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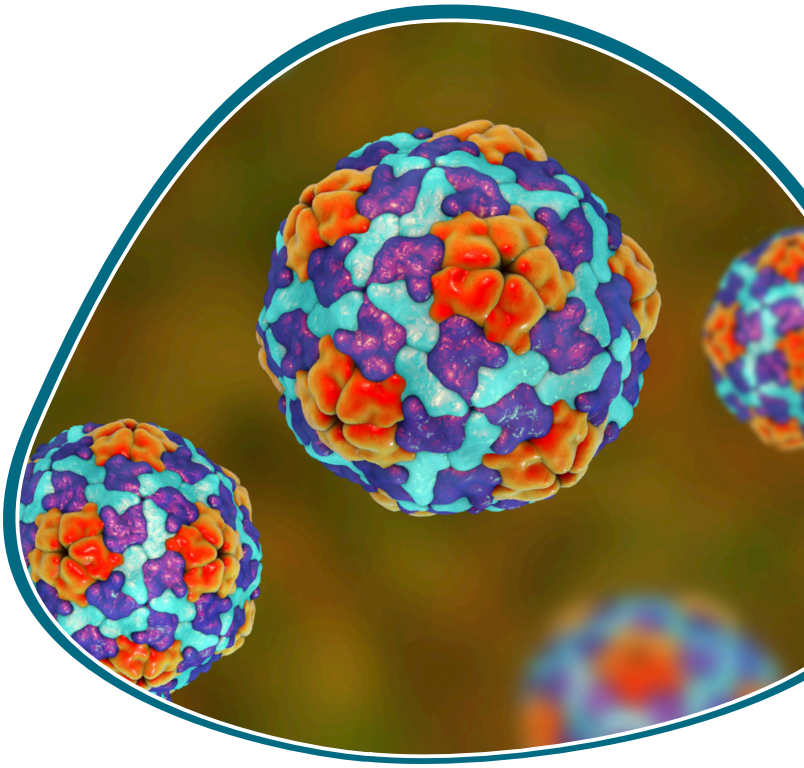
European Study Group on the Molecular Biology of Picornaviruses



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ORAL ABSTRACT BOOK

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Virus epidemiology and emergence

1

Seroepidemiology of Enteroviruses: opportunities and challenges

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Abstract

Serological markers give us information on previous exposure to a pathogen and can be used to infer population-level processes such as past changes in transmission and population immunity. For pathogens such as Enteroviruses, for which a large number of infections are asymptomatic, circulation patterns cannot be revealed using case data only, as reporting of cases is affected by their severity, the willingness of clinicians to submit biological samples for testing, and of labs to report their data, among others. Serological data thus offers new opportunities to infer Enterovirus circulation dynamics. In this talk, I will give an overview of recent studies that have generated and used serological data to further our understanding of the epidemiology of Enteroviruses. Along the way, I will also discuss some of the challenges that we encounter when it comes to interpret or analyse these data.

2

Re-emergence of enterovirus D68 in Europe after easing the COVID-19 lockdown in 2021

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Abstract

Between 31st July and 14th October 2021, the European Non-Polio Enterovirus Network (ENPEN) reported a rapid increase of enterovirus D68 (EV-D68) infections. A total of 139 cases were reported from eight European countries. The upsurge is in line with the seasonality of EV-D68, but high numbers presumably spurred by the widespread reopening after COVID-19 lockdown. During the study period most cases were identified in September and no cases of acute flaccid myelitis (AFM) were identified. After the report cases continued to be reported from these 8 and additional countries. Furthermore, a total of 6 cases with AFM were reported. A complete epidemiological and phylogenetic analysis of the 2021 upsurge in Europe is being conducted and can be presented in meeting. Reinforcement of clinical awareness, diagnostic capacities and surveillance of EV-D68 is urgently needed in Europe.

3

Human parechovirus (HPeV) circulation and epidemiology across the Europe Union (EU) and European Economic area (EEA), 2015-2021

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Abstract

Background: Human parechoviruses (HPeV) infections can range from asymptomatic to life threatening, affecting mainly children. Since the absence of a systematic HPeV surveillance, it is difficult to estimate HPeV-burden in Europe.

This study aimed to asses HPeV circulation, seasonality and epidemiology in Europe.

Methods: A retrospective-descriptive analysis was conducted in 2018 by requesting to EU/EEA national focal points to collect aggregated information on number of HPeV detected by month, HPeV-type, clinical symptoms and age-group, from January-2015 to December-2017. Data up to December-2021 and on “no testing” information were collected in March-2022.

Results: 943 HPeV positive samples were reported from January-2015 to December-2017, with Ireland (n=332), Norway (n=202) and England (n=150) reporting the highest numbers of HPeV-positive samples, followed by Spain (n=89), Denmark (n=85), Italy (n=45), Scotland (n=17), Slovenia (n=15), Austria (n=6) and Luxemburg (n=2). Of the 181 samples typed, HPeV3 was the most common (n=126), followed by HPeV1 (n=33), HPeV6 (n=13), HPeV4 (n=7) and HPeV5 (n=2). Clinical data for 414 subjects were reported, of which 61% were males <3 months (n=277). Fever was the most reported symptom (n=115), followed by neurological signs (n=70), respiratory symptoms (n=39) and sepsis (n=14). To the new call 7 additional countries participated, of which 5 reported “zero testing” information; other analyses are ongoing.

Conclusion: Although these results demonstrate the wide circulation of HPeV in Europe, which tends to target infants <3 months and cause neurological infections, HPeV testing remains heterogeneous. Improve awareness on HPeV is needed to enable systematic testing and surveillance in Europe.

4

Environmental surveillance for poliovirus in the UK between 2017-2022. Recurrent isolation of type 1, 2 and 3 vaccine-like polioviruses

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Abstract

Environmental surveillance is a sensitive method for detecting human enterovirus circulation, and it is used worldwide to support global polio eradication. We use both virus isolation and direct detection molecular methods in combination with Next Generation Sequencing analysis for the detection and identification of poliovirus strains in wastewater samples collected in sewage plants. We report recurrent isolation of type 1, 2 and 3 vaccine-like poliovirus strains from wastewater samples collected in two UK sites between 2017 and 2022. Poliovirus isolates from sewage specimens contained a very low number of mutations from the Sabin vaccine strains indicating a very short period of replication/transmission in humans, from several days to few weeks after vaccination, with these PV strains possibly having been excreted by just one or few recent vaccinees and/or their immediate contacts. Several isolates showed recombinant structures, very common among enteroviruses, including a type 2 vaccine-like poliovirus that had recombined with an unidentified non-polio enterovirus strain. A follow-up study was conducted following isolation of a type 2 poliovirus showing 5 VP1 nucleotide differences from Sabin 2 vaccine strain in October 2021. This PV2 strain was not detected in any of the 140 sewage samples analysed from the area spanning few weeks before and after the positive sample from October 2021 was collected. We conclude that no widespread poliovirus transmission occurs in the UK and that the vaccine-like poliovirus isolates found in wastewater samples are likely derived from people travelling from countries still using oral polio vaccine.

5

Rapid, direct sequencing of poliovirus from stool samples for routine AFP surveillance in the Democratic Republic of the Congo

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Abstract

Poliovirus can be sequenced directly from stool without intermediate culture steps via the Direct Detection by Nanopore Sequencing (DDNS) protocol. This method allows poliovirus serotype determination in as little as 3 days after samples arrive at a laboratory and produces a full-length VP1 sequence required for identification of vaccine-derived poliovirus (VDPV).

DDNS was implemented in parallel to the current culture-based algorithm at the Institut National de la Recherche Biomédicale in the Democratic Republic of Congo (DRC) from November 2021 to January 2022. Over 150 prospective stool samples from acute flaccid paralysis (AFP) cases and their contacts were processed each week, with 2,089 samples tested over the three-month period.

We found comparable sensitivity of DDNS and cell-culture methods (DDNS versus cell-culture as gold standard: 92%, 95% CI: 74-99%. Cell-culture versus DDNS as gold standard: 92%, 95% CI: 74-99%) for the detection of serotype 2 VDPVs (n=25). Both methods displayed high specificity. Where a Sanger sequence was available through the culture-based algorithm (n=16), DDNS consensus sequences showed a mean similarity of 99.97%. DDNS provided these sequences a median of 13 days after sample collection (median 6 days collection to receipt, 7 days receipt to sequence) compared to a median 39 days for the culture-based algorithm.

Stool testing by DDNS allowed early detection of a cVDPV2 emergence in DRC and has the potential to greatly increase the speed of poliovirus detection and response in routine AFP surveillance. Once trained in DDNS, these skills can be applied to the rapid sequencing of other targets.

