enteroviral 2A is not critical for triggering NCTD, as EMCV-$2A^{m}$ infection induced NCTD in both wt and $SETD3^{KO}$ cells, albeit slightly delayed. This result challenges the previous notion that NCTD is critically dependent on the $2A^{pro}$-mediated cleavage of nucleoporins during enteroviral infection. Our results provide important new insights in the functioning of enteroviral 2A proteins.

P35

Microdomain Organization of Enterovirus Replication Organelles

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Abstract

While the activation of the cellular GTPases Arf by GBF1 is essential for the enterovirus RNA replication, the mechanistic role of Arfs in the replication process is not understood. We used super-resolution microscopy, proximity biotinylation, and other methods to investigate the distribution of Arfs on the replication organelles and their biochemical environment. We found that Arfs form isolated microdomains and that viral antigens are localized in both Arf-enriched and Arf-depleted microdomains. These data also imply that there is a mechanism of specific delivery of viral proteins to Arf-enriched domains. We established a HeLa cell line stably expressing Arf1 fused to a peroxidase APEX2 to biotinylate the proteins in the vicinity of Arf1. We found that 2C and 3CD were biotinylated as early as 3hpi and that the non-cleaved fragments of the viral polyprotein were overrepresented in Arf1-enriched domains. The co-IP experiments indicated that 2C directly interacts with activated Arfs. We also observed a significant change in the biotinylation pattern of the cellular proteins GBF1, OSBP, ACBD3, and PI4KIIIbeta, known to be important for the development of the replication organelles upon infection. Together, these data support a model that recruitment of GBF1 to the replication organelles generates foci of activated Arfs on the membranes, which further differentiate into specific microdomains through the recruitment of a specific complex of viral proteins and cellular Arf effectors, and that complexes of viral proteins in Arf-enriched and Arf-depleted domains likely support different stages of the viral life cycle.

P36

Characterisation of new mechanisms of host subversion by Rhinovirus: Identification of a novel phosphomimetic-based interaction between RV and 14-3-3 proteins

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Abstract

Rhinovirus (RV) infections are responsible for causing over 50% of all upper respiratory tract infections (URI's) that occur annually, and are known to exacerbate chronic pulmonary conditions such as chronic obstructive pulmonary disease (COPD), cystic fibrosis and asthma. Despite this significant socioeconomic impact, there are currently no approved antiviral drugs. An alternative strategy to developing antivirals is to inhibit RV/host interactions essential for viral replication. However, RV/host interactions are not fully understood, as the vast majority of host cell proteins essential for RV replication remain unidentified. One family of interest is the 14-3-3 proteins; a family of proteins that regulate biological processes by binding to phospho-Ser and phospho-Thr motifs on cellular proteins. Unpublished mass spectrometry analysis conducted in our lab shows that these proteins interact with a number of non-structural RV proteins. Moreover, 14-3-3 proteins have been implicated in playing important roles in the replication cycle of some flaviviruses such as dengue virus (DENV), West Nile virus (WNV) and Zika virus (ZIKAV). Bioinformatics analysis conducted on 160 RV strains revealed that RV contains a conserved Rx[E/D]P phosphomimetic similar to that employed by DENV, WNV and ZIKAV to bind 14-3-3 proteins. Additionally, siRNA knockdown of two 14-3-3 isoforms, 14-3-3-ε and 14-3-3-η results in delayed RV replication; suggesting these novel host proteins may play an essential pro-viral role during RV replication.

P37

Human intestinal organoids as a platform for investigating virus-host interactions in enterovirus infections.

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Abstract

Enteroviruses are among the most ubiquitous human pathogens. Typically, infection occurs at the intestinal epithelium; symptoms can range from sub-clinical to more severe diseases including acute flaccid paralysis, myocarditis, and meningitis, thus enteroviruses continue to present a significant threat to public health. Recent developments in culturing intestinal stem cells have led to the generation of intestinal organoids that mimic the structural and physiological characteristics of the in vivo intestinal epithelium. These advances allow us to use intestinal organoids to study viral disease through simulation of the viral infection dynamics seen in vivo, therefore revolutionising our ability to further understand enterovirus infections.

