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ABSTRACTS

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The World Society for Virology (WSV) is a non-profit organization established in 2017[1] to connect virologists around the world with no restrictions or boundaries, and without membership fees. The WSV brings together virologists regardless of financial resources, ethnicity, nationality or geographical location to build a network of experts across low-, middle- and high-income countries.[2] To facilitate global interactions, the WSV makes extensive use of digital communication platforms.[3-8] The WSV's aims include fostering scientific collaboration, offering free educational resources, advancing scientists' recognition and careers, and providing expert virology guidance. By fostering cross-sectional collaboration between experts who study viruses of humans, animals, plants and other organisms as well as leaders in the public health and private sectors, the WSV strongly supports the One Health approach. The WSV is a steadily growing society with currently more than 1,480 members from 86 countries across all continents. Members include virologists at all career stages including leaders in their field as well as early career researchers and postgraduate students interested in virology. The WSV has established partnerships with The International Vaccine Institute, the Elsevier journal Virology (the official journal of the WSV) and an increasing number of other organizations including national virology societies in China, Colombia, Finland, India, Mexico, Morocco and Sweden.

Abdel-Moneim AS, Varma A, Pujol FH, Lewis GK, Paweska JT, Romalde JL, Söderlund-Venermo M, Moore MD, Nevels MM, Vakharia VN, Joshi V, Malik YS, Shi Z, Memish ZA (2017) Launching a Global Network of Virologists: The World Society for Virology (WSV) Intervirology 60: 276–277.

2. Abdel-Moneim AS, Moore MD, Naguib MM, Romalde JL, Söderlund-Venermo M. WSV 2019: The First Committee Meeting of the World Society for Virology (2020) Virologica Sinica 35: 248-252.

Keynote Speakers

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SARS-CoV-2: Evolution and Control

From SARS-CoV To SARS-CoV-2, Understanding Of Interspecies Infection Of Bat Coronaviruses

Zhengli Shi
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SARS and COVID-19 are severe human respiratory diseases caused by two SARS-related coronaviruses (SARSr-CoV), SARS-CoV and SARS-CoV-2, respectively, in a 17 year interval. Although these two viruses share 79% genomic sequence identity, they use the same receptor, angiotensin converting enzyme (ACE2), for cell entry. The discovery of SARSr-CoVs in bats suggests that bats are natural reservoirs of SARS-CoV, probably SARS-CoV-2. Phylogenetic analysis based on RNA-dependent RNA polymerase divides the SARSr-CoVs into 2 lineages. SARS-CoV-1 lineage includes SARS-CoV from humans and civets and SARSr-CoVs from bats in China and Japan. SARS-CoV-2 lineage includes SARS-CoV-2 and SARSr-CoVs from pangolins captured by Chinese customs and from bats in China, Cambodia and Thailand. Protein sequence alignment based on the spike receptor binding domain (RBD) indicates that theses SARSr-CoVs can be divided into two clades, clade 1 has similar size as SARS-CoV, clade 2 has shorter size due to one or two peptide deletions. By RBD-ACE2 binding assay, pseudovirus or virus infection assays, it was demonstrated that the clade 1 can use human ACE2 at different efficiency, while clade 2 can't. But clade 2 with one deletion uses bat ACE2 as a receptor, but with a low efficiency. These results suggest that some SARSr-CoVs have acquired the capability to deploy the human ACE2 as a receptor. Thus the future long-term surveillance is urgently needed to prevent the next emerging infectious disease caused by this group of viruses.

Searching For The Origins Of SARS-CoV-2

Marion Koopmans
Erasmus Medical Centre, Netherlands

The first cases of COVID-19 were recognized in the city of Wuhan, where a clinician alerted the Wuhan CDC of an unusual case of pneumonia December 26th. Within a matter of days, it became clear that a larger cluster of cases occurred and that the cause was a new virus from the Sarbecovirus genus. Since then, the virus spread globally, causing a pandemic that will continue to challenge the world in the months and years to come. SARS COV 2 is genetically closest to RatG13, a virus that had been identified in faeces from bats in Yunnan province, but the genetic distance precludes a direct etiological link. In order to understand the origins of the pandemic, the WHO convened a joint

scientific mission that designed and conducted a series of studies to further our understanding of the first phase of the pandemic. The results of the first phase of these studies will be presented.

How Vaccines And Variants Are Shaping Epidemiology And Policy In The COVID-19 Pandemic

Neil Ferguson
Vice-Dean (Academic Development), Faculty of Medicine, Imperial College London

Abstract unavailable.

COVID-19 Vaccines: Taking A Shot Beyond Efficacy

Jerome Kim
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Since the identification of COVID-19 and the SARS-CoV-2 pathogen, 15 months ago, 10 vaccines of various platforms have been shown to be safe and efficacious. Additional vaccines are anticipated in 2021 and 2022. Beyond the proof of efficacy and safety, a remarkable challenge lies in a series of additional challenges between the proof of efficacy and impact – successful mitigation of the burden of COVID-19 disease globally. To achieve 70% global coverage, we will need 10-14 billion doses, just for COVID-19, of high quality, safe and efficacious vaccines. We will need to vaccinate demographics not traditionally in the target population for vaccines in general use globally, which implies that the logistics and human capacity to ship, store, and administer COVID-19 vaccines will need to be strengthened globally. Finally there are a number of post efficacy scientific issues – dose and schedule optimization, correlates of protection, effectiveness studies, surveillance for variants, and pharmacovigiliance that may have profound implications for policy and the eventual control of SARS-CoV-2 worldwide.

COVID-19: pathogenesis and immune responses

Animal Models Of COVID-19 And Immune Responses

Stanley Perlman
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As the COVID-19 pandemic continues around the world, greater understanding of the immune response to vaccines and natural infections are required. Here, we describe the immune response in humans who survived the natural infection and were immunized with one or two doses of one of the LNP-mRNA vaccines. We measured virus-specific antibody and T cell responses before the first vaccination and after each of the two vaccinations. The first vaccine dose greatly increased the virus-specific antibody and T cell responses. In contrast, the second vaccination often resulted in a decrease in antibody titer, while effects on the T cell response were variable. These results suggest that vaccine regimens for previously vaccinated individuals need to be carefully evaluated for

determining optimal vaccine timing. Experimentally infected animal models of COVID-19 are required to understand pathogenesis and for the evaluation of therapeutics and additional vaccine candidates. Over the past few months, we have developed several mouse models for COVID-19. Most recently, we isolated a mouse-adapted SARS-CoV-2 that causes several diseases in young and old BALB/c mice, and in aged C57BL/6 mice. Disease is confined to the lungs. Mice infected with this virus are not only useful for studies of pathogenesis of COVID-19 in the lungs, but also for other manifestations, including anosmia and ageusia.

Pathogenesis Of SARS-CoV-2

Bart Haagmans

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On 31 December 2019, the World Health Organization (WHO) was informed of a cluster of cases of pneumonia of unknown cause in Wuhan City, Hubei Province of China. Subsequently, a new SARS-CoV like virus was identified and characterized. SARS-CoV-2 infection is characterized by a range of symptoms, including fever, cough, dyspnea and myalgia. In severe cases, SARS-CoV-2 infection can be complicated by acute respiratory distress syndrome leading to respiratory insufficiency and multi-organ failure. Single cell RNA sequence analysis of human tissues has revealed new insights in the pathogenesis of this novel coronavirus. Using alveolar and airway organoids additional data can be obtained on the molecular mechanisms of the pathogenesis of SARS-CoV-2. Most importantly,combined, these studies may also provide biomarkers new opportunities to block the replication of SARS-CoV-2 by modulating the host response involved in the pathogenesis of this virus.

Reactive Immune Responses To COVID And Its Vaccines

Kari Nadeau

Stanford University, USA

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected millions of individuals globally and has resulted in over 1 million deaths. FDAauthorized messenger RNA (mRNA) vaccines against SARS-CoV-2, Pfizer- BioNTech's BNT162B2 mRNA and Moderna's mRNA-1273, provide great promise for restraining the spread of infection. With the rollout of mRNA vaccines, we sought to interrogate whether the immune responses influenced by vaccines are different between individuals who never had infection, those who had prior natural SARS-CoV- 2 infection, or those who had allergic reactions to mRNA vaccines. Recent study (PMID: 33931567) suggested that after one dose of mRNA vaccine, the individuals with prior infection showed enhanced T cell immunity and antibody secreting memory B cell response to spike protein compared to those without prior infection. In another recent study (PMID: 33795870), spike-specific-IgG antibody levels in individuals with prior infection after one dose of mRNA vaccine were similar to those without prior infection after two doses of vaccine, and the levels of the antibody measured before vaccination in individuals with prior infection were similar to those without prior infection after one dose. Our results also showed that the infected patients with long term symptoms (>30 days) had higher IgG levels than those who had symptoms for

The Post-COVID-19 Condition: From Clinical Evaluation To Pathophysiology Through The Researcher's Lens.

Emilia Liana Falcone
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It is estimated that at least 10% of all COVID-19 survivors will develop post-acute sequelae of SARS-CoV2 infection (a.k.a. the post-COVID-19 condition). This implies that at least 17 million individuals worldwide may be affected, including adults and children, whether they had mild or severe illness during the acute phase of COVID-19. The post-COVID-19 condition can involve several organ systems including the brain, lungs, heart and skin. However, our current understanding of the potential mechanisms underlying the post-COVID-19 condition are limited. Our objectives will be to review the signs and symptoms associated with the post-COVID-19 condition while discussing some of the currently proposed pathophysiological mechanisms underlying the most common sequelae. We will also discuss the post-COVID-19 research clinic model and the importance of combining research protocols and biobanking with clinical follow-up to improve our understanding of the post-COVID-19 condition and uncover novel treatments.

Take home message:
The post-COVID-19 condition is complex and requires a multidisciplinary approach combining the clinic and laboratory to improve our understanding and treatment of this disease.

From Small To Giant Through The Ages

Enteroviruses And Childhood Paralysis

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A main focus of our laboratory is the study of neurotropic enteroviruses. These single stranded (+) sense RNA viruses of the Picornaviridae family include enterovirus D68, A71, and D94. a unique enterovirus, as its physical and genetic properties are similar to both human rhinoviruses and poliovirus. Originally isolated from the nasopharyngeal cavity of children with pneumonia or bronchiolitis in California during the 1960s, few cases of EV-D68 infections were diagnosed until the late summer of 2014. Yet, most adults are seropositive for EV-D68 serum antibodies in the absence of disease. Since 2014 EV-D68 infections re-emerge biennially, and spread pandemically throughout the US, Europe, Australia and Asia. Virus infection associates with severe respiratory complications, including pneumonia and bronchiolitis, especially in patients with chronic obstructive pulmonary disease, cystic fibrosis and asthma. In some individuals, virus infection can progress to paralysis and AFM. With each subsequent biennial outbreak of EV-D68, the number of infected children and confirmed cases of AFM has grown. The association of AFM with EV-D68 infection in some children suggests that the virus can reproduce in neural tissue. To study whether EV-D68 is neurotropic, murine brain slice cultures from neonatal mice were infected with viral isolates from 1962-2014. These cultures retain tissue and circuitry integrity as well as appropriate vascularization and immune composition of the brain. All isolates examined reproduced in organotypic brain slice cultures generated from C57/B6 mice, in both neurons and astrocytes. To elucidate the mechanism by which EV-D68 invades the CNS from the respiratory tract, a mouse model of infection is being developed using mice of the collaborative cross

The Concept of Virus In The Giant Virus Era

Chantal Abergel
CNRS, France

In the mind of most biologists, a “virus” remains the most reduced and optimized vehicle to propagate a nucleic acid molecule at the expense of a cellular host, an ultimate parasite at the frontier of the living world. With genome sizes and gene contents larger than many bacteria, as well as particle sizes of the order of half a micron the Megaviridae have clearly made the point that being small and simple should no longer be considered fundamental properties of viruses, nor a testimony of their evolutionary origin. More recently, the discovery of the Pandoraviruses, with amphora shaped virions over a micrometer in length and genome sizes up to 2.8 Mb, surpassing the complexity of the smallest eukaryotic cells, raised a number of fundamental questions about giant viruses’ origin and their mode of evolution.

Finally, Pithovirus sibericum is an even larger virus in terms of particle size, but despite its amphora shaped particle, this 30,000 years old virus genome only encodes 460 proteins and is much closer to large icosahedral DNA viruses than to the Pandoraviruses. The convergence between the discovery of increasingly reduced parasitic cellular organisms and that of giant viruses exhibiting a widening array of cellular-like functions may ultimately abolish the historical discontinuity between the viral and the cellular world. I will finally discuss the biodiversity of giant DNA viruses in the light of some recent discoveries.

The RNA Virosphere: From Ecosystems To Emergence

Edward Holmes
University of Sydney, Australia

Virology has entered a discovery phase. Bulk RNA shotgun sequencing – metatranscriptomics – provides a uniquely powerful means to rapidly reveal the microbial composition of any sample without bias, with major implications for the diagnostic sciences and understanding how viruses move across the human-animal interface. Metagenomic techniques also provide important new information on the composition of the virosphere, the fundamental patterns and mechanisms of virus evolution and emergence, and are able to determine disease agents on clinically actionable timescales. Herein, I show how meta-transcriptomics is providing new insights into key aspects of virus evolution, ecology and emergence, focusing on systems as diverse as soil, fish, birds and bats, with the aim of revealing the key rules that underpin the structure of the virosphere, the frequency of host jumping, and the mechanisms that underpin virus emergence. I will conclude by considering how best to prevent the next pandemic and illustrate how meta-transcriptomics and other surveillance tools are leading to the development of potentially transformative next-generation biosecurity and surveillance systems.

Contagium Vivum Fluidum: Virus Taxonomy From The Origins of Virology Until The 21st Century

Murilo Zerbini
ICTV, Brazil

The ICTV (International Committee on Taxonomy of Viruses), a committee of the International Union of Microbiological Societies, is governed by an Executive Committee that supervises approximately 120 specialist Study Groups. The ICTV is a voluntary, self-regulated, non-profit organization responsible for developing taxonomy, including the official classification of viruses, viroids and satellites, as well as the nomenclature of approved taxa. The ICTV maintains several resources that serve the virology community. First, the taxonomy database lists all existing taxa in a hierarchical format that includes the complete history of each taxon. The database is also used to generate an annual spreadsheet, the "Master Species List". Second, the "Virus Metadata Resource" includes the names, abbreviations, isolate-designations, GenBank accession numbers and the hosts of viruses for each virus species. And third, the "ICTV Taxonomy Report", which describes the properties of viruses by taxon and the demarcation criteria used to define species. All of these resources are available free of charge online (www.ictv.global). Recent developments in virus taxonomy include: (i) the classification of viruses identified in metagenomic studies; (ii) the establishment of a 15-rank taxonomic hierarchy to accommodate the full spectrum of virus divergence; and (iii) the establishment of a binomial style of nomenclature for virus species. Although the most challenging aspect of taxonomy is the establishment of taxa that reflect the evolutionary relationships among viruses, nothing leads to more controversy than nomenclature. The adoption of a binomial nomenclature provides, for the first time, a uniform, standardized approach to naming virus species. It is important to note that this system applies to the names of virus species, and not viruses, which will make it much easier to differentiate between the species taxon and the viruses that are assigned to that taxon.