6

Implementing World Health Organization (WHO) Global Action Plan III Containment Safeguards for United States (U.S.) Poliovirus Type 2 Laboratories

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Abstract

Since 2019, wild polioviruses type 2 (PV2) and type 3 were declared eradicated. WHO published containment requirements in the Global Action Plan III (GAPIII) for poliovirus-essential facilities (PEFs) with three safeguards to mitigate risks of a facility-associated release. U.S. PV2 facilities were identified in national poliovirus inventories. We developed and audited 35 containment measures as enhancements to biosafety level 2 (BSL-2) standards during transition to GAPIII (primary safeguard, laboratory containment). National population vaccination estimates were based on annual National Immunization Survey-Child data (2016-2019), including estimates for counties within a 100 km radius of PEF zip codes (secondary safeguard, inactivated polio vaccine third dose [Polio3]). A standardized sanitary sewage system questionnaire was used at three site visits to PEFs and associated wastewater treatment plants (WWTP) [tertiary safeguard, secondary effluent treatment].

Twenty-six PV2 PEFs were identified and reduced to 15 PEFs following the destruction or transfer of PV2 materials (58% decrease). Fifteen PEFs implemented NAC containment measures (median=22/35), with 13/15 endorsed as PEFs (2018-2021). National Polio3 coverage estimates were >90%, with county estimates ranging from 89.9 – 97.5%. Environmental assessments found WW catchment areas served populations of 35,000, 750,000, and 2,300,000 residents for PEFs A, B, and C, respectively. The average WW flow for these WWTP was 2 million gallons per day (MGD), 43 MGD, and 300 MGD. All three WWTP have common WW treatment methods, redundancy in treatment processing/treatment trains, and achieve secondary treatment.

The U.S. made substantial progress in implementing GAPIII containment safeguards to support the global polio eradication effort.

7

Comparison of evolutionary trajectories of Sabin 2 and nOPV2 polio vaccines

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Abstract

We have previously described the design and characterisation of a new live-attenuated poliovirus type 2 vaccine strain (nOPV2) which is predicted to be more genetically-stable than the current oral vaccine strain (Sabin 2), hence less likely to evolve into cVDPVs. nOPV2 is currently being rolled out under WHO Emergency Use Listing following successful clinical trials.

Many aspects of the evolution of Sabin 2 in humans are well known, including the rapid selection for reversion of the key attenuating determinant in domain V of the 5'UTR. Extensive passage experiments under selective conditions have repeatedly shown that the modifications introduced into this region of nOPV2 are genetically stable. However, these experiments and studies of the evolution of nOPV2 in vaccinees suggest there are other positions where mutations are selected on long-term replication. We have constructed viruses with combinations of these mutations and tested their neurovirulence by intraspinal inoculation of susceptible transgenic mice. From this it is clear that the evolutionary trajectories of Sabin 2 and nOPV2 are quite different in terms of their acquisition of virulence, both extent and rate.

Polio vaccine strains also evolve through recombination, with each other and with non-polio enteroviruses. We have constructed synthetic nOPV2 recombinants modelling those that might arise in the field and will present data on the effects of exchanged segments on neurovirulence, growth and relative fitness in cell co-culture and evolution on passage.

These analyses will be useful in interpreting genetic data from the sequencing of isolates obtained following field use of nOPV2.

8

Impact and effectiveness of novel type 2 oral poliovirus vaccine during emergency use listing

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Abstract

Background

Over 228 million doses of novel type 2 oral poliovirus vaccine (nOPV2) were used during supplementary immunisation campaigns (SIAs) in 2021 to stop outbreaks of circulating type 2 vaccine-derived type poliovirus (cVDPV2).

Methods

To estimate the effectiveness of nOPV2, we conducted a matched case-control analysis in Nigeria between 2016 and 2021. To estimate impact, we calculated the relative risk of cVDPV2 incidence and odds of detection in environmental surveillance (ES) after nOPV2 SIAs versus before in nine countries. We compared nOPV2 with monovalent type 2 oral poliovirus vaccine (mOPV2), another vaccine used to stop cVDPV2 outbreaks .

Results

As of February 2022, we estimate low effectiveness of nOPV2 and mOPV2 in Nigeria (using test-negative controls nOPV2 11% [95% CI -9-32%]; mOPV2 12% [95% CI -8-28). We find significant reductions in cVDPV2 incidence (RR 0.83 [95% CI 0.72-0.96]) and prevalence (OR 0.81 [95% CI 0.68-0.96]) following nOPV2 SIAs, and no significant difference between nOPV2 and mOPV2 impact apart from in northern Nigeria, where nOPV2 SIAs have had little effect on incidence (RR 0.95 [95% CI 0.84-1.07] versus 0.60 for mOPV2 [95% CI 0.46-0.77].

Conclusion

We found that incidence and prevalence of cVDPV2 were generally reduced following nOPV2 SIAs, comparable to that following mOPV2 SIAs. In addition, with lower risk of reversion to neurovirulence compared to mOPV2, nOPV2 SIAs are expected to have lower risk of seeding new outbreaks. Protection to individuals was not apparent, possibly because our analysis relies on reported immunisation history.

Translation and Replication

9

Early events during enterovirus replication

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Abstract

The nasal and gastrointestinal epithelium is the primary entry site of many picornaviruses. We are using air-liquid interface (ALI) cultured primary nasal epithelial cells to better understand how enteroviruses traverse the nasal mucus and periciliary mucin layers to infect the nasal epithelium. We are examining the role of host cell and viral structures on virus entry and spread. Also, we have isolated thermostable enteroviruses with dramatic particle structural and mechanistic alterations.

Next, viruses must suppress the first interferon (IFN) responses to establish a successful infection. We hypothesize that capsid proteins, in addition to their structural role in virus particles, are critical to modulate antiviral responses at the early stage of infection and pathogenesis. Genetic analyses indicate that VP4, an internal capsid protein, can modulate IFN responses both in cell culture and animal models. Proteomic analyses uncovered that VP4 interacts with host factors implicated in innate immunity.

During the immediate next step following entry enteroviruses must hijack the cellular translation machine to initiate virus protein synthesis. We used proteomics to compare polysome composition during infection with enteroviruses and flavivirus. These viruses dramatically remodel similar aspects of translation to generate specialized viral polysomes. They employ convergent strategies to implement common changes in mRNA surveillance and canonical translation initiation. They also induce virus-specific recruitment of non-canonical translation factors and proteostasis components. We propose that defining virus-induced polysome specialization is critical to understanding early events of virus replication and a powerful tool to identify targets for antiviral development.

10

Hsp70 cellular chaperones associate with rhinovirus non-structural proteins in infected cells and are important for the replication of the virus

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Abstract

The way rhinoviruses subvert host cells to facilitate viral replication is still poorly understood. As the viral non-structural proteins (NSPs) play a major role in the subversion of host cell processes, we sought to identify cellular proteins that associate with rhinovirus NSPs and may facilitate the replication of the virus. To identify relevant viral-host protein interactions that occur in the context of an infection, we infected HeLa H1 cells with RV-A16 and pulled-down the NSPs and their interactors by NSP-specific affinity purification. Analysis of the pulled-down proteins by quantitative mass spectrometry revealed a specific enrichment in over 160 cellular proteins, some of which were already known to play an important role in rhinovirus replication, like GBF1, PI4KIIIβ and SETD3, indicating that this approach allows the identification of genuine pro-viral host factors. Interestingly, members of the Hsp70 family of cellular molecular chaperones were also enriched in the NSPs-associated fractions. To analyse if these proteins play an important role in the replication of the virus, we tested the effect of small molecule inhibitors of the Hsp70 family. Both the ATP-competitive inhibitor VER-155008 and the allosteric inhibitor pifithrin-μ blocked viral replication at an early stage, strongly reducing the amount of viral RNA, proteins and infectious virions produced. These results indicate that Hsp70 chaperones play an important role in rhinovirus replication, through mechanisms that seem to be distinct from the role of the Hsp90 chaperone and which we plan to investigate in the future.

11

Human-based models to study genotype dependent infection of Parechovirus A

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Abstract

Parechovirus A (PeV-A) are viruses in the Picornaviridae family. The most prevalent genotype, PeV-A1, causes mild respiratory and gastrointestinal symptoms while the second most prevalent genotype, PeV-A3 results in severe neurological disease in infants. The factors for the differential outcome are poorly understood. Therefore, in this work, we investigated the viral dynamics and tropism of PeV-A1 and PeV-A3 using human-based models of the intestine (enteroids) and the brain (cerebral organoids).

Human cerebral organoids were cultured for 67 days before inoculation with PeV-A1 and PeV-A3. To allow for enteroid infection, cells were seeded into Transwell® inserts to differentiate before infection. After infection, production and infectivity of viral particles were quantified using RT-qPCR and viral titration, respectively. Cell tropism was investigated with immunofluorescence and changes in cytokine expression were quantified by RT-qPCR.

PeV-A1 and PeV-A3 were both able to infect the enteroids and the cerebral organoids, although the kinetics of PeV-A3 were slower. The two genotypes presented a different cell tropism in the intestine, with PeV-A1 infecting enterocytes and Paneth cells, and PeV-A3 targeting goblet cells. After cerebral organoid infection, PeV-A3 showed an increased expression of cytokines TNF-α, IFN-γ and IFN-α2.