In this study, we infected intestinal organoids derived from patient stem cell samples taken from three regions of the intestine: the duodenum, terminal ileum, and sigmoid colon, with a number of virus strains from enterovirus species A, B, and C. We investigated the dynamics of the infections over time revealing significant differences in the intestinal epithelial regions which are permissible to infection by these different viruses. Interestingly, we also saw clear differences in both tropism and cytopathic effects of different virus strains within the same virus species. Our results suggest that the organoid system can be used to investigate the virus-host interactions of enterovirus infections.

In conclusion, using an intestinal organoid system as a platform for enterovirus infection, we are able to provide novel insights into virus-host interactions of enteroviruses across a number of important human pathogens.

P38

FMDV antigen/antibody-fragment interactions

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Abstract

Foot-and-mouth disease virus (FMDV) is the causative agent of a highly contagious febrile disease in cloven-hoofed animals, and outbreaks where the disease is endemic cause tremendous hardship. Current vaccines are highly strain sensitive. Antigen-binding fragments such as antibodies and nanobodies therefore tend to be highly specific. Understanding antibody/antigen interactions at an atomic level is crucial to understanding this specificity.

We compare and contrast high resolution cryo-EM structures for O-serotype virus-like particles (VLPs) with nanobodies M170 and C1, in addition to complexes of SAT1 ZIM/22/89 and Asia1 Shamir ISR/89 virus with INT-FS1-01-37 Fab and M332 nanobody respectively (reported gold-standard FSC = 0.143 resolutions: 2.9 Å, 2.9 Å, 2.8 Å and 2.0 Å).

Structures reveal all paratopes to involve two protomeric units, but differ in proximity to the pentameric interface; while neutralizing M170 nanobody (at 3 mg/ml) straddles across the pentameric subunit interface, non-neutralising C1 nanobody encircles the 3-fold axis of serotype O. More weakly neutralizing M332 (at 37 mg/ml) predominantly interacts with the VP1 C-terminus and DE loops within a pentameric unit of strain Asia1 Shamir. By contrast, Fab INT-FS1-01-37, which binds close to the site of nanobody C1, engages in a non-symmetric manner, such that only one Fab may be accommodated per three-fold axis.

These results enhance our understanding of epitope conservation and neutralising epitope distribution between serotypes.

Note:The first two authors contributed equally to this work

P39

RNA secondary structure in the foot-and-mouth disease virus genome shields the virus from sensing by ZAP

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Abstract

Many cellular and viral processes are highly dependent on specific RNA structures and/or sequences. RNA viruses are detected by several cellular ‘sensors’ or pattern recognition receptors (PRR), which trigger antiviral responses. One such PRR is the recently discovered zinc-finger antiviral protein (ZAP), activated by high frequencies of CpG dinucleotides in RNA sequences.

The interrelationship between viral RNA structure/sequence and the cellular antiviral response was investigated using foot-and-mouth disease virus (FMDV) engineered with secondary structure minimised genome sequences. This reduction of RNA secondary structure did not have an attenuating effect on FMDV replication in immortalised cell lines. However, we observed significantly reduced replication of such engineered viruses, in primary cell culture. This attenuated phenotype was reversed to almost WT levels by the inhibitor of interferon signalling, Ruxolitinib. To explore the potential mechanism of this attenuation, we used sub-genomic FMDV replicons and a ZAP KO cell line to show that the attenuated replication of secondary structure minimised genomes was also restored to wildtype levels in cells which lacked ZAP. To directly monitor the interaction between ZAP and viral RNA we immunoprecipitated ZAP from replicon transfected cells and quantitated the bound RNA. This showed that ZAP bound significantly more secondary structure minimised viral RNA relative to WT RNA. Together these data provide evidence that RNA secondary structure shields the FMDV genome from being sensed by ZAP. This attribute may provide a potential mechanistic explanation for the extensive RNA structure present in the genomes of many positive stranded RNA viruses.