One Health, One World

Unveiling The Virosphere

Curtis Suttle
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Viruses are by far the most abundant lifeform on Earth, encompass much of its biodiversity, and are major players in regulating populations and catalyzing global biogeochemical cycles. Without doubt, viruses are critical elements contributing to and helping maintain biodiversity and ecosystem function; yet, our knowledge of the viruses populating the virosphere and quantitative information on their ecosystem effects remain scant. Here, I take a brief stroll through the virosphere, and outline some of our work that tries to unravel the nature and impact of viruses in ocean ecosystems.

Fighting The Beast - A Vaccine Against Ebola Virus

Andrea Marzi
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Ebola virus (EBOV) moved into public focus after the 2001 terror attacks in the United States of America (USA) as a potential bioterror agent. In the aftermath, the funding for research focusing on EBOV countermeasure development surged and several vaccine and therapeutic approaches were developed. From 2001-2014 most EBOV vaccine candidates were stuck at the preclinical to clinical interphase on the pathway to licensure caused by funding issues, lack of interest by pharmaceutical companies and more pressing issues for public health. However, when the unprecedented extent of the West African EBOV epidemic was recognized in 2014, finally, countermeasures for this rare, emerging zoonotic pathogen were accelerated into clinical trials towards the end of the active outbreak response. While those trials did not majorly impact the epidemic, they provided invaluable data on vaccine safety, immunogenicity and, to a limited degree, even efficacy in humans. Finally, in 2019, as a result the first EBOV vaccine, rVSV-ZEBOV (Ervebo), was approved for human use in Europe and the USA and has been utilized in the Democratic of the Congo to contain several EBOV outbreaks since 2018. Filovirus research in the Laboratory of Virology is supported by the Intramural Research Program, NIAID, NIH.

COVID-19 and Global Emerging Coronaviruses Of Humans And Animals

Linda Saif
The Ohio State University, USA

The precipitous global spread of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) ignited a devastating and ongoing pandemic. It was preceded by the deadly SARS and MERS betacoronavirus (CoV) zoonoses. All the mammalian CoVs likely originated in bat reservoirs, with some infecting intermediate animal hosts (SARS: civet cats, MERS: camels) prior to spillover into humans or other animals. Notably betaCoVs from wild ruminants experimentally infect cattle, which historically transmitted them to other species (humans, pigs, dogs, poultry). Multiple factors influence interspecies and zoonotic CoV transmission: the environment (e.g., wet markets with animal/human contact); the pathogen (dose, stability, transmissibility); and reservoir-host interactions (receptors, type/frequency of exposure, superspreaders, etc). For example, live animal markets were implicated in the SARSCoV and possibly SARS-CoV-2 outbreaks. Besides the high transmissibility of SARSCoV-2 in humans and the emergence of variants, a major concern for viral persistence is its spillover and adaptation to animals (reverse zoonoses). SARS-CoV-2 infects pets (cats, dogs) via humans in COVID-19 positive households. More concerning is the spread of SARS-CoV-2 in mink farms with evidence for reciprocal transmission between infected mink and humans. To date at least 14 species, many of them wildlife, are susceptible to SARS-CoV natural or experimental infections. A major concern is that if SARS-CoV-2 becomes established in an animal reservoir(s), the virus can mutate and continue to evolve in the new host species, extending its host range and its potential to reinfect humans or other species. Based on historical precedent and the continued presence of SARS-related CoVs in bats, novel coronaviruses are likely to continue to emerge in animals and humans. A One Health approach encompassing global disease surveillance with international

collaboration and trans-disciplinary research teams is critical to discover and control future pandemic threats, such as the impending Disease X pandemic projected by WHO.

HIV: Yesterday, Today and Tomorrow

Robert Gallo
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Epi-and Pandemics come and go but often the lessons from them are forgotten. The magnitude and modern media attention to SARS-2 may change that but remarkably talks by experts listing recent pandemics have omitted the most recent one (HIV/AIDS) while including epidemics as pandemics.
A common thread of both epi-and pandemic origin is that they involve some change in society or in the genome of the virus, and all over the past100 years have been due to RNA viruses. We want to predict when a new epidemic begins and to prevent their potential explosiveness. This needs attempting, but for some, it may be impossible (consider HIV and AIDS), and though public health lessons from one may be valuable to all others the virus science may dramatically differ (again consider HIV vs. SARS-2) I believe our approach must change. We cannot mainly rely on governments (whose positions vary). Though necessary for funding and oversight we can do much better with scientific leadership. I suggest organizations like the Global Virus Network(GVN) working under WHO leadership and with other private organizations may be a way forward. Finally, I will discuss a possible means of blunting epidemics early on by stimulation of our innate immunity.

Avian and aquatic viruses

Interspecies Transmission of Avian Influenza Viruses

Kanta Subbarao
University of Melbourne, Australia

An influenza pandemic occurs when an influenza virus with a novel haemagglutinin subtype appears and spreads in the human population, which has little or no immunity to the novel haemagglutinin (HA). Reports of direct transmission of avian influenza viruses to humans underscore the need for pandemic preparedness efforts. The influenza hemagglutinin (HA) is a critical determinant of the ability of avian influenza viruses to cross the species barrier and of the virulence of these viruses for the new host. Vaccination is the most effective method for prevention and control of influenza but the diversity of avian influenza viruses creates a challenge for vaccine development. Conventional approaches to develop pandemic influenza vaccines have been actively explored for the past decade and will provide strain-specific immunity. However, a vaccine that induces broadly cross-protective immunity or a universal influenza vaccine could provide protection against seasonal influenza as well as pandemic strains. Several options that are being explored to achieve this goal will be discussed.

Repurposing Engineered Newcastle Disease Virus In Modern Vaccinology

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Newcastle disease virus (NDV) is an economically important avian Orthoavulavirus that naturally infects nearly all birds including domestic poultry. Although the virus is controlled by mass vaccination, sporadic outbreaks of the disease still occur. Using reverse genetics, it is possible to produce a highly stable avirulent vaccine by modifying the virulence factor of the circulating virus to that of the vaccine strain. Moreover, genetically engineered NDVs are known for their incredible specificity and efficiency in antigen delivery as well as the induction of robust immune responses in the vaccinated hosts. As the entire replication occurs in the cytoplasm, the risk of the viral genome integrating into the host genome is avoided. The engineered NDV vaccine has the potential to treat human cancers as it has a propensity to selectively lyse human cancer cells with high specificity and sensitivity, making it a potential candidate for oncolytic virotherapy. It can be exploited to enter and replicate in cancer cells while leaving the normal cells unaffected. To increase the efficiency of the oncolysis, the virus can be engineered to carry immunomodulatory genes to attack the cancer cells thus opening up challenging yet exciting opportunities for the development of vaccines.

Infectious Bursal Disease Virus: Deep Understanding of Viral Molecular Biology Supports Controlling The Disease

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Infectious bursal disease virus (IBDV) is causing an immunosuppressive disease in young chickens, infectious bursal disease. The virus is globally spread and strains with different antigenic makeup and diverse levels of virulence have been described. Depending on the virulence of the strain and the genetics of the chickens up to 100% mortality is observed. The primary target organ of IBDV is the bursa of Fabricius. Due to lytical infection of developing B lymphocytes the humoral immune response is suppressed, and chickens do not respond well to vaccination programs. The virus, a major antigen in poultry vaccines, is causing global losses to the poultry industry. The virus is non enveloped, encodes for five viral proteins (VP) on two segments of double-stranded RNA. Determination of the full-length sequences of both segments was needed for the establishment of a reverse genetics system (RGS). Using the RGS several viral functions were investigated. Among them, the functionality of VP2 was analyzed. First, amino acids of VP2 were determined which enable IBDV to infect primary chicken embryo fibroblasts and permanent cell lines. This knowledge would enable the adaptation of any IBDV to cell culture to enable scalable production of vaccines. Interestingly, these amino acids are in the tip region of the virus capsid as indicated at its crystal structure. Next, the RGS in combination with the determined crystal structure of the viral capsid was used to identify amino acids in the VP2 which caused changes in its structural depended antigenicity using anti-genotype specific neutralizing monoclonal antibodies. This might help to adapt vaccines to changes in the field. Furthermore, it was shown that analysis of the antigenic phenotype did not correlate with classifications of IBDV based on phylogenic profiles. This might have an impact on epidemiological analysis of surveys just based on phylogenic analysis of sequencing data.

Fish Viral Vaccines for Global Aquaculture

Øystein Evensen
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Viral infections in aquaculture cause economic losses to the farmer, have welfare and ecological impacts, and occur across species and geography. There are a multitude of different viral diseases seen under farming conditions where fish are kept at high density and in a confined environment. I will present examples of important diseases in Atlantic salmon, tilapia, groupers, koi, flatfish and rainbow trout. The virus infections span across different viral species and examples will include those where vaccines are currently available and where none exist as of today. Focus will be on totivirus-like virus and infectious pancreatic necrosis virus, alphavirus and orthomyxovirus in salmon, megalocytiviruses of groupers and tilapia, tilapia lake virus of tilapia, cyprinid herpesvirus of koi carp, and novirhabdoviruses of trout and flatfish. Vaccination is used primarily in high value species, like salmon and trout, but are needed in many of the other species and environments, like tilapia, for groupers and flatfish/flounder. Existing vaccines in the market or under development, are inactivated whole virus vaccines (IWV), recombinant subunit vaccines, live attenuated vaccines, and DNA and mRNA vaccines. Delivery modalities are by injection (intraperitoneal), intramuscular or through water (immersion vaccination). For IWV vaccine, oil-adjuvants are used and vaccines are delivered intraperitoneally while DNA vaccines are given by intramuscular route. DNA vaccines are for salmonids, and two vaccines are commercially available against novirhabdovirusand alphavirus-infection in Canada and Europe for salmonids, respectively. Attenuated vaccines based on reverse genetics have been used to develop vaccines against novirhabdovirus infection in flounder, and several are under development for use in groupers and . The immune responses underlying protective immunity in fish in general are not well understood but for some, circulating neutralising antibody responses are good correlates of immunity. Further, antibody repertoire analysis of trout have shown generation public antibody responses shared between outbred fish

Plant viruses

RNAi In A Drum: Can It Work For Viruses?

Neena Mitter
University of Queensland, Australia

An emerging and promising alternative is the topical application of RNAi. Spraying double stranded RNA, the key trigger molecule of RNAi, has been shown to provide protection without the need for genetic modification. Consequently, development of RNA-based biopesticides is gaining momentum as a transformational strategy. Series of papers have shown that topical application of dsRNA can induce RNAimediated defence against viruses. However, a major obstacle in the commercialisation of RNA based biopesticides is the instability of topically applied dsRNA limiting the window of protection. BioClayTM technology using degradable layered double hydroxide clay particles as carriers of dsRNA has opened the window of opportunity to deliver RNA based biopesticides as a commercially viable platform for sustainable crop protection. RNAi effectors delivered as BioClay are stable, do not get washed off and provide protection to the sprayed and unsprayed leaves against the targeted virus for up to 20days post spray. BioClay platform is now being developed targeting both viruses and insect vectors. Real world application of RNA based biopesticides or ‘RNAi in a drum’ will be governed by

factors such as cost-effective production of dsRNA, stable delivery, risk identification and mitigation strategies, regulatory landscape and community acceptance.

A Viral Ribonucleoprotein Complex Guards Potato Virus A RNA Genome All The Way From Replication To Stable Particle Formation

Kristiina Mäkinen
University of Helsinki, Finland

Potyviruses comprise a very important plant virus group agriculturally, economically and biologically. We focus on the identification and functional characterization of host factors promoting potyvirus infection. Our studies on potato virus A infection in Nicotiana benthamiana have revealed an infection-associated protein complex, which is essential for viral RNA silencing suppression, translation and virion encapsidation. It consists of several viral and host proteins. Incompatibility between viral protein genome-linked (VPg) and eukaryotic initiation factor 4E/iso4E (eIF4E/(iso)4E) underlies natural recessive resistance against potyviruses in many plant species. Our data show that interaction of VPg with eIF(iso)4E via its YXXXXLΦ-binding motif is a key interaction for PVA RNA stability and we propose that it is essential for the successful assembly of the protective protein complex around PVA RNA 5'end. Helper component proteinase (HCPro) is the main potyviral RNA silencing suppressor that is involved in many host-virus interactions. It enables transfer of PVA RNA to the RNA silencing suppression pathway, which involves potyvirus-induced RNA granules (PGs). We have demonstrated that HCPro recruits antiviral host proteins VARICOSE (VCS) via a WD-domain -binding motif and ARGONAUTE1 (AGO1) via a WG/GW motif and redirects them to pro-viral functions. AGO1 and VCS accompany HCPro to the PGs and all three proteins associate with polysomes during viral translation. These interactions are also crucial for the accumulation of stable virus particles, which are required for systemic infection. In particular, the deterioration in the stability of viral particles during impaired binding of HCPro-VCS and HCPro-AGO1 may be of great practical importance for resistance breeding.

Continued Threat Of Tospoviruses: New Insights Into Virus-host Interactions And RNAi Strategies

Hanu Pappu
Washington State University, USA

Tospoviruses continue to be one of the major production constraints to a wide range of legumes, ornamentals and vegetable crops in many parts of the world. Transmitted by several species of thrips in a persistent and propagative manner, the viruses have a wide host range that include numerous weeds making disease management difficult. Among more than 32 tospoviruses, Tomato spotted wilt virus (TSWV) is the first recognized tospovirus and is one of the most prolific and economically important viruses. An integrated disease management approach is needed that includes vector management, cultural practices and growing virus resistant cultivars. As in case of many other viruses, growing virus resistant cultivars offers the most effective and eco friendly tactic for tospovirus management. Much progress has been made in developing resistant cultivars of tomato and pepper against Tomato spotted wilt virus, the most prolific member of the tospovirus group. The resistance is governed by *Sw5* and *Tsw* in tomato and pepper, respectively. However, over the years, resistant

breaking TSWV strains have emerged in several parts of the world. The search for new and effective sources of resistance should continue to ensure the sustainability of crop production and to minimize the damage by tospoviruses.