We demonstrated PeV-A infection of iPSC-derived cerebral organoids and fetal-derived enteroids. The differences in cell tropism observed in the intestine may explain the lower replication kinetics and the associated disease in humans. PeV-A3 CNS related illness maybe explained by an increased pro-inflammatory response.

12

Development of a replicon-based strand-specific qPCR assay to investigate FMDV genome replication

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Abstract

The aphthovirus, foot-and-mouth-disease virus (FMDV), is the etiological agent responsible for foot-and-mouth disease (FMD) in cloven hooved animals. FMD is a major burden to agriculture globally, impacting production in endemic regions and resulting in severe financial loss associated with eradication from non-endemic regions following outbreaks. Replication of the single-stranded RNA genome involves synthesis of a negative sense intermediate that in turn acts as a template from which nascent positive sense genomes are produced. Despite earlier attempts to define features of picornavirus genomes essential for RNA replication, the process remains poorly defined. We have previously used FMDV replicons to examine viral RNA and protein elements essential to replication. However, replicon-based systems require transfection of high levels of RNA that overload sensitive techniques such as qPCR for detection of specific strands. Here we describe a method that couples nascent RNA labelling and purification to strand-specific qPCR, permitting the investigation of the impact of mutations in the viral genome on the synthesis of both the negative sense intermediate and new positive strand genomes. Through comparison of mutated and wild-type replicons, we can investigate changes in viral RNA synthesis to better understand replication of the FMDV genome. This technique facilitates the tracking of strand production to better characterise replication complex initiation and structure and could be applied to other replicon-based systems.

13

Hepatitis A virus re-wires lipid metabolism to promote very-long chain fatty acid (VLCFA) synthesis required for viral RNA replication

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Abstract

Although picornaviruses are known to usurp lipid metabolic pathways to promote their replication, such studies have not been carried out with hepatoviruses. Using a genome-wide CRISPR screen strategy, we identified two lipid-related genes as candidate HAV host factors: acetyl-CoA carboxylase (ACC1, encoded by *ACACA*), which catalyzes the first and rate-limiting step in fatty acid synthesis, and 17b-hydroxysteroid dehydrogenase 12 (17b-HSD12, encoded by *HSD17B12*), a crucial enzyme in the fatty acid elongation cycle (Das *et al.*, Nat Microbiol, 2020). Targeted CRISPR knockout confirmed both are essential for HAV replication, yet dispensable for poliovirus. 17b-HSD12-KO cells also failed to support replication of a subgenomic HAV replicon RNA. Lipidomics analysis demonstrated a severe deficiency of very-long chain fatty acids (VLCFA, C ≥22) in 17b-HSD-KO cells, in which HAV replication was partially rescued by supplementation with hexacosanoic acid (C=26). Global lipidomics revealed marked increases in VLCFA in infected hepatoma cells: VLCFA represented 13.6 ±1.1 SD of lipids identified in mock-infected cells, but 22.3% ±1.7 SD 14 days post infection (p=0.003). Lipids with VLCFA tails were increased in multiple lipid classes, including cholesterol esters, phosphatidylcholine, diacylglycerides, and triglycerides. RNAseq analysis of comparably infected cells demonstrated infection-related increases in mRNAs encoding ACC1, fatty acid synthase (FASN), and fatty acid elongase 5 (ELOVL5), potentially explaining increases observed in VLCFA abundance. Thus, although the signaling mechanism remains obscure, HAV infection appears to re-wire lipid metabolism to enhance the production of VLCFA required for assembly of replication organelles and synthesis of new viral RNA.

14

Illuminating picornavirus replication and virus-host interactions

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Abstract

Although picornaviruses have been studied extensively, little is known about the processes that occur during the first several hours of infection because of a lack of sensitive assays. We developed a single-molecule imaging assay, virus infection real-time imaging (VIRIM), to study translation and replication of individual picornaviruses in live cells. VIRIM uncovered a striking heterogeneity in replication dynamics between cells and revealed extensive coordination between translation and replication of single viral RNAs. Furthermore, we identified the replication step of the incoming viral RNA as a major bottleneck of successful infection and identify host genes that are responsible for inhibition of early virus replication. VIRIM is a powerful tool to study virus replication and virus-host interactions.

15

The involvement of packaging signals and the poly-C tract in foot-and-mouth disease virus packaging

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Abstract

The mechanisms for RNA packaging in picornaviruses are not well understood. This is particularly true for foot-and-mouth disease virus (FMDV), as early attempts at understanding FMDV encapsidation revealed a significantly stronger bias against packaging in trans compared to other picornaviruses. However, by using recently identified putative packaging signals and exploiting the length of the poly-C tract to influence encapsidation efficiency, we have developed a robust trans-encapsidation assay that can be used to investigate virus packaging.

The length of the poly-C tract was found to be a critical factor for efficient encapsidation, with sub-genomic replicons containing longer poly-C tracts being packaged with significantly greater efficiency. Viruses recovered from transcripts containing truncated poly-C tracts were found to have greatly extended poly-C tracts after only a single passage. We have also characterised several other RNA motifs involved in FMDV packaging, with a critical packaging signal being located in the first pseudoknot of the 5’-UTR. Other packaging signals dispersed across the genome were then shown to have smaller, but cumulative, effects on encapsidation. Replicons with recoded regions to alter the RNA secondary structure showed varying levels of encapsidation efficiency depending on the number of packaging signals affected and the extent of the disruptions.

These results provide evidence for the involvement of specific RNA structures in picornavirus packaging and highlight the importance of the poly-C tract for packaging, suggesting that the rapid extension of the poly-C tract could be a mechanism required to ensure efficient encapsidation.

16

The upstream ORFs in picornaviruses: where, how and why?

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Abstract

Numerous members of the family Picornaviridae encode additional proteins in alternative open reading frames (ORFs). We recently characterized an upstream ORF (uORF) in enteroviruses, demonstrating that the upstream protein (UP) facilitates virus release in gut epithelial cells. The uORF is present in many but not all enteroviruses, thus raising interest in its conservation, selection pressure, and functionality. Using comparative genomic analysis and available clinical and other metadata, we assess the phylogenetic history of the uORF, the presence and nature of selection within the uORF region, and associations between uORF presence, host species, disease and pathology. Presence or absence of the uORF is interesting from a mechanistic perspective: for example, do viruses lacking a predicted functional uORF still use the conserved upstream AUG for translation initiation? The “spacer” region between the IRES and the polyprotein initiation codon is very short in rhinoviruses resulting in ubiquitous absence of the uORF, consistent with respiratory tract tropism that does not rely on UP’s role in gut epithelial cells. Using ribosome profiling of enterovirus A-C and rhinovirus-infected cells, besides IRES reporter systems, we demonstrate contrasts in the usage of the uORF and polyprotein AUG codons between different viruses. We extend our observations to another picornavirus, Theiler's murine encephalomyelitis virus, that has a different alternative ORF with initiation site downstream of the polyprotein AUG codon, while an upstream AUG is present but not used for translation initiation. Our results provide functional and mechanistic insight into the translation regulation and conservation of additional ORFs in picornaviruses.

17

Sexual RNA Replication Among Picornaviruses

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Abstract

Picornaviruses, like many organisms, have both asexual and sexual replication strategies. Asexual RNA replication, with one parental template, is fast and efficient, producing vast amounts of virus in a short period of time. Sexual RNA replication (aka recombination), with two parental templates, is infrequent and inefficient; however, it can remove lethal mutations from viral genomes, counteracting negative consequences of asexual replication. We hypothesize that sexual RNA replication involves a polymerase-RNA interface near the active site where nascent RNA products, via complementarity with a second parental template, specify homologous crossover sites. An L420A polymerase mutation at the polymerase-RNA interface specifically inhibits sexual RNA replication, but has no effect on asexual RNA replication. Poliovirus containing an L420A polymerase replicates (asexually) at wildtype magnitudes in HeLa cells; however, it cannot recombine normally with homologous RNA templates and it is exquisitely sensitive to ribavirin-induced error catastrophe (Kempf et al. 2016, 2019 & 2020). In human populations, poliovirus recombines with related group C enteroviruses, especially subspecies C2 and C3 viruses (Brouwer et al. 2020; Amona et al. 2020). Co-infection of HeLa cells with poliovirus (enterovirus subspecies C3) and Coxsackievirus A21 (enterovirus subspecies C2) produces recombinant virion RNA. Deep sequencing methods reveal the frequency and characteristics of crossover sites. Altogether, our data substantiate long held theories regarding the advantages and disadvantages of asexual and sexual RNA replication strategies (Chao, 1997; Barton & Charlesworth, 1998; Simon-Loriere and Holmes, 2011). Sexual RNA replication counteracts error catastrophe and mediates homologous recombination among related viral (sub)species members.