Pathogenesis

P40

Identification and functional consequences of recombination events occurring in the 5' non-coding region of group-B Coxsackievirus genomic RNA in cultured human cardiomyocytes

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Abstract

Background. Recently, major group-B Coxsackievirus (CV-B) strains presenting genomic 5’ terminal deletions up to 50 nucleotides associated with minor full-length CV-B RNA forms have been characterized in cardiac tissues from patients with acute or chronic cardiomyopathies. These deletions could explain how the virus can drive the development of acute and chronic CV-B infections leading to human cardiomyopathies. Viral molecular mechanisms involved in the development of cardiomyocytes infection could be linked to molecular recombination events occurring in the 5' non-coding region (NCR). We investigated the existence of recombination events occurring in the 5’NCR between 5’ terminally deleted and full-length viral populations.

Methods. Synthetic non-replicating CV-B3 and CV-B6 RNA forms were transfected into human cardiomyocytes to investigate recombination events occurring in the 5’NCR. Nanoluciferase-tagged CV-B RNA forms were used to investigate the consequences of recombination mechanisms onto translation activities.

Results. Our experimental recombination model allowed us to map two major hot spots located into the spacer 1 and 2 sequences of the 5’-terminal and identified homologous (H=24%) and non-homologous (NH=76%) recombinants. These recombinant forms (H, NH) were characterized by a significant loss of viral genomic replication activities (Mann-Whitney, $p<0.001$). Using Nanoluciferase-tagged synthetic CV-B RNAs, we demonstrated a helper effect of the full-length RNA forms on the deleted forms and a braking effect of the deleted forms on the full-length forms on genomic replication and viral translation activities.

Conclusion. These molecular mechanisms could significantly contribute to the development of acute and chronic CV-B cardiomyocytes infections in some patients with Enterovirus-induced cardiomyopathies.

P41

Efficient replication of enterovirus-D68 in human skeletal muscles leads to cell damage and production of new infectious viruses

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Abstract

Enterovirus-D68 is a respiratory virus that can also cause neurological complications, especially acute flaccid myelitis (AFM). AFM is characterised by limb paralysis, but the cause of this paralysis in humans remains elusive. Paralysis possibly happens due to direct damage to the skeletal muscles as a result of viral replication, or due to infection of skeletal muscles and subsequent virus spread to motor neurons via the neuromuscular junction. Both mechanisms have been studies in mice, but not in humans. To investigate which mechanism potentially leads to the development of EV-D68-associated paralysis, we studied the susceptibility and permissiveness of human skeletal muscles for different EV-D68 clades. We inoculated human inducible pluripotent stem cell (iPSC)-derived myotubules with EV-D68. The presence of intracellular viral capsid protein VP1, the replication of viral RNA and the production of infectious virus particles were monitored daily up to 72 hours post-inoculation (hpi). The infected myotubules showed cytopathic effect as early as 24 hpi. At this time, presence of VP1 and increase of viral RNA were detected. The infected myotubes also produced new infectious virus particles and the viral titre increased over time. Ongoing studies aim to get more insight into functional changes of the skeletal muscle cells and clade-specific differences. Based on these data, we concluded that human iPSC-derived myotubes are susceptible and permissive to EV-D68 infection and the infection leads to the destruction of these cells, suggesting that direct infection of skeletal muscles potentially leads to AFM in humans.

P42

Replication efficiency of different enterovirus-D68 isolates in hiPSC-derived spinal motor neurons and cortical neurons co-cultured with astrocytes.