One potentially useful approach is to use genomics and transcriptomics to understand the interaction between tospoviruses and their hosts and use this information to identify potential markers and then subsequently genes that could confer resistance. Identification of susceptibility genes could be useful in genome editing approaches such as CRISPR-cas. With this long term goal, we have been using next generation sequencing to determine the small RNA profiles of TSWV in TSWV infected resistant and susceptible tomato cultivars. This approach provided insights into the processing of the TSWV genome different tomato genotypes. The virus-specific small RNAs were used to mine the tomato transcriptome to identify the potential targets among the host genes and pathways.

Next generation sequencing combined with genomic and transcriptomic profiling is being increasingly used to gain insights into host-virus interactions and has started to yield practical information that could be useful in identifying new resistant genes leading to their incorporation into existing cultivars or development of new cultivars.

Characterization Of Novel Chlorovirus Glycosyltransferases That Synthesize Atypical Glycans

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Unlike most viruses, the prototype chlorovirus PBCV-1 encodes most, if not all, of the machinery required to glycosylate its major capsid protein (MCP). The structures of the four N-linked glycans do not resemble any other glycans in the three domains of life. We are currently identifying and characterizing the chlorovirus-encoded glycosyltransferases (GTases) and methyltransferases (MTases) involved in the glycosylation of the viral MCP. The virus encoded protein A064R has three functional domains: domain 1 is a β-L-rhamnosyltransferase, domain 2 is an α-L-Rhamnosyltransferase, and domain 3 is a MT that methylates the C-2 hydroxyl group of the terminal α-L-rhamnose unit (α-L-Rha). Methylation of the C-3 hydroxyl group of the terminal α-L-Rha is achieved by A061L. Genetic, structural, and hydrolytic analyses indicate that protein A111/114R, which is conserved in all chloroviruses, is a GT with three domains: galactosyltransferase, xylosyltransferase, and fucosyltransferase.

New winds in virus diagnostics

FRET-POC – A Revolutionary Immunodiagnostic Concept

Klaus Hedman
University of Helsinki, Finland

(A) We have invented a new concept for point-of-care serodiagnosis of infectious and autoimmune diseases. The approach, based on Time-Resolved Förster Resonance Energy Transfer (TR-FRET), converts the immunodiagnostic environment homogenous, i.e. wash-free, rapid and facile. Based on this concept, we have designed several different approaches for highly specific and sensitive

immunodiagnostics (IgM, IgG and/or antigen detection) so far of Puumala hanta, B19 parvo, Zika and SARS-CoV-2 virus infections and coeliac disease (IgA).
(B) We moreover wish to transform the sampling environment of infectious and autoimmune diagnostics from invasive (blood) to non-invasive (urine) – thanks to our very recent observation of antigen-specific free-light-chains (FLCs) of immunoglobulins occurring ubiquitously in urine. We are substantiating this fact in molecular detail, and are setting up the methods required for its diagnostic application in clinical medicine.

Identification Of The SARS-CoV-2 By Mass Spectrometry

Evgeny Nikolaev
Skolkovo Institute of Science and Technology, Moscow, Russia

Detection of viral RNA by PCR is currently the main diagnostic tool for COVID-19. The PCR-based test, however, shows limited sensitivity, especially at early and late stages of the disease development, and is relatively time consuming. Fast and reliable complementary methods for detecting the viral infection would be of help in the current pandemic conditions. Mass-spectrometry is one of such possibilities. We have developed a mass-spectrometry based method for the detection of the SARS CoV-2 virus in our approach that shows confident identification of the N protein in patient samples even with the lowest viral loads and a much simpler preparation procedure. Our main protocol consists of virus inactivation by heating and adding of isopropanol and tryptic digestion of the protein sediment from the swabs followed by MS analysis. A set of unique peptides, produced because of proteolysis of the nucleocapsid phosphoprotein of SARS-CoV-2, is detected. The obtained results can further be used to create fast parallel mass-spectrometric approaches for the detection of the virus in the nasopharyngeal mucosa, saliva, sputum and other physiological fluids.

Plant Virus-Based Nanotools: Novel Functionality And Shapes For Biosensing

Christina Wege
University of Stuttgart, Germany

Plant viral nanoparticles with multivalent protein shells can serve as advantageous carrier scaffolds for displaying biomolecules at highest surface densities and in predetermined arrangements. As we found that upon immobilization on tobacco mosaic virus (TMV), enzymes may gain strongly increased stability and reusability, TMV 'adapters' were evaluated in distinct enzyme-based biosensor layouts. Both the indirect and direct (label-free, electrochemical) detection of glucose or antibiotics profited considerably from TMV scaffolds: These conveyed highest sensitivities, broadest detection ranges, shortest response times and reduced noise. Penicillin sensors retained near-constant performance over a year of repeated uses, whereas their counterparts with conventionally bound enzymes functioned a few weeks only. This seems highly promising also for other biomolecule-assisted detection strategies including long-term stable and easily calibratable point-of-care virus sensors (Poghossian et al. 2020, Front Plant Sci., doi: 10.3389/fpls.2020.598103). For advanced layouts, various straight, kinked and branched nanotubes are accessible through RNA-guided in vitro self-assembly of TMV coat proteins (CPs). Origin of Assembly (OAs) sequences in RNA molecules nucleate the bidirectional growth of TMV-like particles (TLPs) with 700 CPs/100 nm, d: 18 nm, and the enclosed RNA defining the length of the robust final nucleoprotein helix. RNAs with multiple OAs may generate angular shapes, and 3'-terminally immobilized RNAs TLP arrays - forest-like on flat substrates, nanostars on beads, or tetrahedrally expanded on adamantane-based cores. Distinct CP

types with selectively addressable reactive groups combined in single TLPs enable the installation of cooperating molecules, or units for multitasking, either in blends or in longitudinal domains. These versatile opportunities provided by TMV-based hybrid structures point at many potential uses in novel 'smart materials', due to the easy handling and high availability of the natural, plantmade building blocks (review: Wege, C. & Koch, C. (2020), WIREs Nanomed Nanobiotechnol., doi: 10.1002/wnan.1591).

Massively Multiplexed CRISPR-Based Viral Diagnostics

Cameron Myhrvold
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Inexpensive, scalable technologies for pathogen detection and surveillance are crucial to address the COVID-19 pandemic. Here, we introduce Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic acids (CARMEN), a technology that enables parallelized CRISPR-Cas13 detection with up to 5,000 crRNA-target pairs tested in a single assay. CARMEN increases multiplexing and throughput while simultaneously decreasing the reagent cost per test by >300-fold. Using CARMEN-Cas13, we designed and extensively tested a 169-plex assay that simultaneously differentiates all human-associated viruses with ≥10 available genome sequences. CARMEN-Cas13 also enables comprehensive subtyping of influenza A strains and multiplexed identification of dozens of HIV drug-resistance mutations. More recently, we have developed a CARMEN respiratory virus panel (RVP) for the differential diagnosis of SARS-CoV-2 and other respiratory viruses. In addition, we have developed SHINE, a technology for point-of-need detection of SARS-CoV-2. SHINE, the CARMEN RVP, and other such approaches can catalyze the rapid molecular diagnosis and characterization of wide-ranging pathogens, greatly benefiting patients and public health.

Animal viruses

Bats as Reservoirs for Henipaviruses

Anne Balkema-Buschmann
Friedrich-Loeffler-Institut, Germany

Soon after the emergence of the first Hendra virus (HeV) infections in horses and humans in Eastern Australia in 1994, and the first cases of Nipah virus (NiV) in pigs and humans in 1998 in Malaysia and Singapore, bats of different Pteropus spp. Have been identified as the reservoir hosts for these highly pathogenic zoonotic viruses. While in these outbreaks, transmission to horses and pigs mainly occurred indirectly via contaminated fruits or pasture, humans mainly contracted the diseases via direct contact to infected pigs or horses. In contrast, direct transmissions from bats to humans after ingestion of contaminated date palm zap or fruits has been established in Bangladesh and India since 2008.

Interestingly, virus excretion levels in bats are generally very low, rendering spillover transmission of extremely rare events. However, viral shedding fluctuates with the reproductive cycle, which explains the seasonal fluctuation of reported NiV and HeV cases. Besides the initial description in South-East Asia, molecular and serological evidence have also confirmed the presence of henipaviruses in numerous African countries, mainly in bats of Eidolon or Rousettus spp., as well as in pigs, horses

and humans.So far, there are no indications of a noticeable pathogenic potential of African henipaviruses. However, their molecular and serological differentiation from the highly pathogenic NiV and HeV are crucial for a risk assessment regarding the possible transmission of highly pathogenic henipaviruses to livestock or humans in Africa.
To allow a thorough investigation of the virus tissue distribution after infection, the pathogenesis and the viral shedding in relevant fruit bat species, breeding colonies of Rousettus aegyptiacus and Eidolon helvum fruit bats have been established at the Friedrich-Loeffler-Institut (FLI). This allows the establishment of serological assays for the screening and differentiation of bat serum samples, as well as the implementation of in vivo challenge studies in these species.

One Health Investigations Of West Nile Virus Lineage 2 In South Africa

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University of Pretoria, South Africa

Background: West Nile virus (WNV) is endemic to South Africa but remains underreported.
One Health surveillance in animals with neurological signs detected a large outbreak of WNV across the country in 2017. This prompted us to collect human cerebrospinal fluid (CSF) from hospitals, in Gauteng province (arbovirus season, January-June 2017) and CSF and blood through systematic surveillance from patients with acute febrile disease of unknown cause (AFDUC) and/or neurological signs at 3 hospitals in Gauteng and Mpumalanga provinces (ANDEMIA study)(2019- 2020). Mosquito vectors were collected at sentinel sites in 3 provinces(2011-2018). We investigated the epidemiology and phylogenetic relationship of WNV in humans, animals and vectors. Animal surveillance(2017-2020): 18/1078(1.7%) specimens tested WNV positive by RTPCR(3.0% equine, 3.0% wildlife species, 1.8% livestock, 2.5% avian species and 8.3% domestic animals) plus 68/657(10.4%) additional equines by IgM ELISA (2017- 2020). WNV accounted for 10.2% of all submissions (2017), 2.8% (2018), 7.0% (2019) and 7.2% in 2020. In 91.0% of WNV cases neurological signs were present, 34.0% fatal. Human surveillance(2017-2020): In 2017, 2/158(1.27%) CSF samples tested WNV positive by real-time PCR while 8/123 (6.50%) were positive by WNV specific IgM. In ANDEMIA patients (2019-2020) 25/347(7.20%) tested positive for WNV IgM only: (11/212, 5.19%) 2019 and (14/135, 10.37%) 2020. Half of WNV cases presented with neurological signs. In total 5.6% of acute undiagnosed neurological cases and 7.20% of AFDUC were WNV positive. Mosquito vectors(2011-2018): WNV was identified in 16(1.08%)/1471 pools(40731female mosquitoes)(1.09%, MIR=0.39) collected in Gauteng, Limpopo and Mpumalanga provinces, in mainly Culex spp. Phylogenetic analysis confirmed WNV lineage 1 in 1 animal case while all other animal, human and mosquito samples clustered with South African lineage 2 strains. Conclusion: WNV contributed to severe neurological and febrile disease in humans and animals in 2017-2020 in South Africa. Culex vectors commonly distributed in all sentinel sites are the likely vectors.

FMDV Modulation Of The Host Immune Response

Teresa de los Santos
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Foot-and-mouth disease (FMD) is an acute and highly contagious disease that affects cloven-hoofed animals often resulting in economic devastation and impacting agricultural development and sustainability. Although its etiologic agent, FMD virus (FMDV), was discovered over a century ago, disease control still poses great challenges. A small genome characteristic of picornaviruses has

allowed FMDV to particularly overcome many aspects of the host innate immune response. Here, we will summarize the multiple mechanisms FMDV has evolved to become such a successful pathogen, with particular emphasis in the role of the leader protease, a viral factor of exquisite properties.

Insights In Viral Uncoating And Fusion

Covadonga Alonso
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African swine fever virus (ASFV) infectious cycle starts with the viral adsorption and entry into the host cell. After attachment to an unknown receptor, the virus is internalized via clathrin/dynamin mediated endocytosis and macropinocytosis. Then, as several other viruses, ASF virion is internalized and incorporated into the endocytic pathway. Endosomal maturation entails luminal acidification and the lowering of pH acting on the multilayered virion structure dissolves the outer capsid. Upon decapsidation, the inner viral membrane is exposed and could interact with the limiting membrane of the late endosome for fusion. Egress from the endosome is related to cholesterol flux but it remains an intriguing process albeit essential for infection for the viral nucleic acid exit to the cytoplasm for replication. Another related large DNA virus, vaccinia virus (VACV), has a multiprotein fusion complex. ASFV proteins E248R and E199L with structural homology to the VACV proteins of the fusion complex are the protein candidates from the virus side. We have observed a direct interaction between these ASFV proteins and cholesterol transporter protein NPC1 (Niemann-Pick C type 1), as their counter partner at the endosomal membrane and this suggests the implication of these proteins that are crucial for membrane traffic. CRISPR NPC1KO-Vero cells lacking NPC1 protein that were resistant to Ebola virus infection, reduced ASFV infection levels significantly but could not block infection completely. In fact, we observed a compensatory effect in NPC1 KO cells, elevating NPC2 levels. NPC2 is a luminal endosomal protein that acts by tethering cholesterol to NPC1 for transport. Silencing NPC2 in Vero cells with shRNA NPC2, also reduced ASFV infection. The role of these proteins in the membrane viral fusion step for several viruses will be discussed.

Clinical virology

Enteroviruses And Diabetes

Heikki Hyöty
Tampere University, Finland

Type 1 diabetes is caused by an immune-mediated destruction of insulin-producing cells in the pancreas. Several genes modulate the susceptibility for the disease but the rapid increase in type 1 diabetes indicates that environmental factors play an important role in the pathogenesis. Virus infections have been among the most suspected ones, since they cause diabetes in animals and some of them have also been linked to human type 1 diabetes. Recently, considerable progress has been achieved in studies evaluating the possible role of enteroviruses. They have been linked to type 1 diabetes in several epidemiological studies and detected in the pancreatic islets of diabetic patients. Enteroviruses are common including more than 100 different subtypes, the most well known being the polioviruses. Another group of enteroviruses, the group B coxsackieviruses, has linked to type 1 diabetes in epidemiological studies, they can infect pancreatic beta-cells in vitro and in vivo, and their receptor, the coxsackie and adenovirus receptor CAR, is strongly expressed in beta-cells. Viral persistence in insulin-producing cells has been suggested and prolonged infections precede the

initiation of the autoimmune process. This has led to a need for intervention trials to evaluate possible causality. Effective vaccines are available against certain enteroviruses (polioviruses and enterovirus 71) but not against the group B coxsackieviruses. We started a project aiming at developing a new vaccine against these viruses and to test its efficacy in human trials. Experimental coxsackie B virus vaccines have been effective and safe in animal models, and the first human vaccine trial is in progress. In addition, the first trial with antiviral drugs among diabetic patients is in progress. The coming years will show whether these efforts will eventually generate antiviral strategies that could be used to prevent type 1 diabetes.