Virus entry and structure

18

Enterovirus stability and uncoating

Varpu Marjomäki
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Abstract

Members of the enterovirus B group share many details of their infectious entry pathway. They are stabile viruses, remaining infectious outside cells for long time periods. However, they can be relatively quickly triggered to uncoat in optimal spatiotemporal location, in cytoplasmic endosomes. Detailed studies on echovirus 1, coxsackievirus A9 and echovirus 30 show that they stay largely intact on cell surface but start uncoating within 15 min in vesicles which mature into multivesicular endosomes. These vesicles do not acidify demonstrating that uncoating/genome release does not require low acidity. In contrast, our recent studies have suggested that ambient conditions both outside cells and inside endosomes trigger expansion of the virions thus preparing the virions for genome release. Remarkably, albumin, which is abundant in serum and present in high enough amounts in extracellular space, have a high capacity to bind and steal fatty acids from the hydrophobic pockets. Instead of leading to immediate RNA release, virions stay expanded and infectious. Interestingly, ionic changes suggested to occur in endosomes, lowering sodium and calcium levels and increasing potassium levels also lead to expansion but show additional changes in virion structure and higher vulnerability for RNA release, suggesting that progressive ionic changes in endosomes could be the final switch for RNA release. Our recent results show that docking of suitable molecules to several sites on the virion capsid, not just the hydrophobic pocket, leads to strong stabilization and loss of infectivity suggesting further that several sites on the capsid are contributing to RNA release.

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Characterization of cross-neutralizing antibodies to poliovirus serotypes 1, 2, and 3

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Abstract

Despite concentrated efforts, total poliovirus eradication remains elusive. Additional tools are needed to respond to outbreaks. Here we characterize the binding and epitope recognition of a panel of human antibodies with specific emphasis on cross neutralizing antibody 9H2. Using high resolution cryo-EM we have determined the interactions between the antibody fragments and poliovirus serotypes 1, 2, and 3 using inactivated and live capsids. The resulting 3-D maps ranged in resolution from 2.4-3.8 Å. In addition to traditional icosahedral averaging reconstruction techniques, subparticle extraction and classification were used to resolve the binding interface at high resolution and resolve some fab interactions due to steric collision at icosahedral symmetry axes. The 9H2 Fab structure was predicted by SAbPred ABodyBuilder and used to initiate the build of the Fab into each 9H2-capsid complex map. Our data indicate that 9H2 blocks poliovirus receptor binding. The biochemical and structural characterization of cross-neutralizing antibodies is a first step toward the development of biologics or antivirals to help global poliovirus eradication efforts.

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Enterovirus genome delivery

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Abstract

Enteroviruses, the causative agents of diseases ranging from the common cold to poliomyelitis, are one of the largest groups of non-enveloped viruses. To initiate infection, enteroviruses enter cells by receptor-mediated endocytosis. However, how enterovirus particles release their genomes and ensure that the virus RNA reaches cytoplasm remains unknown. Here, we show that conformational changes and expansion of enterovirus RNA genomes, induced by acidic pH, trigger the opening of enterovirus particles. The exit of the RNA from the enterovirus particle results in a loss of one, two, or three adjacent capsid-protein pentamers. The opening in the capsid, which is more than 120 Å in diameter, enables the release of the genome without the need to unwind its putative double-stranded RNA segments. Furthermore, we used cryo-electron tomography of infected cells to show that endosomes containing enterovirusesdeform, disintegrate, and release the virus particles into the cytoplasm. Endocytosis of very-low-density lipoprotein, the natural substrate of rhinovirus 2 receptor, results in disruption of endosomes and release of their content into the cytoplasm. In combination, our results show that enterovirus receptor binding and endocytosis activate a cellular membrane remodeling pathway that disrupts the virus-containing endosomes and thus releases the virus particles into the cytoplasm.

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Membrane interactions and uncoating of Aichi virus, a picornavirus that lacks a VP4

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Abstract

Kobuviruses are an unusual and poorly characterized genus within the picornavirus family and can cause gastrointestinal enteric disease in humans, livestock, and pets. The human kobuvirus Aichi virus (AiV) can cause severe gastroenteritis and deaths in children below the age of 5 years; however, this is a very rare occurrence. During the assembly of most picornaviruses (e.g., poliovirus, rhinovirus, and foot-and-mouth disease virus), the capsid precursor protein VP0 is cleaved into VP4 and VP2. However, kobuviruses retain an uncleaved VP0. From studies with other picornaviruses, it is known that VP4 performs the essential function of pore formation in membranes, which facilitates transfer of the viral genome across the endosomal membrane and into the cytoplasm for replication. Here, we employ genome exposure and membrane interaction assays to demonstrate that pH plays a critical role in AiV uncoating and membrane interactions. We demonstrate that incubation at low pH alters the exposure of hydrophobic residues within the capsid, enhances genome exposure, and enhances permeabilization of model membranes. Furthermore, using peptides we demonstrate that the N terminus of VP0 mediates membrane pore formation in model membranes, indicating that this plays an analogous function to VP4.

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Chemical evolution of Rhinovirus identifies capsid-destabilizing mutations driving low pH-independent genome uncoating

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Abstract

Rhinoviruses (RVs) cause recurrent infections of the nasal and pulmonary tracts, life-threatening conditions in chronic respiratory illness patients, predisposition of children to asthmatic exacerbation, and large economic cost. RVs are difficult to treat. They rapidly evolve resistance, and are genetically diverse. Here, we provide insight into RV drug resistance mechanisms against chemical compounds neutralizing low pH in endo-lysosomes. Serial passaging of RV-A16 in presence of the vacuolar proton ATPase inhibitor bafilomycin A1 (BafA1) or the endo-lysosomotropic agent ammonium chloride (NH4Cl) promoted the emergence of resistant virus populations. We found two reproducible point mutations in the viral proteins 1 and 3 (VP1, VP3), A2526G (serine 66 to asparagine; S66N), and G2274U (cysteine 220 to phenylalanine; C220F), respectively. Both mutations conferred cross-resistance to BafA1, NH4Cl, and the protonophore niclosamide, as identified by massive parallel sequencing and reverse genetics, but not the double mutation, which we could not rescue. Both VP1-S66 and VP3-C220 locate at the interprotomeric face, and their mutations increase the sensitivity of virions to low pH, elevated temperature and soluble intercellular adhesion molecule-1 receptor. These results indicate that the ability of RV to uncoat at low endosomal pH confers virion resistance to extracellular stress. The data endorse endosomal acidification inhibitors as a viable strategy against RVs, especially if inhibitors are directly applied to the airways. Parts of the work have been published in the Journal of Virology, doi: 10.1128/JVI.01060-21. Epub 2021 Oct 27.

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Characterising the mechanism of enterovirus VP0 maturation cleavage

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Abstract

Enterovirus (EV) assembly occurs when pentamers composed of repeating VP0, VP3 and VP1 subunits assemble around the viral genome. This genome packaging initiates the cleavage of VP0 into VP4 and VP2. This cleavage event is essential for viral maturation and infectivity, although the precise details describing the mechanism of VP0 cleavage remain unclear. Unlike the current model of VP0 cleavage, which suggests a catalytic role for RNA in VP0 maturation, we suggest that genome packaging initiates conformational changes which facilitate the formation of a proteinaceous catalytic pocket.

In an attempt to elucidate the VP0 cleavage mechanism we apply a combination of bioinformatic, molecular, evolutionary and structural approaches. We have identified several critical regions of the EV capsid which are highly conserved across the family. Directed mutagenesis of key residues results in the formation of VP0 cleavage-defective particles (provirions), which package genome but fail to process VP0 into VP4 and VP2. Serial passage of cleavage-defective mutants selected for additional mutations at distal sites within the assembled virion which restore VP0 cleavage and viral infectivity. Finally, structural studies of mutationally stabilised provirions has allowed us to better understand the conformational changes which occur upon genome packaging, potentially providing details of the catalytic site and the location of VP0 maturation cleavage.

We propose that the mechanism of VP0 maturation cleavage is conserved across all EVs, and that understanding the VP0 cleavage process will inform the development of future vaccines and anti-viral therapies.

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Mechanistic insights into the anti-enteroviral activity of fluoxetine

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Abstract

The enterovirus genus encompasses many clinically important human pathogens such as poliovirus, coxsackieviruses, echoviruses, numbered enteroviruses and rhinoviruses. These viruses are the etiological agents of several human diseases, including hand-foot-and-mouth disease, neonatal sepsis, encephalitis, meningitis, paralysis and respiratory infections. There is an unmet need for antivirals to treat these diseases. The non-structural protein 2C is a AAA+ helicase and plays a key role in viral replication. As such, it is an attractive target for antiviral drug development. Repurposing screens with FDA-approved drugs have identified 2C-targeting compounds such as fluoxetine and dibucaine, but the molecular basis of 2C inhibition has remained enigmatic. Here we present the crystal structure of the soluble fragment of coxsackievirus B3 2C protein in complex with (S)-fluoxetine (SFX), revealing a conserved, hydrophobic drug-binding pocket which is distal to the ATP binding site. To decipher the molecular mechanism of inhibition by fluoxetine and other 2C-targeting compounds, we engineered a soluble, hexameric and ATPase competent 2C protein. Using this system, we show that SFX, dibucaine, HBB and guanidine hydrochloride inhibit 2C ATPase activity in a dose-dependent manner. Moreover, using cryo-EM analysis, we demonstrate that SFX and dibucaine lock 2C in a defined hexameric state, rationalizing their mode of inhibition and allowing us to generate the first reconstruction of the oligomeric complex. Taken together, these results provide important structural and mechanistic insights into 2C inhibition and provide a robust engineering strategy which can be used for structural, functional and drug-screening analysis of 2C proteins from current or future enteroviruses.