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Abstract

Enterovirus D68 (EV-D68) is causing outbreaks of respiratory disease since 2014 and has been associated with the development of acute flaccid myelitis (AFM). Besides AFM, EV-D68 is also associated with other neurological complications, such as encephalitis. Although multiple clades have been circulating since 2014, neurological complications are not associated with a certain clade. However, whether viruses from different clades or those circulating before 2014 and after 2014 differ in their cell tropism or replication efficiency in cells of the CNS remains unknown. Here, we determined the infection efficiency and replication kinetics of EV-D68 clades on human inducible pluripotent stem cells (hiPSC)-derived spinal motor neurons (sMN) and cortical neurons co-cultured with astrocytes. Viruses from clades isolated before 2014 (A, B2039, B1) and after 2014 (A2, B3) were included. All viruses replicated efficiently in cortical neural co-cultures and infected predominantly cortical neurons rather than astrocytes. In sMN, isolates from subclade A2 and B1 replicated more efficiently than other isolates. Immunofluorescence for viral antigen suggested differences in infection efficiency. Infection of sMN and cortical neuron co-cultures did not result in visible cell death or the induction of apoptosis based on the expression of cleaved caspase-3. Our studies suggest that both cortical and motor neurons are susceptible and permissive to EV-D68 infection. However, there are no clear differences in replication efficiency between viruses isolated before 2014 and after 2014 in these hiPSC-derived neural cells. Ongoing studies focus on how EV-D68 infection could result in neuronal dysfunction.

P43

Human cerebral organoids to study neurotropism of different enterovirus D68 clades.

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Abstract

Enterovirus D68 is a positive strand RNA, non-polio enterovirus that has caused several outbreaks of respiratory disease in the last decade. In recent outbreak, EV-D68 infection can cause polio-like acute flaccid myelitis (AFM) in children. One possible explanations for the rise in neurological symptoms in recent outbreaks, is that neurovirulence is a clade specific feature, another possibility is that changes in the receptor usage lead to increased replication or neurotropism. In neuronal cell lines, EV-D68 strains that have acquired the ability to use heparan sulfate proteoglycans (HSPG) as an additional receptor besides sialic acid, have increased neurotropism compared to EV-D68 strains that are dependent on sialic acid for infection. We tested both of these hypotheses in cerebral organoids, which are induced pluripotent stem cell derived 3D cultures that mimic the developing human brain. We infected cerebral organoids with EV-D68 strains from multiple clades (clade A, B2, B3 and D), and we compared the infection of a sialic acid dependent strain to sialic acid independent strains. We show that EV-D68 replicates in cerebral organoids and that infection of cerebral organoids is not clade specific. Additionally, our data suggests that the dual sialic acid and HSPG binding strains do not have increased neurotropism compared to the sialic acid dependent strain, as all strains replicated in the cerebral organoids. Therefore the increase in AFM caused by EV-D68 is unlikely dependent on the EV-D68 clade or on HSPG binding. Further research using clinical EV-D68 isolates from AFM patients is needed to confirm these results.

Treatment and prevention

P44

Willow bark-derived antivirals kill infectivity through stabilization of enteroviruses

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Abstract

Enteroviruses can cause an array of diseases ranging from common cold to more serious acute and chronic infections. Currently, no vaccines (except for poliovirus and EV71) or clinically approved antivirals are available against these viruses. In addition, these viruses are relatively resistant to commonly used disinfectants. Hence, it is imperative to find novel ways to reduce the viral load on the surfaces and in the environment. Here, we report the efficacy of extracts isolated from the bark of Salix L. species against coxsackievirus A9 (CVA9). Leaves and barks of willows are known in herbal medicines for their ability to reduce fever and pain. Bioactive compounds isolated from willows have shown to have bactericidal, antioxidant and anti-cancer properties. Screening of several willow clones using cytopathic effect (CPE) inhibition assay, showed their effectiveness in protecting the A549 cells from CVA9 infection. None of the reference compounds (salicin, salicylic acid, picein and triandrin) tested showed any antiviral activity, suggesting presence of bioactive agents other than these compounds, responsible for Salix spp. antiviral activity. The time and temperature studies showed that the pretreatment of virus with the extracts at room temperature for 45 seconds was sufficient to kill the virus infectivity. Mechanistic studies performed using dynamic light scattering, transmission electron microscopy and particle stability assay revealed that the tested extracts prevented infection mainly through clustering and stabilization of the virions. Studies are on the way to find the bioactive compounds and the mechanisms behind their action.