The Path Towards A Cure Of Chronic HBV Infection

Fabien Zoulim
Lyon University, France

HBV affects more than 250 million people worldwide, and is a main cause of liver cirrhosis and hepatocellular carcinoma. In spite of universal vaccination programs, HBV infection remains a public health problem, with only suppressive strategies available implying lifelong therapy. Therefore, HBV infection emerges as an unmet medical need that requires the development of new individual molecules and combinations to achieve the goal of HBV elimination. Functional cure defined by the sustained clearance of HBsAg in serum after treatment cessation is considered as the main attainable goal. Major progress has been made in our understanding of HBV persistence, and has paved the way for the development of novel cure strategies. Several approaches are being explored with direct acting antivirals to deplete directly or indirectly the intrahepatic pool of HBV cccDNA, or strategies to restore adaptive immune responses to eliminate infected cells and control the infection. Direct acting antivirals include, beside nucleos(t)ide analogues, entry inhibitors, viral RNA targeting agents (SiRNA, antisense oligonucleotides, and other small molecules), capsid assembly modulators, and HBsAg release inhibitors. Drugs targeting cccDNA are explored in pre-clinical models. Since cccDNA eradication remains challenging, the antivirals in current development will likely be combined with immunotherapy to maintain the antiviral effect on the long term. Immunotherapeutics in development include strategies to invigorate immune responses through the stimulation of innate immunity with TLR7 or TLR8 agonists, or the inhibition of immune checkpoints with PD1 or PDL1 blocking agents. They also include strategies to stimulate the HBV specific adaptive responses. The development of novel biomarkers to assess target engagement and predict treatment endpoints are also needed to assist drug development. We are thus entering a very exciting time for HBV cure programs and several clinical trial programs are ongoing to assess the efficacy and safety of the emerging combination therapies.

Tumor Suppression By Oncolytic Parvoviruses: From Bench To Bedside And Back

Jean Rommelaere
DKFZ, Germany

Oncolytic virotherapy using viruses endowed with natural or engineered capacity to recognize and destroy tumors has emerged as a promising treatment modality in modern oncology. Rodent proto parvoviruses, in particular the rat H-1 parvovirus (H-1PV), have shown broad-range tumor-suppressive properties in various preclinical cancer models. In addition to inducing selective cancer cell death, these viruses are also able to exert immunostimulatory effects, prompting establishment of an antitumorigenic proinflammatory tumor microenvironment. Parvoviruses therefore

hold potential for enhancing the efficacy of other immunotherapies. Initial encouraging in-human observations from clinical trials and compassionate virus uses are presented and speak in favor of further H-1PV clinical development as partner drug in combined immunotherapeutic protocols against cancer.

Host And Microbial Regulation Of Norovirus Pathogenesis

Stephanie Karst
University of Florida, USA

Noroviruses are a leading cause of severe childhood diarrhea and gastroenteritis outbreaks across the globe yet there are major gaps in knowledge about how they cause disease and interact with their hosts. The lack of information about norovirus pathogenesis and immunity stems from their notorious intractability to in vitro propagation and strict species specificity. Our work on murine norovirus infection has revealed that noroviruses infect intestinal immune cells, a finding that has significant implications for pathophysiological and immunological outcomes of infection. By using small animal models of norovirus infection, we have identified host and environmental factors that regulate infection. Of particular importance, bile acids are metabolites biotransformed by commensal bacteria in the intestinal lumen. They play regionally distinct and opposing roles in norovirus infections, inhibiting viral infection of the proximal intestine via priming an antiviral immune response and promoting viral infection of the distal intestine by enhancing virus binding to its host receptor. I will discuss consequences of immune cell infection and bile acid interactions on norovirus pathogenesis.

Phage And Insect Viruses

Antiviral Defences in Insects: The Impact of miRNA

Karyn Johnson
University of Queensland, Australia

Insects host a variety of viral infections and can act as vectors of virus diseases. The small RNA pathways can be important regulators of the outcome of infection in insect hosts. miRNAs are recognised as widespread regulators of gene expression across many biological functions including antiviral defence and immunity. miRNAs are ~22 nucleotide non-coding RNAs that are transcribed from the genome and bind in a sequence-specific manner to target messenger RNAs (mRNA). miRNAs act as guides for effector complexes, enabling modulation of gene expression primarily via mRNA decay or translational inhibition. Using the Drosophila model, we have demonstrated that two miRNAs (miR-956-3p and miR-8-5p) can have a considerable impact on virus infection, one being proviral and the other antiviral. However, the influence of the vast majority of miRNAs on virus infection remains untested and it is not yet known whether there is generality in the influence of miRNAs across diverse viruses. The recent development of genetic tools in Drosophila made the possibility of functional analysis of a large number of miRNAs in whole flies achievable. We conducted an unbiased screen of Drosophila miRNA loss-of-function lines. We challenged the flies with viruses from different virus families to answer questions about the proportion of miRNAs that impact virus infection, and to what extent the impact of the miRNAs is conserved across diverse virus families.

Densoviruses For Insect Biocontrol: Something Old, Something New

Myléne Ogliastro
University of Montpellier, France

Densoviruses are small DNA viruses infecting invertebrates that belong to the family *Parvoviridae*. Discovered in the 1960's as insect pest pathogens, densoviruses have been considered as potential tools for biocontrol, although they received little attention at the time of success of chemical insecticides. The loss of insect diversity and the rapid emergence of resistances among pests and vectors put denso viruses "back in the future" as alternative biocontrol solutions. Developing a safe and sustainable use of viruses to control insects requires a comprehensive understanding of virus-host interactions at different scales, i.e. from the molecular mechanisms driving virus specificity and evolution, to their diversity and function in (agro)ecosystems. I will describe here the research done on denso viruses, in the lab and in the wild, to understand their pathogenesis, explore the mechanisms driving viral evolution and survey their diversity in ecosystems, highlighting the prospects that agrosystem viral metagenomics open today.

The Ongoing Battle Between Phages And CRISPR-Cas Systems

Sylvain Moineau
Université Laval, Canada

Fighting viruses is no easy task. Bacteria use an array of sophisticated defence strategies to thrive in virus-rich ecosystems. CRISPR-Cas is one of these mechanisms. Bacterial CRISPR-Cas type II systems function by first incorporating short DNA 'spacers', derived from invading defective phage genomes, in the CRISPR array mostly located in their genome. The bacterial CRISPR array is then transcribed and matured into short RNAs, which, by recruiting Cas9 endonuclease, act as surveillance complexes that recognize and cleave subsequent invading matching DNA sequences. The cleavage occurs near a short PAM motif, adjacent to the sequence targeted by the spacer. Phages have evolved counter-tactics to thwart such mechanisms, leading to a so called biological arms race. For example, phages can bypass CRISPR immunity through point mutation or deletion of the CRISPR target or PAM in their genome as well as by the production of anti-CRISPR proteins (ACRs). Using the Gram-positive dairy bacterium Streptococcus thermophilus as a model, I will recall the roles played by virulent phages in the understanding of CRISPR-Cas systems and the development of industrially-relevant phage-resistant bacteria. The emergence of ACR-containing phages illustrates the ongoing battle between phages and their hosts and the need for additional anti-phage approaches in industrial settings.

Phage Therapy: Experience And Perspectives

Mzia Kutateladze
Eliava Institute, Georgia

Discovery of bacteriophages, particularly their ability to replicate and lyse pathogenic bacteria may have been among the most important milestones in the history of biomedical sciences. In the

pre-antibiotic era of the early 20th century, phage therapy was becoming a powerful weapon against infectious diseases of bacterial etiology in many different countries. Unfortunately, phage treatment and research was largely forgotten in the Western world as antibiotics became widely available. In contrast to its decline in the West, phage therapy remained a standard part of the healthcare systems in Eastern Europe and the USSR during the second half of the 20th century. Phage preparations were used for diagnostic, therapeutic and prophylactic purposes to combat various bacterial infections. Nowadays, the rapid propagation and spread of multi-drug resistant bacterial strains is leading to renewed interest in phage therapy. Public perception and awareness of phage application to treat infections is changing rapidly in the world. More and more publications (scientific and popular) of results of phage treatment indicate the growing interest among the patients and doctors as well. George Eliava Institute of Bacteriophages, Microbiology and Virology (Tbilisi, Georgia) is perhaps the most famous institution in the world focused on the study and application of bacteriophages. Study of causative bacterial agents of infection and elaboration of phage-based preparations against infectious diseases are the main focus of the Eliava Institute today. Main application of the Eliava phages is directed for treatment and prophylaxis of human bacterial diseases. Phages are successfully used to treat acute, as well chronic infections caused by antibiotic-resistant bacterial strains. The author will present several case reports after application of bacteriophages for treatment of various infectious complications.

Submitted Talks

SARS-CoV-2: Disease & Pathogenesis

440893

A 65-year-old Woman With A History Of Type 2 Diabetes Mellitus And Hypertension And A 15-day History Of Dry Cough And Fever Who Presented With Acute Renal Failure Due To Infection With SARS-CoV-2

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Acute kidney injury is one of the most common complications in patients infected with SARS-CoV-2, occurring in up to 7% of cases and increasing to 23% in patients treated in the Intensive Care Unit (ICU). This report's objective was to describe the clinical case of a patient infected by SARS-CoV-2 who developed acute renal injury, probably secondary to this infection.
On 1 April 2020, a 65-year-old woman presented to the emergency service of the National Institute of Respiratory Diseases, Mexico City, with a 15-day history of dry cough and subjective fever. Finally, the following diagnoses were integrated: Acute renal injury of etiology to be determined (acute chronic kidney disease secondary to T2DM vs. acute renal injury by SARS-CoV-2) and COVID-19. The patient had a typical presentation of severe COVID-19, evidencing all the risk and severity factors for this disease. However, after being admitted to the hospital, she showed evidence of acute renal injury. Although the renal injury may have been due to microangiopathic damage caused by chronic

hypertension and diabetes, it is imperative to consider the possibility that such exacerbation contributes to SARSCoV-2 infection or synergy of multiple factors. Every aspect of this pandemic remains unclear. The formulation of hypotheses to explain the physiopathological mechanisms by which this new virus can cause mortality in infected patients may help reduce mortality rates and control the pandemic.

445664

The Spectrum Of Gastrointestinal Symptoms In Patients With Coronavirus Disease-19: Predictors, Relationship With Disease Severity, And Outcome

Ghoshal Uday C, Ghoshal Ujjala, Mathur Aakash, Singh Ratender K, Nath Alok, Singh Dharamveer Singh Sanjay, Singh Jasmeet, Pandey Ankita, Rai Sushmita, Vasanth Shruthi, Dhiman Radha Krishan
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INTRODUCTION: We prospectively studied the frequency, spectrum, and predictors of gastrointestinal (GI) symptoms among patients with coronavirus disease-19 (COVID-19) and the relationship between GI symptoms and the severity and outcome. METHODS: Consecutive patients with COVID-19, diagnosed in a university hospital referral laboratory in northern India, were evaluated for clinical manifestations including GI symptoms, their predictors, and the relationship between the presence of these symptoms, disease severity, and outcome on univariate and multivariate analyses. RESULTS: Of 16,317 subjects tested for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in their oropharyngeal and nasopharyngeal swabs during April–May 2020, 252 (1.5%) were positive. Of them, 208 (82.5%) were asymptomatic; of the 44 symptomatic patients, 18 (40.9%) had non-GI symptoms, 15 (34.1%) had a combination of GI and non-GI symptoms, and 11 (25.0%) had GI symptoms only. Thirty-three had mild-to-moderate disease, 8 severe, and 5 critical. Five patients (1.98%) died. On multivariate analysis, the factors associated with the presence of GI symptoms included the absence of contact history and presence of non-GI symptoms and comorbid illnesses. Patients with GI symptoms more often had severe, critical illness and fatal outcomes than those without GI symptoms. DISCUSSION: Eighty-two percent of patients with COVID-19 were asymptomatic, and 10.3% had GI symptoms; severe and fatal disease occurred only in 5% and 2%, respectively. The presence of GI symptoms was associated with a severe illness and fatal outcome on multivariate analysis. Independent predictors of GI symptoms included the absence of contact history, presence of non-GI symptoms, and comorbid illnesses.

440815

Functional And Molecular Impact Of SARS-CoV-2 Infection On Cerebral Activity

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The current COVID-19 pandemics put the world in an unprecedented situation, with a wide impact on our daily lives. Increasing evidence indicates that short- and long term neurological complications are associated with SARS-CoV-2 infection. Yet, minimal information regarding the impact of the virus onto the central nervous system is available, and the lack of molecular and functional studies dampen our understanding of these COVID-19-induced neuropathological features (neuroCOVID). Here, we highlight important neurological impacts of SARS-CoV-2 at the molecular and functional levels using human stem cell-derived cerebral organoids or an ex vivo model of cultured post-mortem human brain slices. We showed that exposure to SARS-CoV-2 significantly decreases the amplitude of the electrical signal detected by three-dimensional microelectrode arrays, suggesting that the virus modulates functional brain activity. To gain further molecular insights into this phenomenon, we performed transcriptomic and proteomic analyses, comparing non-infected and SARS-CoV-2-infected cerebral organoids. We found that the virus induces very subtle changes that could not be readily classified according to gene ontology. Nevertheless, a small subset of proteins was significantly modulated, including a transmembrane surface protein known to promote excitatory synapse formation. The upregulation of this protein was also confirmed in human brain slices using western blot and immunostaining and further investigations are ongoing to identify whether this protein is involved in SARS-CoV-2- induced electrical impairment. In conclusion, the use of animal-free physiological models allowed us to demonstrate that SARS-CoV-2 perturbs electrical brain activity and to identify potential molecular partners involved, which may be important to develop innovative treatments against neuroCOVID.

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445407

Clinical And Serological Findings Of COVID-19 Patients In The Region Of Makkah, Saudi Arabia.