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Using cryo-electron microscopy to understand aphthovirus replication

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Abstract

Foot-and-mouth disease virus (FMDV) must be handled in a high containment laboratory, thus making imaging in its native state difficult. Equine rhinitis A virus (ERAV) belongs to the same aphthovirus genus of the picornavirus family and shares many features with FMDV, without requiring high containment. Here we aim to investigate the localisation of viral induced membrane rearrangements during its replication cycle. Initially we employed transmission electron microscopy (TEM) of thin sections of virus infected cells to verify that FMDV and ERAV infection induce comparable membrane structures in cells. In ERAV infected cells, double membrane vesicle were observed, similar to those seen in cells infected with FMDV, verifying ERAV as a general model for the study of Aphthovirus replication. Subsequently, we have visualised ERAV-infected cells in more detail and at multiple time points using a combination of cryo focussed-ion-beam (FIB) milling and cryo-electron tomography (cryoET). Our data show that, as expected the production of fully packaged virus peaks at the later time points during the virus infection cycle. Interestingly, earlier time points revealed particles in the act of packaging and unexpectedly, later time points showed viruses appearing to begin the process of uncoating within the cell. Finally, we will use sub-tomogram averaging to generate an intermediate resolution structure of the different intracellular virus particles, into which existing X-ray crystal structures can be fitted.

Evolution and diversity

26

The evolution of HAV from animal reservoirs

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Abstract

For decades, hepatitis A virus (HAV) was considered a predominantly human pathogen. Recent years have witnessed a plethora of discoveries of previously unknown HAV-related viruses from diverse non-primate hosts. Those discoveries have allowed re-visiting the evolutionary origins of HAV and explain how a virus engendering life-long immunity after infection may have survived in large populations of small mammals such as bats and rodents during millennia. I will summarize the available data on HAV-related viruses in non-primate hosts, discuss the likely zoonotic origin of HAV and illustrate the possibilities to use the newly gained insight into HAV ancestors to investigate the features that make HAV unique among picornaviruses.

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Classification and Taxonomy of the *Picornaviridae*: New Species, Genera and Subfamilies

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Abstract

The family *Picornaviridae* belongs to the order *Picornavirales* along with seven other small positive-sense RNA virus families (*Caliciviridae*, *Dicistroviridae*, *Iflaviridae*, *Marnaviridae*, *Polycipiviridae*, *Secoviridae* and *Solinviviridae*). Members of the *Picornaviridae* are currently divided into 158 species which are grouped into 68 genera. Recently, five subfamilies (*Caphthovirinae*, *Ensavirinae*, *Heptrevirinae*, *Kodimesavirinae* and *Paavivirinae*) have been approved, grouping all but two of the currently designated genera. However, there is an ever-growing list of picornaviruses awaiting formal classification. Many new viruses can be provisionally assigned to the new subfamilies while awaiting genus assignments. In addition, the International Committee on Taxonomy of Viruses (ICTV) has introduced binomial species nomenclature. We will therefore be expected to rename all existing 158 picornavirus species to fit into this system which will consist of the genus name followed by a freeform species epithet, e.g. the species *Foot-and-mouth disease virus* could become *Aphthovirus vesiculae* (of course, the virus common name, foot-and-mouth disease virus, would not change). Some suggestions for the new species names will be presented as well as future directions for the taxonomic structure of the *Picornaviridae*.

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Evolution of Nidoviral Polymerases Via Key Adaptations of the Picornaviral Polymerase Active Site

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Abstract

Positive sense RNA viruses replicate their genomes using virally encoded RNA-dependent RNA polymerase (RdRP) enzymes. These utilize an active site closure mechanism based on a movement of conserved motif A in the palm domain that forms a platform upon which viruses can readily evolve their replication speed and fidelity to optimize fitness. The coronaviral SARS-CoV polymerase shares this mechanism, but has unusually fast elongation rates compared to the structurally similar picornaviral enzymes. To address the mechanistic basis for the high speed and fidelity of the SARS-CoV-2 replicase we specifically addressed the roles of two non-canonical residues in the active site: Ala547 replacing a canonical glutamate in motif F located above the active site and SDD replacing the canonical GDD sequence in the palm domain motif C that sits under the priming base. Our data show that Ala547 allows for a doubling of replication rate over the glutamate used by most RdRPs, but this comes at a fidelity cost that is mitigated by using a SDD sequence instead of GDD within motif C. Comparison with comparable poliovirus polymerase mutations indicate the coronaviral enzymes have been tuned for high-speed replication, and in the process they have shifted a key fidelity control point within the active site to maintain genome integrity. The SDD motif is present in all nidoviruses while the motif F alanine is present only in the large genome nidoviruses that also have error correcting ExoN domains, providing a link between these two residues and the evolution of longer genomes.

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Demonstration of co-infection and trans-encapsidation of viral RNA in vitro using epitope-tagged foot-and-mouth disease viruses.

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Abstract

Foot-and-mouth disease, caused by foot-and-mouth disease virus (FMDV), is an economically devastating disease affecting several important livestock species. FMDV is antigenically diverse and exists as seven serotypes comprised of many strains which are poorly cross-neutralised by antibodies induced by infection or vaccination. Co-infection and recombination are important drivers of antigenic diversity, especially in regions where several serotypes co-circulate at high prevalence, and therefore experimental systems to study these events in vitro would be beneficial. Here we have utilised recombinant FMDVs containing an HA or a FLAG epitope tag within the VP1 capsid protein to investigate the products of co-infection in vitro. Co-infection with viruses from the same and from different serotypes was demonstrated by immunofluorescence microscopy and flow cytometry using anti-tag antibodies. FLAG-tagged VP1 and HA-tagged VP1 could be co-immunoprecipitated from co-infected cells, suggesting that newly synthesised capsids may contain VP1 proteins from both co-infecting viruses. Furthermore, we provide the first demonstration of trans-encapsidation of an FMDV genome into capsids comprised of proteins encoded by a co-infecting heterologous virus. This system provides a useful tool for investigating co-infection dynamics in vitro, particularly between closely related strains, and has the advantage that it does not depend upon the availability of strain-specific FMDV antibodies.

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Molecular evolution of immunodeficiency-associated VDPV type 2 case from Argentina

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Abstract

We studied the genomic evolution of eleven type 2 immunodeficient vaccine-derived poliovirus (iVDPV2) isolates obtained over a 352-day period from a non-paralytic patient from Argentina with X-Linked agammaglobulinemia. The patient received two doses of trivalent oral poliovirus vaccine (tOPV) in November 2015 and February 2016. iVDPV excretion apparently ceased after the first day of treatment with the antiviral pocapavir. The iVDPV isolates were characterized by limited divergence from parental Sabin 2 strain with a range of estimated nucleotide similarity between 99.49% and 99.59% of total open reading frame positions. The two key determinants for the attenuation of Sabin 2 were reverted and represented early evolutionary events. Seven amino acid changes were detected in the capsid region; none mapped to known neutralizing antigenic sites or to the VP1 hypervariable region. Inferred phylogenetic trees yielded similar topologies among distinct genomic regions and were consistent with the detection of a single evolutionary lineage from parental Sabin 2 strain and with stepwise accumulation of substitutions compatible with sampling time. Results from molecular phylogenies inferred under distinct clock models and using different genomic regions yielded date estimates of OPV2 origin compatible with establishment of infection from the first dose of tOPV. The date of the common ancestral node to all iVDPV2s was estimated to be about 4 days before detection of the first VDPV2. Understanding intra-patient evolution of a prolonged iVDPV excretor is important in the context of endgame strategies to achieve the goal of polio eradication.

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Insights into picornavirus biology and evolution from deep mutational scanning analyses

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Abstract

Background: RNA viruses have the highest mutation rates in nature, which facilitate their adaptation to changing environments and complicate their clinical management. However, most mutations are deleterious to protein function, raising the question of how RNA viruses are able to deal with such high mutation rates. Moreover, whether different proteins show altered tolerance to mutations is unclear.

Methods: To address these fundamental questions, we performed the largest analysis of mutational fitness effects in a picornavirus to date, encompassing >90% of all possible single amino acid mutations across two-thirds of the viral protein-coding region, including both structural and non-structural proteins. We integrate the results of this analysis with functional, structural, and evolutionary information to gain insights into the features that influence mutational fitness effects in these diverse viral proteins. In addition, to better understand how the local environment can influence evolutionary processes, we compared mutational fitness effects under different environmental conditions.

Results: Mutations were overall deleterious yet the distribution of mutational fitness effect varied between different viral proteins and was significantly altered by environmental conditions such as temperature. Different structural, evolutionary, and functional attributes correlated with mutational fitness effects across all proteins but were different for the distinct environmental conditions.