P45

Structural and immunological characterisation of stabilised EVA71 VLPs as a potential vaccine candidate.

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Abstract

Enterovirus A71 (EVA71) causes a potentially fatal disease in young children. In common with other picornaviruses both empty capsids (ECs) and infectious virions are produced during the viral lifecycle. While initially antigenically indistinguishable, ECs readily convert to an expanded conformation at moderate temperatures. In the closely related poliovirus these conformational changes result in loss of antigenic sites required to elicit protective immune responses. Whether this is true for EVA71 remains to be determined and is the subject of this investigation.

To this end we selected a thermally resistant genogroup B2 EVA71 population using successive rounds of heating and passage. The mutations found in the structural protein-coding region of the selected population conferred increased thermal stability to both virions and naturally produced ECs. We introduced these mutations into a recombinant expression system to produce stabilised virus-like particles (VLPs) in Pichia pastoris. We then characterised these structurally and immunologically.

Unlike poliovirus, both native and expanded EVA71 particles elicit antibodies able to directly neutralise virus in vitro, suggesting that many of the antigenic sites present on native particles are unaffected by the conformational changes associated with particle expansion. However, unlike wild-type VLPs, stabilised VLPs maintain a native conformation as determined by reactivity with a distinguishing antibody. The immunological consequences (if any) of these differently reactive VLPs are being explored. Interestingly, structural studies of stabilised VLPs suggest an unusual mechanism of stabilisation by destabilising the expanded conformation.

P46

Development of a novel approach to broadly-protective anti-enterovirus vaccines

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Abstract

Enteroviruses are arguably the most numerous human viral pathogens. Due to high antigenic diversity, the development of traditional capsid-targeting vaccines is feasible for only few enteroviruses. Yet, their replication proteins are much more conserved and are essentially interchangeable, as evidenced by the extensive recombination of enterovirus genomes. We observed that upon immunization with live polioviruses of mice and non-human primates, the animals develop antibodies not only against the capsid proteins but also against a number of non-structural proteins, indicating that antigens from conserved non-structural proteins are presented to and recognized by the immune system. We set to investigate if a vaccine based on expression of conserved replication proteins only could be protective, and if that protection could be extended to antigenically diverse enteroviruses. We trans-packaged the poliovirus replicon RNA coding for P2P3 proteins using our previously developed Newcastle Disease Virus (NDV) vectored vaccine expressing poliovirus virus-like particles. This trans-packaging system based on efficiently replicating poliovirus P2P3 RNA and NDV allows cost-effective production and purification of packaged replicons. These replicons can be administered similarly to live poliovirus vaccine, but the capsid proteins are present only in the original inoculum and are not produced upon replicon RNA replication. Preliminary experiments in transgenic mice demonstrate that replicon immunization indeed promote a much higher presentation of non-structural proteins to the immune system, however a significant development of antibodies against capsid proteins was also observed, indicating that an alternative replicon RNA delivery system is required to completely remove the input of immunodominant capsid proteins.

P47

G-quadruplex stabilization in the rhinovirus genome inhibits uncoating

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Abstract

Rhinoviruses (RVs) are the major cause of the Common Cold. RV infection can lead to health complications through comorbidities, and colds are also a huge socio-economic burden. However, there are currently no vaccines or approved drugs for treating RV infections. A new approach to tackling viruses is by targeting structural elements in their genome. G-quadruplexes (GQs) are an attractive target for such therapy. RV genomes contain G-rich sequences, including four strictly conserved predicted GQs. The functional relevance of these sequences remains unclear. Here we demonstrate that a prototypic RV, RV-A2, forms GQs and examine their potential as novel anti-rhinoviral targets. Using biophysical analyses, we show that synthetic ribooligonucleotides representing putative GQ-forming sequences from RV-A2 adopt a GQ structure that is stable under physiological conditions. Notably, one conserved sequence forms an unconventional, two-layer zero-nucleotide loop GQ. In the context of the viral genome, most putative GQ-forming sequences are constrained in alternative metastable structures. The GQ-binding compounds pyridostatin (PDS) and PhenDC3 interfered with viral uncoating in Na^+ but not in K^+-containing buffers. Thermostability studies and ultrastructural imaging of ex-virion RNA suggest that Na^+ keeps the encapsidated genome less condensed, allowing PDS to reach the metastable structures and stabilize GQ formation. The resulting conformational changes in the viral genome impair RNA release from the virion, identifying this critical early step in the viral life cycle as a potential therapeutic target.