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Makkah in Saudi Arabia hosts the largest annual religious event in the world. Despite the many strict rules enacted, including Hajj cancellation, city lockdowns and social distancing, Makkah region has the second highest number of new COVID-19 cases in Saudi Arabia. Public health interventions based on identifying, isolating, and managing new cases could slow the infection rate. While RT-PCR is the current gold standard in SARS-CoV-2 diagnostics, it yields false positive and negative results, which mandates the use of serological complementary tests. Here, we report the utility of serological assays during the acute phase of patients with moderate and severe clinical manifestations of SARS-COV-2. Fifty patients with positive RT-PCR results for SARS-CoV-2 were enrolled in this study. Following RT-PCR diagnosis, sera samples from the same patients were analyzed using in-house Microneutralization Tests (MNT) and ELISA (IgM, IgA, and IgG) for the presence of antibodies. Of the 50 patients analyzed, 43 (86 %) showed a neutralizing antibody titer of >= 20. Using univariate analysis with neutralizing antibodies as a dependent variable and the degree of disease severity and underlying medical conditions as fixed factors revealed that patients with no previous history of non-communicable diseases and moderate clinical manifestation had the strongest neutralizing antibody response (Mean: 561, 11). Patients with severe symptoms and other underlying diseases, including deceased individuals, demonstrated the lowest neutralizing antibody response. However, the observed differences were found to be statistically insignificant according to ANOVA analysis. Conversely, anti-Spike protein antibody responses, as measured by ELISA (IgM, IgA, and IgG), showed a statistically significant correlation with neutralizing antibodies. This shows that serological

assays complement molecular testing for diagnostics, but the previous medical history (anamnesis) of patients should be considered in the interpretation of serological results.

445589

SARS-CoV-2 Gene Products Differentially Regulate Inflammatory Cytokine Production As Potential Mediators Of Severe Disease In COVID-19

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SARS-CoV-2, a novel coronavirus, is the cause of the coronavirus disease 2019 (COVID-19) pandemic, resulting in unmatched worldwide morbidity and mortality. Severe COVID-19 is associated with a cytokine storm marked by innate immune responses and inflammatory cytokine secretion, mediated by several pathways, including the NLRP3 inflammasome. There is an urgent need for understanding the pathogenesis and development of novel therapeutic approaches. SARS-CoV-2 genes, including ORF3a and ORF8, are critical for modulating pathogenicity. Here we sought to probe the role of SARS-CoV-2 genes in mediating inflammatory cytokine signaling. HEK 293T cells were transfected with plasmids expressing the following SARS-CoV-2 genes: OF3a, ORF7A, ORF8, envelope (E) protein. Supernatants were collected and analyzed for secretion inflammatory cytokines, TNF-α, IL-8, CXCL10, IL-10, CCL2, IFN-α, IFN-γ, IL-4, CCL3, IL-12, IL-18, IL-1β by beadbased immunoassay using flow cytometry. Supernatants were also applied to PMAdifferentiated THP-1 macrophages and analyzed by bead-based immunoassay after 4 hours. ORF8 was a potent inducer of IL-1β, and OFR3a was a potent inducer of IL8. ORF7A and envelope expression did not significantly induce inflammatory cytokines, TNF-α, IL-8, CXCL10, IL-10, CCL2, IFN-α, IFN-γ, IL-4, CCL3, IL-12, IL-18, IL-1β. ORF3a expression resulted in significant IL-1β induction in PMA-differentiated THP-1 macrophages. SARS-CoV-2 gene products can differentially regulate inflammatory cytokine production in 293T cells and THP-1 macrophages. These observations implicate ORF3a and ORF8 as mediators of pathogenesis in SARSCoV-2 infection and drivers of cytokine storm in severe COVID-19. Future studies will probe the mechanism of this activation and in therapeutic development in directacting antivirals to reduce the cytokine storm associated with morbidity and mortality of COVID-19.

439559 3min Student

Clinical And Laboratory Findings Of COVID-19 In High Altitude Inhabitants Of Saudi Arabia

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Background: SARS-CoV-2, the causative agent of COVID-19, continues to cause a worldwide pandemic, 1with more than 100 million being affected globally as of this writing. People's responses to

COVID-19 range from asymptomatic to severe, and the disease is sometimes fatal. Its severity is affected by different factors and comorbidities of the infected patients. Living at a high altitude could be another factor that affects the severity of the disease in infected patients.
Methods: In the present study, we have analyzed the clinical, laboratory, and radiological findings of COVID-19 infected patients in Taif, a high altitude region of Saudi Arabia. In addition, we compared matched diseased subjects to those living at sea level. We hypothesized that people living in high altitude locations are prone to develop a more severe form of COVID-19 than those living at sea level.
Results: Age and a high Charlson comorbidity score were associated with increased numbers of intensive care unit (ICU) admissions and mortality among COVID-19 patients. These ICU admissions and fatalities were found mainly in patients with comorbidities. Rates of leukocytosis, neutrophilia, higher D-dimer, ferritin, and highly sensitive C-reactive protein (CRP) were significantly higher in ICU patients. CRP was the most independent of the laboratory biomarkers found to be potential predictors of death. COVID-19 patients who live at higher altitude developed a less severe form of the disease, and had a lower mortality rate, in comparison to matched subjects living at sea level.
Conclusion: CRP and Charlson comorbidity scores can be considered predictive of disease severity. People living at higher altitudes developed less severe forms of COVID-19 disease than those living at sea level, due to a not-yet-known mechanism.

445211 3min Student

Detection Of SARS-CoV-2 In Various Clinical Specimens Of Hospitalized Latvian COVID-19 Patients

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Background and Aims. Even though COVID-19 and SARS-CoV-2 infection is generally associated with respiratory symptoms, this virus is able to cause extrapulmonary manifestations, as the expression of the cell entry receptor of SARS-CoV-2 can be detected in various tissues. We aimed to explore the detection of SARS-CoV-2 in various clinical specimens collected from hospitalized COVID-19 patients. Materials and methods Nasopharyngeal swab, peripheral blood, urine and fecal samples were collected from COVID-19 patients hospitalized at Latvian Centre of Infectious Diseases in the first few days of hospitalization. Blood plasma and peripheral blood mononuclear cells (PBMCs) were isolated. SARS-CoV-2 was detected, and the viral load determined using commercially available quantitative real-time PCR kit. Results Overall, SARS-CoV-2 was detected in all types of clinical specimens collected. Highest number of SARS-CoV-2 positive samples were observed among the swab samples, closely followed by fecal samples (69.6 % and 55.1% respectively). Statistically higher viral loads were detected in fecal samples as compared to the swabs (46204 [IQR: 5424.0-3919660.0] vs 6004 [IQR: 468.3- 32859.0] viral copies/ml, p=0.0009). Few patients even had more than three SARS-CoV-2 positive samples, with one harboring SARS-CoV-2 in all of the analyzed samples. Clinical data analysis revealed this patient to have multiple chronic health problems, and that he had deceased from COVID-19 complications. Interestingly, most of the patients harboring SARS-CoV-2 in PBMC samples, had very low or undetectable IgG and IgM antibody titers. Conclusions SARS-CoV-2 infection seems to have an extrapulmonary distribution, as it could be detected in all of the sample types analyzed. Clinical significance of the multiple sample SARS-CoV-2 detection remains to be elucidated.

445539 3min News

Persistent/Chronic Viral Co-infections In COVID-19 Patients In Latvia

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Objectives. Several SARS-CoV-2 peak protein heptapeptides have been shown to have similarity to human proteome heptapeptides, which apparently explains the autoimmune nature of this SARS-CoV-2. Co-infections have been reported in severe acute respiratory syndrome (SARS) patients but there is limited information on coinfections by other viruses triggering autoimmunity in COVID-19 patients. In view of the above, the impact of existing co-infections on the clinical manifestations of COVID-19 patients cannot be ruled out, therefore we analysed the presence of 12 persistent/chronic viral infection markers in biological samples from COVID-19 patients in Latvia. Materials and Methods. In total 92 [41 (44.6%) male, median age 61 years; 51 (55.4%) female, median age 65 years] hospitalized COVID-19 patients were included. Presence of 12 viral genomic sequences in 92 DNA samples isolated from peripheral blood mononuclear cells (PBMC) and cell-free blood plasma was tested by multiplex PCR (Allplex Respiratory Panel 4, Allplex Meningitis-V1 Assay and Allplex Meningitis-V2 Assay, Seegene Inc., Republic of Korea). Results. In total 30 out of 92 (32.6%) PBMC samples were Epstein-Barr virus positive, 17 out of 92 (18.5%) samples were human herpesvirus 7 positive and one sample – human herpesvirus 6 positive. No plasma samples were positive for any of the viruses tested. For 6 (6.5%) patients, co-infection of Epstein-Barr virus and human herpesvirus 7 was detected, and for one patient – Epstein-Barr virus and human herpesvirus 6. Conclusions. Epstein-Barr virus is the most common chronic/persistent co-infection among hospitalized COVID-19 patients in Latvia, followed by human herpesvirus 7 infection. Given that all these herpesviruses are also triggers for autoimmune diseases, it is possible that their co-infection with SARS-CoV-2 may affect the course of the disease, making it more severe and triggering autoimmune processes.

444074 3 Min News

Cytokine Storm And COVID-19 Severity In Hospitalized And Non-Hospitalized Patients

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Objectives. Cytokine storm in case of COVID-19 is considered to be one of the major causes of acute respiratory distress syndrome (ARDS) and multiple organ failure. It plays an important role in the process of disease aggravation. Aim of the study was to characterize the effect of SARS-CoV-2 on the production of cytokines in patients with severe clinical symptoms and selected sets of samples from patients with mild or no clinical symptoms. Materials and Methods. Cytokine panel (GM-CSF, IFN-γ, IL-1β, IL-6, IL-8, IL-17A, IL-18, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-AB/BB, TNF-α, VEGF-A) was created to determine the differences in cytokine levels in hospitalized and non-hospitalized patients using the Luminex200 system. Plasma samples from 117 (60.7±16.5 years) hospitalized and 61 (43.8±15.2 years) - non-hospitalized patients were analysed. From those patients - 10 hospitalized and 17 non-hospitalized were studied at 2 time points. The obtained results were analysed using

GraphPad Prism 9.0. Results. Comparing hospitalized patients against outpatients results clearly shows that most patients with severe COVID-19 exhibit markedly increased plasma levels of pro-inflammatory cytokines and the statistically significant correlation between hospitalized patients and outpatient for multiple cytokines: IFN-γ, IL-6, IL-8, IL-18, IP-10, MCP-1, TNF-α and VEGF-A. IL-8, IL-19, IP-10, PCP-1, MIP-1β were higher in patients who received supplemental oxygen than in those who did not. Inpatients had higher levels of IL-18 and IL-8 cytokines in men than in women, while PDGF levels were higher in women. No effect of gender on cytokine levels was observed in the non-hospitalized group. Conclusions. Patients receiving oxygen supplementation had higher plasma levels of IL-8, IL-18, IP-10, MCP1, and MIP-1α, indicating not only on virus-induced inflammatory processes but also on possible damage of lung tissue, hart failure, neuroinflammatory processes characterized by neuronal degeneration and on autoimmune processes.

429142 3min News

Impact Of COVID-19 On Psychological Health And Academic Performance Of Medical Students

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BACKGROUND AND OBJECTIVES:COVID-19 pandemic affected mental health and psychosocial conditions of everyone worldwide according to the WHO. Public health emergencies affect college students and are expressed as anxiety, worry, and fear. The aim of this study was to assess the impact of COVID-19 on the psychological health and performance of medical students.
DESIGN AND SETTINGS: a cross-sectional study using an online survey was done. PARTICIPANTS AND METHODS: participants were 1591 Saudi medical students. A predesigned questionnaire included questions on demographic characters, GPA, having a relative got COVID-19, how to face sad news and stressors, and satisfaction with online lectures. The Generalized Anxiety Disorder (GAD-7) scale was used that included seven-items asking about the degree the participant was bothered by feeling anxious in the last two weeks.
RESULTS: 59.4% of students had various degrees of anxiety. Participant age of the, unsteady family income, high GPA, satisfaction with the online lectures and being female was associated with higher GAD-7 scores.
CONCLUSION: mental health of medical students was significantly affected by the COVID-19pandemic. Psychological support programs for medical students during the pandemic and provision of high quality distance learning is essential for psychological well-being during the pandemic.

SARS-CoV-2: Immunity

441268

The Effect Of Repeated Immunizations With Coronavirus Proteins On Immunity And Cross-reactivity Within The Human Coronavirus Family Members

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COVID-19 pandemic has spread throughout the world and as of April 2021 nearly 140 million cases and 3 million deaths have been recorded. COVID-19 vaccine development has proceeded exceptionally fast and dozens of vaccines are under development or in clinical use. In this work we describe the production and validation of polyclonal rabbit and guinea pig antibodies against SARS-CoV-2 N, S1 and RBD proteins as well as MERS, HKU1, OC43, 229E, NL63 N proteins. In addition, antibody cross-reactivity analyses between seasonal coronaviruses and SARS-CoV2, and microneutralization assays against three SARS-CoV-2 variants were carried out. Furthermore, a limited comparison of the efficacy of MF59-like adjuvant and adjuvant system 03 (AS03) was executed. Immunofluorescence results revealed no cross-reactivity of SARS-CoV-2 anti-S or anti-N antibodies against seasonal coronavirus proteins and vice versa. This may indicate that previous seasonal coronavirus infections do not provide protection against COVID-19 infection. The results from immunofluorescence and microneutralization assays suggest that antisera produced in the presence of AS03 adjuvant have higher titers compared to antisera that were generated using MF59-like adjuvant. Although few animals had quantifiable immunofluorescence and microneutralization titers after the first immunization, overall remarkably high antibody levels were seen after the second or third round of immunizations. In some cases the fourth antigen dose leads to reduction of antibody levels. We also show that antisera against SARS-CoV-2 S1 or RBD proteins effectively neutralized the SARS-CoV-2 B.1 and B.1.1.7 (UK) variants, whereas the neutralization activity against the B.1.351 (South Africa) variant was clearly reduced.