Conclusion: Our results provide a comprehensive understanding of mutational fitness effects across multiple picornavirus proteins and the different functional, evolutionary, and structural attributes that correlate with them. In addition, we provide insights into how the local environment can shape mutational fitness effects and, as a result, viral evolution.

Virus-host interactions and immune response

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Foot-and-mouth disease virus carriers: virus persistence in individuals and populations

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Abstract

Foot and mouth disease virus (FMDV) causes a highly contagious acute vesicular disease, resulting in more than 50% of cattle, regardless of vaccination status, and almost 100% of African buffalo becoming persistently infected for long periods (months) of time. Previous studies have shown FMDV capsid proteins and/or genome are localised in the light zone of germinal centres of lymphoid tissue in cattle and African buffalo. The pattern of staining for FMDV proteins was consistent with the virus binding to follicular dendritic cells (FDCs). We have now demonstrated a similar pattern of FMDV protein staining in mouse spleens after acute infection and showed FMDV proteins are colocalised with FDCs. Blocking antigen binding to complement receptor type 2 and 1 (CR2/CR1) prior to infection with FMDV significantly reduced the detection of viral proteins on FDCs and FMDV genomic RNA in spleen samples.

Through a combination of experimental and theoretical approaches, we investigated how highly contagious FMDV persists in African buffalo populations. Viral persistence in a population is unlikely through acute transmission alone. However, occasional transmission from persistently infected carriers reliably maintains the virus in populations.

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A GLOBAL UNDERSTANDING OF HOW PICORNAVIRUS CAPSIDS ESCAPE ANTIBODY NEUTRALIZATION

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Abstract

Background: Picornavirus capsids constitute the key target of host humoral responses and therefore play an important role in viral evolution and pathogenesis. While numerous studies have defined monoclonal antibody neutralization sites across picornavirus capsids, how picornaviruses are targeted by – and escape from- neutralizing antibodies in a physiological context remains unknown.

Methods: To address this gap, we have used a high-throughput approach to map all single amino acid mutations in the capsid of a picornavirus that confer resistance to neutralization by both human and mouse polyclonal sera. For this, coxsackievirus B3 populations harboring extreme diversity in the capsid region were neutralized with human and mouse sera. The mutations enriched in the neutralized populations compared to mock-neutralized populations were then identified using high-fidelity next-generation sequencing.

Results: With this approach, we have obtained for the first time a complete picture of how picornavirus capsids are targeted by polyclonal sera in both the natural host, humans, as well as the most common experimental animal model, mice. Specifically, we define the antigenic profiles of these sera and analyze the breadth of escape from identified epitopes in a structural and evolutionary context.

Conclusion: Our findings help illuminate a key aspect of host-pathogen interaction in picornaviruses and shed light on how natural immunity can shape viral antigenic evolution.

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A Picornavirus that retargets RSK kinases to phosphorylate new substrates

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Abstract

The leader (L) protein of cardioviruses is a small multifunction protein that interferes with innate immunity and notably perturbs nucleo-cytoplasmic trafficking in infected cells. This activity correlates with L-mediated hyperphosphorylation of FG-nucleoporins (FG-Nups) (1). L activities were shown to depend on the ability of L to hijack RSK kinases through a short linear motif shared by some proteins of highly unrelated pathogens. The phenotype of some L-mutant viruses led us to propose the "model of the clamp" whereby such pathogens' proteins recruit RSKs through the conserved linear motif and then recruit specific RSK targets that act as effectors after phosphorylation by RSK (2). Here, we confirmed that nucleocytoplasmic perturbation by cardioviruses L proteins depends on RSK. We then used a biotin ligase (BioID2) approach to identify the "proxeome" of the L/RSK complex in TMEV-infected cells. Interestingly, FG-Nups emerged as targets for BioID2-L as well as for BioID2-RSK, suggesting that FG-Nups are recruited by L to be phosphorylated by RSK. To test whether FG-Nups are direct targets for RSK-mediated phosphorylation in infected cells, we constructed cells that express "analog-sensitive" RSK kinase mutants, following the strategy developed in the group of Kevan Shokat (UCSF). Our results confirm that RSK directly phosphorylates at least two FG-Nucleoporins during TMEV and EMCV infection, thereby confirming the "clamp" model where L interacts with RSK and reshapes the target range of this cellular kinase.

1. Lizcano-Perret and Michiels, 2021, Viruses 13, 1210

2. Sorgeloos et al. 2022, PNAS 119 e2114647119

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Structural analysis of the bovine antibody repertoire: contributions of the heavy and light to antigenic recognition

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Abstract

A fraction of bovine antibodies comprise heavy chains characterised by ‘ultralong’ CDR3H loops of up to 70 residues, which extend above the antigen-binding surface of the heavy and light chain variable domains. These so-called ultralong heavy chains are paired with a highly conserved light chain, raising questions about the role of the light chain in bovine antigenic recognition.

Specificity of antibody secreting cells isolated from animals serially vaccinated with Foot and Mouth Disease Virus-like Particles (VLPs) of multiple serotypes were screened using a fluorescent-based assay, and subsequently sequenced. 9 ultralong antibodies were expressed as Fabs. 7 Fab structures were solved by X-Ray diffraction, and 2 were predicted using AlphaFold. Additionally, 1 ultralong CDR3H identified from a non-immunized animal was produced, along with 2 chain-exchanged variants. Interaction between Fabs and VLPs were measured using Bio-layer Interferometry. Fab-VLP interactions were then studied using cryogenic electron microscopy.

143 unique antibodies were identified from 349 B cells, including 22 ultralong CDR3H. A pair of antibodies sharing an identical ultralong heavy chain gave differing specificity screening profiles, and have differentially encoded light chains. Different light chains induce varying degrees of twist to the CDR3H stalk, which affects relative positions of residues within the knob domain and may explain the observed specificity differences.

Antigenic interaction involving ultralong CDR3H exclusively relies upon the CDR3H, though the light chain and other CDRH loops affect its precise positioning, offering insight to bovine immunological mechanisms. Ongoing investigations hope to further clarify the structural and functional differences between near identical antibodies.

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HAV egress through the apical and basolateral membranes is controlled by the differential use of the hepatocyte traffic network

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Abstract

In this work, we analyzed the expression of genes coding for proteins involved in the syndecan-ALIX–mediated exosome biogenesis and release in HAV-infected undifferentiated and differentiated hepatocytes, using two strains differing in their replication capacity: the HM175 strain (L0) and the fast-replicating HM175-HP strain (HP). This latter strain harbors a mutation in the VP2 protein, which facilitates capsid interaction with ALIX.

The syndecan-ALIX mediated exosome biogenesis was found to be very active in the Huh7-AI cells, independently of its polarization status. Among several RAB proteins involved in the hepatocyte traffic network, RAB11 was highly expressed, followed by RAB35 and RAB7. During HAV infection, expression of ALIX, syntenin, RAB7 and RAB35 coding genes was increased. Confocal microscopy and silencing of RAB proteins were used to identify the HAV traffic pathway in unpolarized cells. HP capsids clearly co-localized with RAB35, and to a lesser degree with RAB11 and RAB7. In contrast, L0 capsids preferentially co-localized with RAB7 and to a lesser extent to RAB11. Silencing experiments suggest that HAV uses the communicating vessel network controlled by RAB proteins, with preference for RAB7 and RAB11. However, RAB35 silencing specifically reduced HP egress. In polarized Huh7-AI hepatocytes, RAB35 was preferentially located at the basolateral membrane suggesting its involvement in the basolateral traffic pathway, which in turn could explain the more efficient egress through this membrane of HP strain, compared with the L0 strain.

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Heparan sulfate binding mediates Enterovirus A71 virulence in immunosuppressed mice

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Abstract

Enterovirus A71 (EV-A71) is the most neurotropic EV after poliovirus, causing major hand, foot and mouth disease outbreaks together with neurological complications and deaths. We have previously isolated an EV-A71 variant from an immunocompromised patient treated with rituximab, in which a leucine was substituted by an arginine on the virus capsid. The mutation was absent in the patient's respiratory tract and emerged as a mixed population in the gut, to become dominant in the blood and cerebrospinal fluid. We demonstrated that this mutation, together with a cell-associated compensatory mutation (VP1-E167G), increases the virus affinity for heparan sulfate (HS), an EV-A71 attachment receptor. We introduced these mutations in a mouse-adapted EV-A71 backbone (MP4) and showed that MP4-VP197L (WT) does not bind HS, while MP4-VP197R and MP4-VP197R167G bind HS with weak and strong affinity, respectively. We next orally inoculated these variants into hSCARB-2 transgenic mice treated with anti-CD20 Abs, to suppress humoral immunity and recapitulate the patient immune status. While MP4-VP197R167G was attenuated, MP4-VP197L showed a similar virulence in immunocompetent and immunosuppressed mice and MP4-VP197R showed an enhanced virulence in immunosuppressed mice. This increased virulence was not observed upon direct inoculation in the mice brains. We also demonstrated that, in contrast to MP4-VP197L, MP4-VP197R was effectively neutralized by sera collected from MP4-VP197L or MP4-VP197R infected mice. Our results shed light on the original clinical observation, demonstrating that, in absence of B cell immunity, variants with low HS affinity can emerge and confer greater in vivo fitness and virulence.