P48

Hepatitis A RNA contamination of routine serum samples with RNA present in the reagents of the commercial anti-HAV serological assay

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Abstract

Background

Diagnosis of hepatitis A virus (HAV) infection relies on serology and, in specific cases, on the HAV RNA detection. In our laboratory, HAV RNA-positive cases were repeatedly identified, unspecific to patients' serological profile/medical history.

Methods

Several rounds of HAV RNA external quality assessment (EQA) schemes and contamination checks of laboratory surfaces were performed, questionable samples assayed with different HAV RNA tests, and additional serum/stool samples collected from puzzling HAV RNA-positive patients and healthy employees.

Results

All contamination-check-samples and employees' samples tested HAV RNA-negative and laboratory successfully passed all EQA schemes. Positive results were reproducible with different HAV RNA assays. None of the HAV RNA-positive patients seroconverted to support HAV infection and their stool samples were consistently HAV RNA-negative. Finally, an association between HAV RNA-positivity and preceding anti-HAV testing on Roche Cobas e411 automated serological analyser was observed. When HAV RNA-negative samples from healthy controls were first tested on Cobas e411 and subsequently for HAV RNA, all samples tested HAV RNA-positive, confirming preceding serological testing as contamination source. HAV RNA was also detected in Cobas e411 anti-HAV reagents, further proven by sequencing. Initial anti-HAV serological testing using two other automated serological analysers followed by HAV RNA testing from the same aliquot did not result in HAV RNA contamination.

Conclusion

Because Cobas e411 uses a single pipette tip for sampling anti-HAV reagents and samples, reflex HAV RNA testing should not be performed from same sample aliquot. Serological and molecular testing should be always performed from different sample aliquots.

P49

Occurrence and elimination of contaminant extracellular vesicles from insect cell cultures

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Abstract

Species of the enteroviruses genus are the causative agent of hand, foot, and mouth disease (HFMD), which in severe cases cause encephalitis, meningitis, and polio-like flaccid paralysis. Recent efforts in vaccination against Enterovirus A71, which is the major causative virus of HFMD, lead to a significant increase of the HFMD-causing enterovirus species coxsackievirus A6, A10, and A16. This disease burden demands the rapid development of an alternative vaccination approach, desirably with broad-spectrum applicability. Virus-like particles (VLPs) are prime candidates for such broad-spectrum vaccines due to their non-infective and non-replicative nature.

During the effort to create multivalent enterovirus VLPs by the baculovirus expression vector system, we observed the emergence of extracellular microvesicles (EVs). Little is known about the evolutionary origin and function of EVs, with hypotheses ranging from modulation of virulence to cell signaling. With EVs measuring between 10-80 nm in diameter, the identification and purification of VLPs of similar dimensions become challenging, especially as conventional purification techniques for viruses and VLPs (e.g. sucrose cushions, sucrose density gradients, and PEG (polyethylene glycol)-precipitation) can be inadequate for the complete separation of the EVs from either VLPs, expressed proteins, or the baculovirus vector. Here, we present an alternative methodological approach for the elimination of these extracellular EVs that does not impair viral protein production or VLP assembly that is based on the disruption of polar and hydrophobic interactions that underlie the formation of EV envelopes. This methodology facilitates the purification of VLPs by eliminating a major contaminant.