441284

COVID-19 mRNA Vaccine Induces Antibody Responses And Neutralizing Antibodies Against SARS-CoV-2 Variants B.1.1.7 And B.1.351

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As SARS-CoV-2 has been circulating for over a year, dozens of vaccine candidates are under development or in clinical use. The BNT162b2 mRNA COVID-19 vaccine induces spike protein-specific neutralizing antibodies associated with protective immunity. The emergence of the B.1.1.7 and B.1.351 variants has raised concerns of reduced vaccine efficacy and increased re-infection rates. Here we show, that after the second dose, the sera of BNT162b2-vaccinated health care workers (n=180) effectively neutralize the SARS-CoV-2 variant with the D614G substitution and the B.1.1.7 variant, whereas the neutralization of the B.1.351 variant is five-fold reduced. Despite the reduction, 92% of the vaccines have a neutralization titre of >20 for the B.1.351 variant indicating some protection. The vaccines' neutralization titres exceeded those of recovered non-hospitalized COVID-19 patients. Our work provides strong evidence that the second dose of the BNT162b2 vaccine induces efficient cross-neutralization of SARS-CoV-2 variants currently circulating in the world.

441280

Seasonal Human Coronavirus Nucleoprotein Antibody Prevalence In 1-, 2- And 3-year-old Children

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Respiratory infections caused by seasonal human coronavirus (229E, HKU-1, NL63 and OC43) are common in children during early life. We analyzed serological follow-up samples to estimate the rate of primary infection and reinfection(s) caused by seasonal coronaviruses in early childhood. Sera were collected from 141 children at ages of 13 months, 2 years and 3 years and antibody levels against seasonal coronavirus nucleoprotein were measured using enzyme immunoassay. Seroprevalence for the seasonal coronaviruses increased by age. Altogether, 82% (115 / 140) children were seropositive for at least one seasonal coronavirus by the age of 3 years. The seroprevalence at 1, 2 and 3 years were 14%, 21% and 34% for 229E; 6%, 18% and 18% for HKU-1; 19%, 29% and 54% for NL63; 13%, 24% and 24% for OC43. The mean antibody levels were higher for 229E and NL63 than for HKU-1 and OC43. Based on repeated increases in antibody levels the number of reinfections of 229E, HKU1, NL63 and OC43 were observed in 12, 0, 22 and 6 children, respectively, by the age of 3. In children seropositive at age 1 year and no reinfection the antibody levels decreased 50-74% by the age of 3 years. The serological study provides additional information on the epidemiology and disease burden of seasonal human coronaviruses.

439138

Antibody Responses Against SARS-CoV-2 Are Similar In Art Suppressed HIV Infected And HIV Uninfected Individuals

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Sub-Saharan Africa has reported high seroprevalence of the ongoing COVID-19 pandemic and yet there are limited studies focusing on understanding disease pathogenesis in this region. There is evidence that HIV may adversely impact COVID-19 clinical outcomes, but the underlying mechanisms are unknown. Here, we followed 72 study participants with polymerase chain reaction confirmed SARS-CoV-2 infection in Durban, South Africa using a longitudinal observational cohort study design. Participants enrolled were mostly women (55/72,76.4%). The median age was 43.4 (IQR: 33.5-51.0). 30/72(41.7%) were people living with HIV (PLWH), 25/30(83%) of the PLWH showed an antiretroviral therapy (ART) suppressed HIV viral load in the plasma. Hypertension (17/72,23.6%) and diabetes (15/72,20.8%) were the other leading co-morbidities. 8/72(11.1%) participants had a history of TB. Blood samples were collected weekly up to 28 days and at 3-month post diagnosis (PD) and subjected to a receptor binding domain (RBD) antibody immunoassay, detecting anti-SARS-CoV-2 RBD-specific IgM, IgG, and IgA. A live virus SARS-CoV-2 focus-forming assay was used to determine neutralization potency.
SARS-CoV-2 seroconversion rates within 28 days of diagnosis differed across antibody isotypes (IgM:52/72,72.2%, IgG:71/72,98.6%, and IgA:43/72,59.7%). Most participants remained seropositive at 3-months PD for IgG(86%) but not IgM(18.6%) or IgA(34.2%). Loss of IgA was significantly faster in participants with a history of TB(p=0.015). HIV uninfected individuals and PLWH with ART suppressed

viremia had similar seroconversion rates and titers of IgM, IgG, and IgA as well as plasma neutralization potency against a strain of SARS-CoV-2.
We demonstrated similar antibody kinetics, durability, and neutralization potency in PLWH on ART versus HIV negative people in an African setting. The data suggest that immune responses to SARS-CoV-2 infection or vaccines are potentially similar in PLWH versus their HIV uninfected counterparts. The loss of IgA individuals with history of TB, as well as the antibody response in viraemic PLWH warrants further investigation.

445743

Obtention And Characterization Of Pseudovirions To Develop An Immunosensor For SARS-CoV-2

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The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the most critical public health problem in the last century. Since the beginning of this pandemic, the development of diagnostic methods is of high priority. In this study, we aimed to produce viral particles that express the Spike protein of this emergent coronavirus and significantly reduce biological risk requiring only a BSL-2 facility. The pseudovirions were obtained by co-transfecting HEK-293 cells with three plasmids that express Murine Leukemia Virus Gag and Pol, Spike protein of SARSCoV-2 and a luciferase reporter gene. HEK-293 cells were harvested 72 hours post transfection (h.p.t). The pseudovirions were characterized by flow cytometry, indirect immunofluorescence, Western Blot and luciferase expression. The spike protein was detected on the pseudovirions' surface by indirect immunofluorescence and flow cytometry using an anti-SARS-CoV-2 Spike polyclonal antibody and an anti-Rabbit IgG secondary antibody cross-linked with allophycocyanin. Pseudovirions infectivity was assessed by transducing the VEROE6 cell using 10-fold serial dilutions of the supernatant obtained from transfected HEK-293. The luciferase activity was determined at 72 h.p.t. The title was estimated based on the endpoint dilution and relative luciferase level expression (7,76 x 108 Relative Luciferase Units per milliliter). Furthermore, Spike protein expression was demonstrated in transfected HEK-293 cells by Western Blot using the anti-SARSCoV-2 Spike polyclonal antibody and horseradish peroxidase (HRP) conjugated secondary antibody. The pseudovirions will be used in a system that uses a thiolated ACE2-reactive peptide linked to maleimide-coated magnetic particles. The electrochemical reaction will be followed by chronoamperometry with a biotinylated anti-ACE2 antibody conjugated with streptavidin-HRP. The pseudovirions will enable future studies to improve and develop new diagnostic methods and general knowledge on this virus.

446716

SARS-CoV-2 Antigenic Variation In India

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing Coronavirus disease 2019 (COVID-19) pandemic is the serious global threat until we identify the effective vaccines. Resurgence of newer variants of SARS-CoV-2have drastically increased the number of severe infections and deaths in India. We are continuously grappling with the newer mutants of SARS-CoV-2 specifically the E484Q and L452R mutations in the Spike glycoprotein. In addition to this, newer variants have been found to exhibit one additional mutation V382L, which is the sublineage of B.1.617. These variants are

a reason for concerns due to presence of mutations specifically in the RBD region of Spike glycoprotein which is likely to reduce the efficacy of current vaccines due to compromised level of inhibition exhibited via neutralizing antibodies.Thus, identification of potential consensus epitopes exhibited in spike glycoprotein may help us to design potential vaccine candidate which may protect from these newer variants of SARS-CoV-2. Therefore, we analyzed the antigenicity of SARS-CoV-2 Spike glycoprotein via predicting its B and T cell epitopes and their potential interactions with major histocompatibility complex (MHC) I and II to understand their immunogenic repertoire. Our data demonstrated the presence of various novel consensus epitopes, which exhibit strong affinity towards the peptide binding cleft of MHC molecules. These peptides were further investigated for immune simulation to understand the level of B lymphocyte, CD4+ and CD8+ T lymphocyte response upon antigen exposure. Our data exhibited that several of the novel peptides induces robust lymphocyte response identified as antigen specific CD4+ Th cells, CD4+ Treg cells and CD8+ T effector and memory cells over a month of time, suggesting the strong immune response during SARS-CoV-2 infection. Prominently, booster dose of these peptides resulted in long lasting memory cell response which is crucial for designing ideal vaccine candidates against these newer variants of SARS-CoV-2. Collectively, our study revealed novel epitopes of SARS-CoV-2, which may impart opportunities for the development of multi-epitope-based vaccines for the prevention of COVID-19.

445219 3min Student

Characterization Of Antibody Responses To SARS-CoV-2 Infection In COVID-19 Patients In Latvia

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Objectives The coronavirus disease 2019 (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Antibody testing plays a big role in understanding a virus's epidemiology and it reveals whether a patient's immune system has developed antibodies against the virus. The main goal of this study was to evaluate antibody response to SARS-CoV-2 in dynamics. Materials and Methods In this study 155 patients (both hospitalized and outpatients) were analysed for the presence of virus-specific (IgG NCP, IgG S1 and IgA) antibodies using EUROIMMUN semi-quantitative ELISA. 168 patients' plasma samples for virus specific antibodies were quantified using quantitative ELISA (ANTIBODIES) for antiSARS-CoV-2 IgG and IgM class antibodies. Results In 123 (79.35%) COVID-19 patients' plasma samples anti-SARS-CoV-2 IgG NCP, in 122 (78.71%) - anti-SARS-CoV-2 IgG S1 and in 117 (75.48%) - anti-SARS-CoV-2 IgA class antibodies were detected. 23 of 155 patients had been tested for antibodies repeatedly. The highest number of IgA positive plasma samples were among those collected after 5-12 days, the lowest number – after 63 – 85 days from the disease onset. There are slight differences in the IgG antibody response to the two SARS-CoV-2 antigens (against S1 and NCP) tested. 168 patients had been tested for antibodies in dynamics. IgM class antibody titters decreased after about 2 months of observation. Also, a decreasing tendency of IgG class antibody titters in patients' plasma samples was observed after about 2 months, but in some patient plasma samples IgG class antibodies remain detectable until ~6 months. In one patient sample collected after two months, IgM antibodies had reappeared. Conclusion IgM titter decreased after about 2 months when a decreasing tendency in some of the cases was also observed for IgG. In some cases, IgG antibodies remain detectable until ~6 months. Repeated IgM production can indicate a possible COVID19 reinfection/reactivation in some patients.

SARS-CoV-2: Diagnosis & Intervention

445268

Validated 2nd Gen. MARIPOC® SARS-CoV-2 Allows Rapid And Automated Antigen Testing At Least Up To Day 9 From The Symptom Onset

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Background: Rapid antigen testing has undergone a renaissance due to the needs enforced by COVID-19 pandemic and, in parallel, scientific studies showing that it can be as accurate as viral culture in identifying contagious individuals. The automated, rapid, and high capacity 2nd gen. mariPOC® SARS-CoV-2 antigen test has been designed to target conserved epitopes of the SARS-CoV-2 nucleocapsid protein ensuring robustness against strain variation. The test reports 80-90%, on average, of positives in 20 minutes and confirms negative results at 55 minutes. We validated the test for diagnostic and serial testing of infected individuals. Materials/methods: Limit of detection (LoD) of the mariPOC SARS-CoV-2 test was verified with gamma-irradiated SARS-CoV-2 (BEI Resources, NR-52287). The clinical sensitivity of the test was validated in a retrospective study against qRT-PCR with frozen UTM samples (N=45, Ct range 16.3-33.8) and dry nasopharyngeal swab specimens (N=13) from individuals with COVID-19 suspicion. The specificity of the test was validated prospectively (N=201). Cross-reactions were studied against microbes commonly found in the nasal cavity. One infected subject was followed by serial testing up to 14 days from the onset of symptoms. Results: LoD for mariPOC® SARS-CoV-2 test was 2.7 TCID50 per test. The test showed 100% (13/13) and 84% (38/45) sensitivity with dry nasopharyngeal swab specimens and UTM samples, respectively. For UTM samples, the test showed 92% sensitivity below Ct 30. Specificity of the test was 100% (201/201). The test detected SARS-CoV-1 but showed no cross-reactivity with other microbes. Follow-up of the infected individual showed positive antigen test results up to 9 days from the symptom onset. Conclusions: mariPOC is a highly sensitive and specific test for COVID-19 diagnostic and serial testing. The quantitative results provide insightful data on the pathogenesis of COVID-19 and the positivity up to day 9 further validates the high sensitivity of the test.

441402 3min News

Concordance Analysis Of Diagnostic Tests For Molecular Detection Of SARS-CoV-2 In The Public Health Laboratory Of Tolima-Colombia

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Introduction: Molecular detection of SARS-CoV-2 is the only diagnostic test currently available in acutely or recently infected individuals. Molecular-based testing is the only diagnostic assay format authorized by the FDA, but it is still only qualitative, and can vary greatly in assay sensitivity.
Objetive: Estimate the analytical concordance for the E gene of the AccuPower SARS-CoV-2 Real-Time RT-PCR Kit; Detection Kit for 2019 Novel Coronavirus (2019-nCov) RNA (PCR-Fluorescence Probing) with Light Mix Sars CoV (Berlin-Roche) tests used for the diagnosis of SarsCoV2 in the Public Health Laboratory of Tolima-Colombia.
Results: The different PCR methodologies 33 respiratory swabs were analyzed and their results compared with those obtained by the Berlin protocol (Roche) with a Kappa 0.71 (CI 95%: 0.33-1.09)
Conclusions: The two techniques analyzed in this study obtained good agreement when compared with the Berlin protocol established by the CDC during the SarsCoV2 pandemic in the world.

441400 3min News

Molecular Detection Of Influenza A, Influenza B And SARS-CoV-2 In Deaths During The First Epidemic Peak In The Year 2020 The Department Of Tolima-Colombia

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Introduction: The emerging and reemerging respiratory virus infections pose a continuing threat to human life. Acute respiratory viral diseases claim over 4 million deaths and cause millions of hospitalizations worldwide every year. In the last century, swine and avian influenza infection, severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) were the most damaging respiratory infections for human beings all over the world. However, even leaving aside outbreaks of the novel respiratory viral infections, 294, 000 to 518, 000 deaths annually are associated with seasonal influenza and a significant number of deaths occur despite antivirals, especially in A(H1N1) pdm09 virus infection which continues to circulate as a seasonal FluA or FluB (Influenza A or B).
Objective: Detected by real time PCR Flu A, FluB and SarsCoV2 in respiratory swab samples from patients who died during the first peak due to COVID19 in the department of Tolima-Colombia.
Results: Sars-CoV2, FluA and FluB viruses were identified using the FLU-COVID RTPCR Vitro master diagnostica kit. SarsCov 2 in 109 respiratory swabs from deceased 60.55% men and 39.45% women with age ranges (25-92 and 14-96) respectively. Were identified by real-time PCR using the was identified in 48.62% of the samples. FluA and FluB were not detected in the analyzed samples.
Conclusions: As shown by several authors worldwide in the cases of deaths with respiratory illness in the pandemic peak of SarsCoV2, there may be a marked competition of this current pandemic virus with other viruses previously described in this type of disease.