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Primate interferon stimulated genes encode potent foot-and-mouth disease virus restriction factors

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Abstract

The cellular antiviral response is part of the mammalian innate immune system and has species-specific components which may contribute to the restriction of viral tropism to particular hosts. The picornavirus foot-and-mouth disease virus (FMDV) infects multiple species of domestic livestock and wild animals, however many other mammalian species such as primates and horses are non-susceptible to FMD. The virus can use integrins of all these species as receptors to enter cells, suggesting that the species-specific tropism of FMDV is determined by a post-entry restriction in the virus life cycle. Interferon-stimulated gene overexpression library screens from human and non-human primate (macaque) identified primate proteins which act as potent restriction factors to FMDV replication. Expression of a single such protein in a highly susceptible porcine cell line, rendered the culture refractory to FMDV infection. The genes encoding these restriction factors appear to exist only in FMDV non-susceptible species, thus providing a novel potential mechanism for species-level tropism of FMDV.

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Structural and functional repurposing of human proteins apparently acquired by picornaviruses in their 2A locus

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Abstract

While the conserved genome organization is one of the defining features of picornaviruses, the 2A proteins are amongst the most divergent. To date there are at least 5 different types identified, with varying roles during infection. Remarkably, there is a group of picornaviruses, including Aichi Virus (AiV) and Parechovirus A (HPeV), whose $2A^{H/NC}$ proteins share conserved H-box and NC-motifs with the human protein PLA2G16, which we previously identified as a picornavirus host factor. These residues are essential for enzymatic activity for the cellular enzyme as well as for the reported enzymatic role of the viral 2A proteins in RNA replication. We set out to discover whether the 2A proteins are active as lipid modifying enzymes, and have been acquired to allow AiV and HPeV to become independent from the cellular host factor. The crystal structures of the AiV-2A and HPeV1-2A proteins show a similar topology to PLA2G16 in the N-terminus, but diverge in the C-terminal half. AiV-2A preserves the active site configuration, but is inactive as a phospholipase. Remarkably, a topological rearrangement of the C-terminus in HPeV1-2A results in a conformation incompatible with catalysis, despite the conservation of the H-box and NC-motif; consistently the HPeV1-2A protein is inactive as a phospholipase too. Intriguingly, however, HPeV1 is independent of PLAG16 for cellular entry, through an as yet unidentified mechanism. Further work is required and ongoing to trace $2A^{H/NC}$ proteins' potential role during virus entry and how might they have been repurposed to fulfil new functions in the viral replication cycle.

Pathogenesis

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Organoid models for human entero- and parechoviruses

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Abstract

Viruses are obligate intracellular pathogens that are entirely dependent on the host for propagation. Traditionally, viruses have been studied using cell lines and animal models but these models do not accurately reflect human disease. Ideally, human models to study human viral pathogenesis are desirable and this has become possible with the advent of organoid models. Stem cell-derived organotypic models or organoids closely recapitulate the in vivo situation in terms of heterogeneity and organization of cell types.

Human gut, airway, and brain organoids have been applied in my laboratory for studying pathogenesis of human entero- and parechoviruses. Application of human airway epithelial cultures and gut epithelial cultures as primary replication sites for EV-A71, EV-D68, and human parechovirus A (PeV-A) will be presented. We studied differences in replication and cell tropism of PeV-A genotypes, and effects of single nucleotide polymorphism on EV-A71 replication in in airway and gut epithelial cultures. Furthermore, application of human brain organoids as (secondary) infection sites for EV-D68 and PeV-A infection will be presented. Taken together, our work will highlight the potential of human organoids as suitable models for studying pathogenesis of human picornaviruses.

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Early emergence and maintenance of major 5' terminally deleted viral RNA forms are associated with the development of acute and persistent CVB3/28 infections in mouse

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Abstract

Major enterovirus-B (EV-B) populations characterized by 5’ terminal genomic RNA deletions ranging up to 50 nucleotides (5’TD) have been associated with the development of acute and chronic myocarditis and type 1 diabetes in mice or humans (Glenet et al. 2020). To date dynamics of emergence of EV-B 5’TD forms and their impact on pathophysiology in target tissues remain unknown. Using RACE-PCR and micro electrophoresis methods (Bouin et al. 2016), we investigated dynamics of emergence of 5’TD enteroviral populations in DBA/2J mouse's organs during a systemic infection with a CVB3/28 strain. At 3 days post-infection (DPI), an emergence of major 5’TD enteroviral populations associated with minor full-length RNA forms was evidenced in all studied organs. At 7 DPI, replication activities of viral populations were maintained with an infectious particles production only in heart, pancreas and liver. Moreover, acute inflammation and histological lesions were observed only in heart and pancreas. Other organs were otherwise normal and viral clearance was observed at 7 DPI. At 28 DPI, a persistent infection characterized by low 5'TD viral RNA loads, RNA(+)/RNA(-) ratios <5, viral capsid VP1 expression and an absence of detectable infectious particles (PFU) was observed in heart and pancreas, associated with dystrophin degradation and insulin production impairment, respectively. We showed that the early emergence and maintenance of 5'TD RNA populations are associated with the development of EV-B acute and persistent infections with cardiac and pancreatic cell dysfunctions in DBA/2J mouse.

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Pathogenesis linked to proteinase 2A activity duringpersistent cardiac infection by enteroviruses

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Abstract

Group B enteroviruses, i.e., coxsackie B viruses, exhibit cardiac tropism and cause acute and chronic cardiomyopathies. The pathogenesis of enterovirus-induced cardiomyopathies is linked to the expression and activity of viral 2A proteinase, a small (16 kDa) protein that plays multiple roles in the enterovirus replication cycle. It is primarily involved in the proteolytic cleavage of the viral polyprotein, but it also inhibits viral sensing by the cell. The 2A protein plays a crucial role in redirecting cellular resources to viral RNA translation by cleaving eIF4G and inhibiting cap-dependent translation. During cardiac infections by coxsackievirus, 2A cleaves dystrophin, a major muscle protein involved cardiac function. This cleavage disrupts the dystrophin-glycoprotein complex and promotes myocyte slippage, causing the loss of systolic function. During persistent infections, enteroviruses exhibit impaired replication due to 5’ terminal deletions of viral RNA. Deep sequencing of viral RNA from cardiac samples from dilated cardiomyopathy patients revealed viral populations that were heterogeneous, and genomes harboring 5’ terminal deletions were found together with full length viral RNAs. These viral RNAs all exhibited 2A proteinase activity, even in the absence of viral RNA replication. These results underscore the high-level activity of enterovirus 2A proteinase and its role in both acute and persistent infections. Inhibition of its proteolytic activity would prevent polyprotein maturation and block viral replication. It would also prevent the inhibition of host cap-dependent translation. We have developed models to track 2A activity and are currently generating novel molecules to inhibit 2A catalytic activity as a potential therapeutic strategy.

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Unravelling the role of immune cells in the viremia of enterovirus-D68

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Abstract

Enterovirus-D68 (EV-D68) often causes mild respiratory infections, but can also cause severe respiratory infections, systemic spread and central nervous system (CNS) complications. The mechanism that leads to systemic spread is yet not understood. We hypothesise that immune cells contribute to viremia. To investigate whether immune cells are susceptible to EV-D68 infection, we inoculated human peripheral blood mononuclear cells (PBMC) with EV-D68 belonging to different clades and found that B cells were susceptible to the infection. However, no progeny virus was detected, which might be related to their resting state. To investigate whether activation state plays a role in the permissiveness of B cells, we inoculated Epstein-Barr virus-transformed B lymphoblastoid cell lines (BLCLs) or lentivirus-transduced B cells with EV-D68 and found that these cells were productively infected, without large differences in replication efficiencies among different clades. To understand how EV-D68 can reach activated B cells, which are mostly located in lymphoid tissues, we inoculated dendritic cells (DCs) and the inoculation resulted in a productive infection. Co-culture of infected DCs with uninfected B cells resulted in spread of infection to B cells. Altogether, we conclude that immune cells potentially play an important role in the development of viremia during EV-D68 infection, which is an essential step towards the development of extra-respiratory tract complications. As the incidence of EV-D68-associated CNS complications increased in the last decade, combined with the emerging potential of the virus, it is important to understand the pathogenesis of EV-D68 infection in order to counteract the disease.