P50

Exploitation of Bioreactor technology for consistent high yield production for next generation VLP polio vaccines

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Abstract

The development of vaccines for poliovirus (PV) and their use in subsequent global vaccination programs has reduced the prevalence of the virus to near eradication.

Immunisation will need to continue for the foreseeable future to complete and maintain eradication of PV. However, cultivation of live poliovirus is required for the manufacture of both the oral polio vaccine (OPV) and the inactivated polio vaccine (IPV), which carriers the obvious risk of accidental reintroduction of PV.

The use of virus like particle (VLP)-based vaccines offers the potential to remove the need to cultivate live PV for vaccine manufacture. However, VLPs based on wild-type PV sequences have demonstrated inherent instability.

The particles convert from the native (N or D) conformation to a heated (H or C) conformation at low temperatures, this C confirmation shows greatly reduced I Immunogenicity and is unsuitable for use as a vaccine.

We have addressed this problem by introducing stabilising mutations into the viral capsid sequences and expressed the stabilised VLPs in Pichia pastoris using laboratory scale technology. There remains, however, a need to maximise both the yield and D/C ratio of the recombinant VLPs.

Bioreactors allow improved control of conditions for yeast growth and protein expression and so may be used to increase yields and optimise the ratio of D to C.

In collaboration with CPI, we have shown that this expression system is compatible with fermentation, and that the use of Bioreactors results in higher yields and more favourable ratios of D to C antigens.

P51

A glutathione binding site in polio and other enteroviruses is a target for antivirals and stabilisation of vaccine candidates

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Abstract

Polio virus-like particle (VLP) vaccines have clear advantages over inactivated or attenuated virus vaccines especially after eradication, however, instability can alter their antigenic conformation, compromising their immunogenicity.

We have investigated two potential antiviral pockets in terms of the stabilisation afforded by natural ligands and antiviral molecules. Glutathione (GSH), a crucial co-factor for the morphogenesis of many enteroviruses including poliovirus (PV) has been shown to bind a positively charged pocket at the protomer interface in enterovirus F3, a pocket observed to bind putative antiviral benzene sulfonamide compounds in other enteroviruses. Lipid molecules or natural pocket-factors bind a hydrophobic tunnel in the VP1 beta-barrel accessible from the surface depression surrounding the five-fold axis. High affinity pocket-factor mimics, e.g., pleconaril or GPP3, can replace the lipid, over-stabilising the particle, preventing uncoating.

We report single-particle cryo-EM structures of GSH bound to stabilised PV3 (mutant SC8) VLPs in combination with GPP3 or pleconaril. We also present a structure for the benzene sulphonamide CP17 bound to wt PV2 VLPs.

The effect of GSH on the antigenic state of VLPs was investigated following heating in the presence of various concentrations of GSH. We show that GSH can enhance particle stability in all serotypes. Binding of GSH or an analogous tight-binding antiviral at the interprotomer interface did not impede or alter the ability of the VP1 hydrophobic pocket to bind potential drugs such as GPP3, so effects at the two druggable sites, separated by some 22 Å in the capsid are likely to be additive.

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Mutational stabilisation, structural authentication, recombinant expression (mammalian, insect, plant and yeast) and immunogenicity of poliovirus virus-like-particles (VLPs).

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Abstract

As we approach global eradication of polio there is an imperative need to produce the vaccines that are predicted to be required for the foreseeable future through methods that do not require the cultivation of replication competent poliovirus. Replacement of the current inactivated polio vaccine (IPV) vaccines with virus-like particle (VLP) vaccines could meet this requirement. However, VLPs produced using wild-type viral protein sequences are too antigenically unstable for practical purposes. We have addressed this problem through the introduction of stabilising mutations in the capsid proteins for each of the poliovirus serotypes, resulting in particles with similar or greater stability to IPV. Both high resolution cryoEM structure determination and antigenic analyses have shown that the stabilised VLPs are equivalent to wild-type particles.