440891

SOLAVAX: A Whole Virion Vaccine For COVID-19 Produced Via A Novel Inactivation Method And Preliminary Results Demonstrating The Efficacy In A Hamster Challenge Model

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The COVID-19 pandemic has generated intense interest in the rapid development and evaluation of vaccine candidates for this disease as well as other emerging pathogens with significant global

impacts. Several novel methods for preparing vaccine candidates are currently undergoing clinical evaluation in response to the rapid spread of the SARS-CoV-2 virus. In many cases, these methods rely on new approaches for vaccine production and immune stimulation. Some of these vaccine candidates have already received emergency authorization use in multiple countries. We report on the use of a novel method (SolaVAX) for production of an inactivated vaccine candidate and the testing of that candidate in a well-characterized hamster model for its ability to prevent infection upon challenge with SARS-CoV-2 virus. The studies employed in this work included a long-term evaluation of neutralizing antibody levels post-vaccination, levels of specific antibody subtypes to RBD and spike protein that were generated, evaluation of viral shedding post-challenge, flow cytometric and single cell sequencing data on cellular fractions and histopathological evaluation of tissues post-challenge. The results from this preliminary evaluation provide insight into immunological responses occurring as a result of vaccination with the proposed vaccine candidate and the impact that adjuvant formulations, specifically developed to promote Th1 type immune responses, have on vaccine efficacy and protection against infection following challenge with live SARS-CoV-2. This data may have utility in the development of effective vaccine candidates against emerging viral pathogens. Furthermore, the results of this preliminary evaluation suggest that preparation of a whole virion vaccine for COVID-19 using this specific photochemical method may have potential utility in the preparation of one such vaccine candidate.

445782

Durability And Cross-protective Activity Of An Intranasal Vaccine Against SARS-CoV-2 Infection In Mice

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SARS-CoV-2 variants that attenuate antibody neutralization could jeopardize vaccine efficacy and the end of the COVID-19 pandemic. We recently reported the protective activity of a single-dose intranasally-administered spike protein-based chimpanzee adenovirus-vectored vaccine (ChAd-SARS-CoV-2-S) in animals, which has advanced to human trials. Here, we assessed its durability, dose-response, and cross-protective activity in mice. A single intranasal dose of ChAd-SARS-CoV-2-S induced durably high neutralizing and Fc effector antibody responses in serum and Specific IgG and IgA secreting long-lived plasma cells in the bone marrow. Protection against a historical SARS-CoV-2 strain was observed across a 100-fold vaccine dose range and over a 200-day period. At 6 weeks or 9 months after vaccination, serum antibodies neutralized SARS-CoV-2 strains with B.1.351 and B.1.1.28 spike proteins and conferred almost complete protection in the upper and lower respiratory tracts after challenge. Thus, in mice, intranasal immunization with ChAd-SARS-CoV2-S provides durable protection against historical and emerging SARS-CoV-2 strains.

426802

Multidisciplinary Approaches Identify Repurposing Candidates For COVID-19 That Block Virus/Receptor Interactions And In Vitro Infection

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a recently emerged virus responsible for the coronavirus infectious disease 2019 (COVID-19) pandemic. As of April 2021, there have been close to 140 million cases of COVID-19 worldwide, resulting in the death of over 3 million individuals. To complement the effectiveness of vaccines, antiviral drugs are urgently needed to help contain this pandemic and repurposing existing drugs is the most rapid path to clinical intervention for emerging diseases.
The SARS-CoV-2 spike protein receptor binding domain (RBD) binds to human angiotensin converting enzyme 2 (ACE2) to initiate viral entry to the host cell. Compounds that interfere with this binding event may therefore have strong potential as entry inhibitors of SARS-CoV-2 infection.
Here, we used a dual strategy of in silico screen of 57,641 compounds and biophysical screen of 3,141 compounds by surface plasmon resonance (SPR) to identify those that bind to human ACE2 or the SARS-CoV-2 Spike RBD. These combined screens identified 17 ACE2-binding compounds and 6 SARS-CoV-2 Spike RBD-binding compounds that interact with less than 3 μM affinity to their respective targets, thereby blocking Spike RBD/ACE2 interaction. Importantly, three of these compounds demonstrated dose-dependent antiviral in vitro potency in a Vero-E6 model of SARS-CoV-2 infection and may have utility as repurposed therapeutics.
We are now testing our top hits in ex vivo human airway epithelial cell models and using X-ray crystallography to investigate identified scaffolds that bind to SARS-CoV- 2 Spike RBD for the development of new chemical entities against COVID-19.

440746

Fusion Inhibitory Peptides Block SARS-CoV-2 Infection In ACE2-Transgenic Mice

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent of the coronavirus disease 2019 (COVID-19) which is responsible for the current pandemic. Although several vaccines have been developed so far, SARS- CoV-2 continues to spread globally and effective therapeutic strategies are not yet available. Entry of SARS-CoV-2 into the host cells is mediated by the ACE-2 receptor, which is a component of the angiotensin-regulating system. This virus binds ACE2 via its envelope Spike (S) glycoprotein, leading to the virus-cell fusion and consequent SARS-CoV-2 entry and replication. SARS-CoV-2 S is a homotrimer in which each monomer contains 2 subunits, S1 and S2. S2 is responsible for the fusion and presents two heptad repeat domains (HR) in N and C amino termini. The interaction between these domains (HRC and

HRN) is critical for the membrane fusion. Here, we have analyzed the capacity of fusion inhibitory peptides, derived from the heptad repeat domains in C-amino terminus (HRC) of Spike, to inhibit SARS-CoV-2 infection in a murine model, both ex vivo and in vivo. We observed that the peptide causes significant inhibition of SARS-CoV-2 infection in organotypic cultures prepared from lungs of mice expressing human ACE2 (B6.Cg-Tg (K18- ACE2) 2Prlmn/J, Jackson). In vivo, while SARS-CoV-2 is provoking 100% lethal infection within 10 days post-infection in K18-hACE2 mice, intranasal administration of peptides reduced the body-weight loss and protected between 80% and 100% of mice from the SARS-CoV-2-induced lethality. These results were associated with a high decrease of viral load in lungs at day 2 post-infection compared to the mock- treated group. These findings indicate that fusion inhibitory peptides highly reduce the clinical impact of SARS-CoV-2 infection in the animal model, thus providing a proof of concept for a new complementary approach of antiviral prophylaxis to be developed as part of the global effort against the current SARS-CoV-2 pandemic.

447042 3min Student

A Novel Engineered Water Nanostructure Based Surface Disinfection Technique Against Coronaviruses

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Considering the current CoVID-19 pandemic, we tested out the efficacy of a novel engineered water nanostructure (EWNS) based surface disinfection technique targeted against human coronavirus 229E. Our previous studies have successfully demonstrated the efficacy of this technique against bacteriophage MS2 (which served as a surrogate for the human Norovirus GII.4) by reducing the viral titer by almost 1.4 log after only 5 minutes of treatment. The advantage of using this novel “dry”, nano-aerosol based electrospray method for surface disinfection is that it can deliver different active ingredients which have been generally recognized as safe (GRAS) either singly or in combination at nanogram levels (much less than the OSHA permissible levels). Another advantage of this novel surface disinfection technique is that it does not leave a residue and hence suitable for use in a wide variety of settings such as food industry, hospitals etc. Our initial success with MS2 encouraged us to test the efficacy of this technique against human coronavirus 229E which would serve as a surrogate for SARS-CoV-2. The EWNSs were directed onto a stainless-steel coupon on which 229E suspension was evenly spread and exposed to the EWNSs at different time intervals. We observed that after 5 mins of treatment both 3% Triethylene Glycol and 3% H2O2 were able to produce a 1 log reduction in the viral titer (quantified using a standard viral plaque assay). We also observed a complete inactivation of the virus following 5 mins of exposure to EWNSs delivering 10% H2O2. To summarize, we were able to successfully demonstrate that the EWNS based disinfection technique is highly effective against a SARS-CoV-2 surrogate even when nanogram levels of active ingredients were delivered to the surface.

432510 3min Student

Allosteric Inhibition Of SARS-CoV-2 3CL Protease By Bismuth Drugs

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In December 2019, a seventh coronavirus which could infect humans emerged and later was named as the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS- CoV-2). Within just a few months, it has spread worldwide and global cases have increased rapidly. To date, more than 124 million

people around the world were infected and global deaths have exceeded 2.7 million. The emergence of SARS-CoV- 2 has brought a huge challenge to global public health.
The 3-chymotrypsin-like protease (3CLpro, nsp5) of SARS-CoV-2 is essential for virus replication, which cleaves polyproteins at eleven conserved sites.[1] The polyprotein processing function of the 3CLpro makes it an attractive target for anti- viral therapy.
Previous studies demonstrated that bismuth drugs could inhibit SARS-CoV-2 replication effectively.[2] However, the detailed molecular mechanisms remained unknown. Herein, for the first time we demonstrated that 3CLpro was a highly potent target of bismuth drugs. Biochemical studies revealed that bismuth drug was an allosteric inhibitor for 3CLpro and bismuth binding caused dimeric 3CLpro dissociation. In particular, site-directed mutagenesis studies revealed a unique bismuth-binding site on the dimer interface. Molecular dynamic (MD) simulations further illuminated a step-wise dissociation process of 3CLpro dimer, elucidating the molecular mechanism of the allosteric inhibition. Our work not only provides intensive insights into the anti-SARS-CoV-2 mechanisms of bismuth drugs, but also lays a foundation for the design of allosteric inhibitors to treat COVID-19.
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430524 3min Student

Antiviral Activity Of Vacuolar ATPase Blocker Diphyllin Against SARS-CoV-2

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a causative agent of the pandemic coronavirus disease 2019 (COVID-19), which has resulted in over two million deaths worldwide to date. Diphyllin and diphyllinosides are known as natural blockers of cellular vacuolar ATPases, and so can act as inhibitors of the pH- dependent fusion of viral envelopes with host cell endosomal membranes. Such pH- dependent fusion is a critical early step during the SARS-CoV-2 replication cycle. Accordingly, the anti-SARS-CoV-2 profiles and cytotoxicities of diphyllin, diphyllinoside cleistanthin B, and two structurally related compounds, helioxanthin 8- 1 and helioxanthin 5-4-2, are evaluated here using in vitro cell-based assay systems. Neither helioxanthin exhibits any obvious anti-SARS-CoV-2 effects in vitro. By contrast diphyllin and cleistanthin B do exhibit anti-SARS-CoV-2 effects in Vero cells, with respective EC 50 values of 1.92 and 6.51 μM. Diphyllin displays anti-SARS-CoV- 2 effects also in colorectal adenocarcinoma (CaCo-2) cells. Moreover, when diphyllin is added at various times post infection, a significant decrease in viral titer is observed in SARS-CoV-2-infected Vero cells, even at high viral multiplicities of infection. Importantly, neither diphyllin nor cleistanthin B are found to be cytotoxic to Vero cells in concentrations up to 100 μM. However, the cytotoxic effect of diphyllin is more pronounced in Vero E6 and CaCo-2 cells. Overall, our data demonstrate that diphyllin and diphyllin analogues might be perfected as anti-SARS-CoV-2 agents in future preclinical studies, most especially if nanomedicine approaches may be invoked to optimize functional drug delivery to virus infected cells.

The Replication-Defective Sementis Copenhagen Vector Encoding the SARS-CoV-2 Spike Glycoprotein Induces Broad and Durable Cellular and Humoral Immune Responses After Vaccination

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The ongoing COVID-19 pandemic has highlighted the continued need for vaccines that can generate robust antibody and cellular immunity to provide effective long-term protection in a broad population range and control transmission of the causative agent SARS-CoV-2 and variants thereof. These vaccines should be able to provide stand-alone protection as well as perform as an effective boost vaccination to currently authorised vaccines. Therefore, using the non-replicating vaccinia-based Sementis Copenhagen Vector platform system, a COVID-19 vaccine was constructed to express the full-length, native SARS-CoV-2 spike glycoprotein (SCV-S) and efficacy was tested in a broad age range using young and aging mice, and inbred/outbred strains of mice. A robust Th1-biased spike-specific antibody response was detected as early as day 14 post-vaccination, with a significant increase in antibody levels observed following administration of a booster dose. Neutralizing antibody titres were sustained and effective against the original Wuhan Hu-1 isolate, SARS-Co-V2 B1.1.7 and B1.351 variants in pseudovirus neutralisation assays. Importantly, pre-existing immunity to the vector did not have any impact on the production of spike-specific binding and neutralizing antibodies. Spike-specific cell-mediated immune responses targeting both the S1 and S2- subunit of the spike protein were detected by day 7 post-vaccination, with significant cytotoxic effector activity directed against the receptor binding domain (RBD) located in the S1 subunit of the spike protein. Spike-specific long-lived antibody secreting cells and poly-functional CD8 + T cells were detected at the termination of the study 9 months post-vaccination, demonstrating induction of durable antigen-specific humoral and cell-mediated responses. Together these results demonstrate that SCV-S vaccination generates robust and durable serological and cellular priming, capable of delivering long-term and broadly protective immunity against SARS-CoV-2 across a broad range of ages, and supportive of progressing the candidate vaccine to evaluation in challenge models of disease

SARS-CoV-2: Molecular Virology

40486

Identification Of Cellular Factors Required For The SARS-CoV-2 Replication

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a recently emerged virus that has caused a worldwide pandemic. The clinical image ranges from asymptomatic, mild respiratory tract infections to severe disease accompanying lung injury, multiorgan failure, and death. SARS-CoV-2 is the third animal coronavirus to emerge in humans in the 21st century, and coronaviruses appear to have a unique talent to cross the species border and infect a wide range of species. This is somewhat surprising, as except for the receptor requirement, the complex interplay between cellular and pathogen factors is fine-tuned to closely related species. The insight of these host-virus interactions provides a deeper understanding of the process and means for antiviral drug design and development. This study demonstrates a complex analysis of SARS-CoV-2 infection using a genome-wide CRISPR-Cas9 knock-out system in HeLa cells overexpressing the entry receptor angiotensin-converting enzyme 2 (ACE2). This platform allows for de novo identification of factors required for viral replication. However, the interpretation of the data is uneasy and prone to biases. To comprehensively study the interaction network, we have designed a bioinformatic pipeline allowing for the multifactorial analysis of the data generated in 48 replications. Obtained results provide an interesting insight into the viral replication mechanisms.