Treatment and Prevention

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~~Facing t~~he Oral Poliovirus Vaccine Paradox

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Abstract

Following the global switch from trivalent oral poliovirus vaccine (tOPV; containing types 1 [OPV1], 2 [OPV2] and 3 [OPV3]) to bivalent OPV (bOPV; containing OPV1 and OPV3) in April 2016, circulating vaccine-derived poliovirus (cVDPV2) was detected in Borno state in northern Nigeria in July 2016. This orphan cVDPV2 was genetically linked to 2013 Borno viruses that were part of the Chad-cVDPV-A emergence that originated in Chad in 2012 and spread to Nigeria. Detection of this virus indicated that immunity gaps to poliovirus type 2 remained in high-risk areas and that pockets of children were missed by tOPV immunization campaigns before the global switch. Illustrating this OPV paradox, new emergences of cVDPV2 were seeded by mOPV2 campaigns in northern Nigeria in areas of low population immunity. For example, the NIE-JIS-1 emergence circulated extensively in Nigeria since first detection in 2018 and spread to neighboring countries in the Lake Chad basin and West Africa where it sustained prolonged transmission. With widening type 2 immunity gaps across the continent, cVDPV2 outbreaks were subsequently detected in 28 countries during 2020-2021 (MMWR 70 number 49). To break the cycle of mOPV2 seeding new emergences/ outbreaks, type 2 novel oral poliovirus vaccine (nOPV2) was developed, resolving the OPV paradox. With nOPV2 development efforts culminating in WHO issuance of the first Emergency Use Listing (EUL) in November 2020, nOPV2 use began in March 2021 in Nigeria, with more than 125 million doses administered through October 2021 and subsequent wider use (GPEI_cVDPV2-nOPV2_Factsheet_13-Jan-2022-EN.pdf (polioeradication.org)).

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Initial use of type 2 novel OPV for immunisation campaigns in several countries

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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) with greatly reduced 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. We report the whole-genome characterization of nOPV2 isolates from stools and environmental samples collected in several countries following immunisation campaigns and focusing on key genomic regions responsible for nOPV2 genetic and phenotypic properties. Results so far show that none of the viruses have lost mutations altering base pairing in domain V of the 5'NTR, where the main nOPV2 determinants for attenuation locate. Although mutations are observed that are expected to decrease attenuation slightly, laboratory studies show that none of the mutation combinations identified in nOPV2 isolates could cause the nOPV2 strain to approach the neurovirulence of Sabin 2 with the A481G reversion alone, which is observed almost universally within 14 days of replication in Sabin OPV2 recipients. Early results from Tajikistan strongly suggest that nOPV2 was effective at interrupting a cVDPV2 outbreak that was occurring at the time of vaccination.

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Protease-free production of poliovirus VLPs using Pichia pastoris: Insights into particle formation and potential vaccine candidates

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Abstract

Poliovirus (PV) is the highly transmissible causative agent of poliomyelitis. Infection results in an acute flaccid paralysis in up to 2% of cases and can be fatal. Integrated global immunisation programmes using live-attenuated oral (OPV) and/or inactivated PV vaccines (IPV) have dramatically reduced incidence of the disease with PV now only endemic in Afghanistan and Pakistan. However, to achieve eradication, vaccination will need to continue into the foreseeable future. Nevertheless, there are biosafety concerns for both OPV and IPV, as both have the potential to reintroduce PV into the environment.

These concerns could be addressed by virus-free virus-like particle (VLP) vaccines, which mimic the ‘empty’ capsids (ECs) normally produced in viral infection. Native ECs are antigenically indistinguishable from mature virus particles, however, they readily convert to an alternative ‘non-native’ conformation which no longer induces protective immunity. Within our WHO funded consortium (together with the Universities of Oxford, Reading, Florida, NIBSC, John Innes Centre and Pirbright Institute) we have identified combinations of mutations for each PV serotype resulting in antigenically stable ECs.

Here we show that stabilised PV1 VLPs can be produced in P. pastoris without the need for the protease, 3CD, instead using a 2A peptide sequence to terminate translation. Analysis of all permutations revealed that only VP3 could be tagged with 2A and maintain native antigenicity. Transmission electron microscopy of these VLPs reveals they retain the classic picornaviral icosahedral structure. Furthermore, these particles are thermostable above 37°C demonstrating their potential as next generation vaccine candidates for PV.

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Design of stable Enterovirus A71-C4 virus like particles (VLPs) as a potential approach to vaccine development

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Abstract

We have previously described the design of stable and immunogenic polio virus-like particles (VLPs) as an alternative approach to vaccine production. To do this we devised a pipeline for the identification of stabilising mutations which could then be combined in a single construct; this strategy may have applications for other enterovirus vaccines.

Enterovirus A71 (EVA71) is one of causative agents of hand, foot and mouth disease which is usually mild but in some cases neurological and systemic complications may occur. EVA71 have been classified into 6 genogroups, A-F with genogroups A-C being most prevalent in humans. VLP vaccines might be a useful alternative to inactivated vaccines currently in use or development. We have identified mutations that stabilise EVA71 -C4 empty viral capsids in the native antigenic conformation by (1) incorporating modifications that proved successful in the context of poliovirus and (2) identifying new candidate mutations using an analogous pipeline. Here we will report the characterisation of a range of different modifications that have stabilising and de-stabilising effects on EVA71 particles as well as unexpected effects on morphogenesis. A subset of these have been chosen for recombinant expression in Pichia pastoris and immunogenicity studies.

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Immunogenicity of polio VLPs formulated with adjuvants

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Abstract

Stabilised polio virus-like-particles (VLPs) produced in recombinant expression systems could provide an ideal vaccine to replace IPV (inactivated poliovaccine) in the post-eradication world.

When inoculated into transgenic mice expressing the human poliovirus receptor (TgPVR mice) all such VLPs elicit similar or better neutralising antibody responses compared to an IPV reference and mice are protected against challenge with wild type polioviruses to an equivalent or greater extent. However in rats, the species used for IPV lot release, most VLPs are less immunogenic than IPV suggesting an inherent difference in the immune systems of the two animal models. One possibility is that the viral RNA (present in IPV but absent in VLPs) acts as an adjuvant in rats but is less potent in mice. In support of this VLPs produced in both yeast and insect cells induced a significantly higher antibody response when inoculated in conjunction with aluminium-based adjuvants than IPV alone. This effect was seen whether or not the VLPs were adsorbed to the adjuvant and was equally potent in monovalent and trivalent preparations. Furthermore, adsorption to the adjuvant had no significant impact on the native antigenicity of VLPs.

Immunogenicity of stabilised polio VLPs in humans is not yet known but this observation opens the door to pre-clinical development and scale-up by promising that VLP vaccine preparations are likely to obtain regulatory approval for clinical trials.

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Polyphenols Epigallocatechin Gallate and Resveratrol, and Polyphenol-Functionalized Nanoparticles Prevent Enterovirus Infection through Clustering and Stabilization of the Viruses

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Abstract

To efficiently lower the virus infectivity and combat virus epidemic or pandemic, it is important to discover broadly acting antivirals. Here, we investigated two naturally occurring polyphenols, epigallocatechin gallate (EGCG) and resveratrol (RES), and polyphenol functionalized nanoparticles for their antiviral efficacy. Concentrations in the low micromolar range permanently inhibited infectivity of high doses of enteroviruses (10^7 PFU/mL). Sucrose gradient separation of radiolabeled viruses, dynamic light scattering, transmission electron microscopic imaging, and an in-house developed real-time fluorescence assay revealed that polyphenols prevented infection mainly through clustering of the virions into very stable assemblies. Clustering and stabilization were not compromised even in dilute virus solutions or after diluting the polyphenols-clustered virions by 50-fold. In addition, the polyphenols lowered virus binding on cells. In silico docking experiments of these molecules against 2-fold and 3-fold symmetry axes of the capsid, using an algorithm, developed for this study, discovered five binding sites for polyphenols out of which three were novel binding sites. Our results altogether suggest that polyphenols exert their antiviral effect through binding to multiple sites on the virion surface leading to aggregation of the virions and preventing RNA release and reducing cell surface binding.

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Rhinoviruses in asthma & COPD - importance and treatment options

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Abstract

Acute attacks of asthma and chronic obstructive pulmonary disease (COPD) are major unmet medical needs. New approaches to prevention/treatment are needed.

Respiratory virus infections (RVIs) are detected in 80-100% of asthma and COPD attacks in well-designed studies. All RVIs can precipitate attacks, but rhinoviruses (RVs) precipitate the great majority. People with asthma and COPD have increased severity of upper and lower respiratory illness with RV infections, with greater inflammatory responses driven by increased virus loads. In COPD more severe RV infection is followed by secondary bacterial infections, which prolong attacks.

Impaired innate (type I/III interferon [IFN]) antiviral immune responses are important in asthma and COPD attack pathogenesis. Deficient type I/III IFN induction has been observed in bronchial epithelial cells (BECs) and bronchoalveolar lavage cells in both diseases and in peripheral blood mononuclear and dendritic cells in asthma, in response to RVs and other respiratory viruses.

Clinical trials with inhaled IFN-β in asthma report improved asthma control and lung function, but insufficient severe exacerbation frequency meant that significant reductions in this primary outcome were not observed.

IFN-λs are the most abundant and earliest IFNs produced by virus-infected BECs, constitute the front line of antiviral defense in the lung and are less pro-inflammatory than type I IFNs.

Early treatment with type I & III IFNs has been shown to accelerate virus clearance and improve clinical outcomes in COVID-19, particularly in those with high initial virus load. Thus IFNs have potential to prevent and treat virus-induced asthma & COPD attacks

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