We went on to compare expression of the stabilised VLPs in a variety of recombinant systems to determine their suitability for commercial development of cost-effective industrial scale production of polio vaccines. Mammalian cell, insect cell (via baculovirus), whole plant and yeast (Pichia pastoris) systems were investigated, in addition to production in cell-free extracts.

Finally, the immunogenicity of the stabilised VLPs was investigated using the CD155 Tg mouse model, which allows for live virus challenge, and the approved vaccine batch release assay used to check commercial vaccines. With the inclusion of adjuvants approved for human vaccines the immunogenicity of recombinantly expressed stabilised VLPs was equivalent to, or superior to IPV, thereby highlighting the potential for these recombinant VLPs to become the next-generation PV vaccines.

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An innovative, safe and effective antiviral decontamination glycomaterial for non-toxic removal of viruses and proposed modifications to control picornavirus infections

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Abstract

Background

Many microorganisms use lectins as adhesins to interact with host glycoconjugates and to trigger the first steps of infection. Glycomimetics have become attractive tools to hamper pathogen adhesion to host cells. In order to overcome the shortcomings of current decontamination methods, we developed an antiviral decontamination glycomaterial that is effective against a range of pathogens and can be used for the safe and efficient capture and removal of viruses from skin, as well as surfaces.

Methods

The antiviral decontamination glycomaterial proposed in this study incorporates a biodegradable cellulose matrix, with a mix of irreversibly attached active agents (glycoconjugates). This material mimics natural cell membranes with multiple presentations of predominant binders and effectively captures the pathogen in a hook and loop manner resembling a microbiological Velcro^{TM} system. The binders were carefully selected using cutting-edge microarray technology and bioinformatic research. Efficacy tests were then conducted to demonstrate thecapture and removal of target pathogens from selected surfaces.

Results

An average of 90-99% pathogens (influenza virus and selected bacteria) were removed from contaminated surfaces. During the COVID-19 pandemic, an independent laboratory of expertise has proven the efficiency of the adapted glycopolymer to capture and remove up to 99.99% of SARS-CoV-2 from human skin. Recently, we have conducted bioinformatic searches, reviewed literature and biochemical studies and structurally characterised interactions to nominate candidate glycan receptors that will bind to picornaviruses.

Conclusion

Our results offer an excellent platform for the design of antiviral glycomaterials. The proposed modification of our glycopolymers against picornaviruses remains to be tested.

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Towards a universal rhinovirus vaccine: Characterisation of antibody responses induced by conserved capsid epitopes

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Abstract

Rhinoviruses (RV) are responsible for 70% of respiratory tract infections and are estimated to cause losses of $40 billion annually to the US economy alone. Despite this, no RV vaccines are available due to the approximately 150 serotypes which generate little to no cross-protection. However, antibodies against the highly conserved N-terminus of capsid protein, VP4, have been shown to neutralise multiple serotypes. Here, we investigate the ability of RV VP4 N-terminal peptides (N-VP4) to induce an antibody response. Mice were prime-boost vaccinated with different sized N-VP4 peptides: amino acids 1-15, 1-30, and 1-45 (N15, N30, and N45) using two different peptide display systems (keyhole limpet hemocyanin (KLH) or SpyCatcher VLPs) and bled 2 weeks after each vaccination. Mouse sera were analysed by ELISA to assess response against N-VP4$_{1\text{-}15}$, N-VP4$_{1\text{-}30}$, and N-VP4$_{1\text{-}45}$. Further, reactivity with a series of overlapping peptides was used to identify predominant epitopes recognised by the sera. Our study showed that SpyCatcher- and KLH-conjugation elicit distinct responses that target different parts of N-VP4. SpyCatcher-conjugated N30-vaccination induced a highly consistent response while the KLH-conjugated peptides of different lengths induced a broader range of less consistent responses. Interestingly, responses against the extreme N-terminus of VP4 were not produced in response to longer peptides, suggesting VP4 peptides may adopt a conformation restricting availability of some epitopes. Together, this indicates that N-VP4 peptides can induce an antibody response which can be fine-tuned based on peptide length and choice of presentation method.

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