440312

Syncytia Formation By SARS-CoV-2 Variants

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Virus induced syncytia formation could facilitate viral replication and immune evasion as well as active the host immune response and induce tissue damage. Several corona viruses including the currently pandemic SARS-CoV-2 and the ubiquitous (but understudied) HKU1 common cold virus form syncytia in vivo or in vitro. Indeed, Severe cases of COVID-19 are associated with extensive lung damage and the presence of infected multinucleated syncytial pneumocytes. The viral and cellular mechanisms regulating the formation of these syncytia are not well understood. We previously showed that SARS-CoV-2-infected cells express the Spike protein (S) at their surface and fuse with ACE2-positive neighboring cells. We also find that cells that express the HKU1 S protein fuse with neighbouring cells that express wild type TMPRSS2 serine protease, but not with a catalytically inactive TMPRSS2. Interferon Induced transmembrane proteins (IFITMs), a family of restriction factors that block the entry of many viruses, inhibit S-mediated fusion for both SARS-CoV-2 and HKU1, with IFITM1 being particularly potent. Interestingly, while TMPRSS2 accelerates syncytia formation by SARS-CoV-2 and counteracts the restrictive effects of IFITM1, it does not interfere with IFITM1 restriction of HKU1-S mediated syncytia formation. Finally, we also find that the S protein from different variants of SARS-CoV-2 produce syncytia more rapidly. We are currently further characterizing the syncytia forming potential of the recently emerged UK and South African variants. Our results suggest that the

ability of several pathogenic corona viruses to induce cell-cell fusion is modulated by a variety of cellular proteins that either inhibit or facilitate syncytia formation.

440320

Molecular Barriers To SARS-CoV-2 Replication In Bat Cells

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Bats are natural reservoirs for numerous coronaviruses, including the potential ancestor of SARS-CoV-2. Knowledge concerning the interaction of coronaviruses and bat cells is, however, sparse. There is thus a need to develop bat cellular models to understand cell tropism, viral replication and virus-induced cell responses. Here, we report the first molecular study of SARS-CoV-2 infection in chiropteran cells. We investigated the susceptibility to SARS-CoV infection of a panel established bat cell lines, belonging to the species Myotis myotis, Eptesicus serotinus and Tadarida brasiliensis, as well as novel Nyctalus noctula cells, which was completed with primary cells from Rhinolophus ferrumequinum and Myotis spp. bats. None of these cells were sensitive to infection, not even the one expressing detectable levels of angiotensin-converting enzyme 2 (ACE2), which serves as the viral receptor in many mammalian species including humans. Following transduction with human ACE2, six bat cell lines expressed comparable or higher levels of hACE2 than a permissive human cell line. In three out of these six cell lines, the resistance to infection was overcome by hACE2 expression, suggesting that restriction to viral replication was due to lack of bACE2 expression or absence of bACE2 binding. By contrast, multiple restriction factors to viral replication exist in the three N. noctula cells since hACE2 expression was not sufficient to permit infection. Notably, viral replication was efficiently controlled in E. serotinus cells and correlated with a potent induction of interferon-stimulated genes. Despite a high level of replication, infectious virions were not released from M. myotis cells. Together, our data highlight the existence of species-specific molecular barriers to viral replication in bat cells. Our newly developed chiropteran cellular models are useful tools to investigate the interplay between viruses belonging to the SARS-CoV2 lineage and their natural reservoir, including the identification of factors responsible for viral restriction.

445752

SARS-CoV-2 Endocytosis Into HUH-7 Hepatoma Cells Is Dependent On ARF6

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SARS-CoV-2 entry has been extensively studied since the beginning of the spread of this novel beta-coronavirus. Whereas the viral receptor, ACE2, multiple entry factors and the mechanism of fusion of the virus at the plasma membrane have been extensively investigated, viral entry via the endocytic pathway is less understood. Because of the wide tropism of SARS-CoV-2 and the potential biological impact of the endocytic entry pathway, we sought to study the mechanism of endocytosis mediated entry in a cell line, Huh-7, that is resistant to the antiviral action of camostat (TMPRSS2 inhibitor) and in which SARS-CoV-2 entry does not rely on viral fusion. By means of CRISPR-Cas9 knock out (-KO), we studied replication of SARS-CoV-2 in Huh-7 lacking dynamin, which is required during clathrin- and caveolin-mediated entry, or lacking one of the component of dynamin-independent entry pathways, i.e. the Rho GTPase-activating protein 26 (GRAF-1), flotillin (FLOT-1) and ADPribosylation factor 6 (ARF6), using a combination of TCID50 assay and immunofluorescence. We also used a combination of specific (dynasore, NAV-2729) and broad spectrum (sertraline, chlorpromazine, fluvoxamine, β-methyl-cyclodextrin) inhibitors to complement the finding with -KO cells. Our data showed that in Huh-7 cells SARS-CoV-2 infection is independent of dynamin but dependent on ARF6. Furthermore, the ARF6 inhibitor NAV-2729 also blocked viral internalization in Huh-7 cells and it exhibited antiviral activity during infection in a more physiologic culture model, i.e., kidney organoids. Together, these data suggest that ARF6 plays a critical role during SARS-CoV-2 endocytic uptake and infection of certain cell types, and this finding could aid in the development of antiviral therapies to block systemic spread and extra-pulmonary infections

439141 3min Student

Effective Inhibition Of SARS-CoV-2 Replication Using PAMPS-PAaU Block Copolymers

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Newly identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is associated with a severe, life-threatening respiratory infection. The virus quickly spread around the world and caused a global pandemic that claimed millions of lives. Despite the extraordinary commitment of scientists, pharmaceutical companies, and governments, an effective drug that would save the lives of those infected has yet to be found. Despite the introduction of vaccines, such a drug is still highly desirable, especially in countries more severely affected by the pandemic or where mass vaccination will not be introduced quickly. Biologically active antiviral polymers have attracted more and more attention from scientists in recent years, especially due to their high efficiency, ease of modification, and low toxicity. Antiviral polymers typically block virus attachment or entry into cells by creating a protective molecular barrier on their surface or by interacting with the virion itself. The strategy for improving their pharmacological and physicochemical properties is the formation of block copolymers composed of 2 or more polymers. Thanks to the use of specialized chemical synthesis methods, we can freely modify

the length and arrangement of polymer blocks. Such a modification allows us to create copolymers with the properties best suited to counteracting infection. In our research, we synthesized a series of block copolymers composed of poly(2-acrylamido-2-methyl-1-propanesulfonic acid)(PAMPS) and poly sodium 11-(Acrylamido)undecanoate (PAaU). Obtained PAMPS-PAaU block copolymers were subjected to in vitro tests in Vero cells infected with SARS-CoV-2. PAMPS-PAaU exhibits strong antiviral properties, strongly inhibits SARS-CoV-2 caused cytopathic effect and virus yield in supernatant up to 99.9% at a concentration of 25 µg/ml,compared to untreated control. Additionally, tested copolymers show low toxicity with a CC50 dose above 3000 µg/ml. Summarizing, the obtained results indicate a high potential of the presented copolymers in hampering SARS-CoV-2 infection in vitro.

SARS-CoV-2: Transmission & Epidemiology

441278

Improved Viral Concentration Methods For Wastewater-based Epidemiology

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Wastewater-based epidemiology (WBE) has been shown to be a valuable tool in forecasting clinical presentation of COVID-19 through community-based biosurveillance. Due to shedding in feces and urine early in the infection process, viral increases observed in wastewater (WW) typically precede increases in clinical cases by 4-7 days. To maximize detection sensitivity, the use of a primary viral concentration approach is essential. Concentration approaches currently used include methods such as viral precipitation, electrostatically charged filtration and standard ultrafiltration techniques. However, these approaches are time- and laborintensive, ranging from hours to days to complete before obtaining samples for extraction and PCR-based analysis. In contrast, the InnovaPrep Concentrating Pipette Select system has been demonstrated to be ideally suited for SARS-CoV-2 WBE by providing rapid sample concentration from WW samples with volume concentration factors of 150-500 times the starting concentration and viral recovery efficiencies of 40% or more. During this presentation an overview of the system and description of dead-end hollow fiber filtration and wet foam elution technology will be discussed, along with WBE-specific protocols currently used by analytical laboratories. In addition, new innovations will be presented which allow collection and concentration of virus from raw sewage treatment plant influent samples in as little as 3-4 minutes, with samples ready for standard extraction protocols and PCRbased analysis.

441313

Dose, Distance, And Dilution In Airborne Viral Transmission

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The transmission of airborne pathogens via aerosols is considered to be the main route through which SARS-CoV-2 infects hosts. It is therefore essential to quantify airborne transmission in enclosed spaces and determine which recommendations should be implemented to minimize the exposure to airborne pathogens as students return to schools and employees to their workplaces. We have developed a method to detect viable virus particles from aerosols by using a LacZ-alpha-marked bacteriophage Phi6, a known proxy for SARS-CoV-2 due to its similar shape, size and physiology. Our marked Phi6 exclusively infects the Pseudomonas genus of bacteria, which, when plated on agar containing X-Gal, produce easily identifiable blue plaques. The growing LacZ-alpha producing Pseudomonas phaseolicola then serve as virus detectors, which we place at a range of distances

from the aerosol generating source of phage Phi6. Our method is therefore perfectly suited to study the dose, distance, and dilution effects of airborne viral pathogens in built environments. Although air currents can strongly impact the results, we consistently detect virus particles at distances of 18 feet away from the source within 15-minutes of exposure in classrooms with a state of the art continually operating HVAC system. This method can be used to quantify the exposure to pathogens at various distances from the source for different amounts of time, data which can be used to set safety standards for room capacity, air turnover rate, and the efficacy of additional interventions which aim to reduce pathogen levels in any space of specified size with a particular intended use. We present preliminary and encouraging results on the impact of humidity in aerosol spread and discuss an exposure-reducing intervention that can be easily implemented and used across a variety of spaces and situations.

441357

Detection Of SARS-CoV-2 RNA In Bivalve Mollusks And Marine Sediments

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The presence of SARS-CoV-2 in wastewater poses the question of whether this new pandemic virus could be released into watercourses and potentially continue to finally reach coastal waters. In this study, we employed two bivalve molluscan species from the genus Ruditapes as sentinel organisms to investigate the presence of SARS-CoV-2 signals in the marine coastal environment. Estuarine sediments from the natural clam banks were also analyzed. Viral RNA was detected by RT-qPCR, targeting IP4, E and N1 genomic regions. Positive samples were also subjected to a PMAxx-triton viability RT-qPCR assay in order to discriminate between intact and altered capsids, obtaining indirect information about the viability of the virus. SARSCoV-2 RNA traces were detected in 9/12 clam samples by RT-qPCR, from which 4 were positive for two different target regions. Viral quantification ranged from

441311

Detection Of Mutations Associated With Variants Of Concern Via High Throughput Sequencing Of SARS-CoV-2 Isolated From NYC Wastewater

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Monitoring SARS-CoV-2 genetic diversity is strongly indicated because diversifying selection may lead to the emergence of novel variants resistant to naturally acquired or vaccine-induced immunity. To date, most data on SARS-CoV-2 genetic diversity has come from the sequencing of clinical samples, but such studies may suffer limitations due to costs and throughput. Wastewater-based epidemiology may provide an alternative and complementary approach for monitoring communities for novel variants. We developed a protocol for processing wastewater with the goal of detecting the genetic signal of SARS-CoV-2 and sequencing its RNA. This protocol is one of the safest, cheapest, and most reproducible approaches for the detection and sequencing of SARS-CoV-2 RNA in

wastewater and was adopted by the New York City Department of Environmental Protection in August 2020 to support their efforts to monitor SARS-CoV-2 prevalence in wastewater in all five boroughs of the city. Our targeted sequencing approach revealed the presence of mutations associated with several variants of concern at appreciable frequencies. Our work demonstrates that wastewater-based SARS-CoV-2 sequencing can inform surveillance efforts monitoring the community spread of SARS-CoV-2 variants of concern and detect the appearance of novel emerging variants more cheaply, safely, and efficiently than the sequencing of individual clinical samples.

441392

Investigations Of The Potential Role Of Insect Vectors On SARS-CoV-2 Transmission

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The COVID-19 pandemic, caused by the highly contagious SARS-CoV-2, has drawn the world's attention to the impact of emerging zoonotic viruses on global health and economy. SARS-CoV-2 is transmitted person-to-person via inhalation of infected droplets and aerosols and to a lesser extent by direct contact with contaminated fomites. Arthropods are known to transmit numerous bacteria, viruses, and parasites. Public health guidelines for SARS-CoV-2 state that arthropods play no role in its transmission, despite an absence of experimental scientific data to support this statement. Therefore, we examined the susceptibility of biting insect species to SARS-CoV-2 infection. First, we determined that cell lines derived from C. sonorensis (W8a), Aedes aegypti (C6/36), Culex quinquefasciatus (HSU), and Culex tarsalis (CxTrR2) were unable to support SARS-CoV-2 virus infection and replication. Next, in vivo studies were done with biting midges (Culicoides sonorensis, and two mosquito species (Culex tarsalis and Culex quinquefasciatus. These insects were fed SARS-CoV-2-spiked blood and held for an extrinsic incubation period to allow the ingested virus to replicate, and then processed for viral RNA detection and virus isolation. The results indicated that none of the biting insects supported SARS-CoV-2 replication. In addition, we examined the potential for mechanical transmission of SARS-CoV-2 by house flies (Musca domestica). Two studies were performed to determine if house flies (1) can acquire SARS-CoV-2 from an inoculated food source; and (2) can transmit the virus to a naïve surface. The results indicated that house flies could acquire infectious SARS-CoV-2 virus but could mechanically transmit only viral RNA and not infectious SARS-CoV-2. Overall, these studies support the public health statement that insects do not play a significant role in SARS-CoV-2 epidemiology; however, additional studies on natural virus transmission by house flies or other mechanical vectors may be justified.

Human Viruses: Clinical Virology

441248

Characterization And Clinical Impact Of Human Parvovirus Tissue Persistence

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Parvovirus B19 (B19V) and human bocavirus 1 (HBoV1) are pathogenic human parvoviruses. The B19V genome can persist life-long in various human tissues after primary infection, and the virus enters the cells by antibody-dependent enhancement (ADE). Furthermore, HBoV1 has been shown to persist in tonsillar and intestinal tissues. However, little is known of the host-cell tropisms and the actual clinical impact of parvovirus persistence in tissues. We aim to characterize the tissue site(s), host cells, viral activity and cellular response of parvovirus persistence in these tissues. In pediatric tonsils, we discovered by RNAscope in situ hybridization (RISH) that HBoV1 DNA persists in lymphoid germinal centers (GCs), and that the cell types harboring the virus are mainly naive, activated, and memory B cells and monocytes. Moreover, we showed, in B- cell and monocyte cultures and ex-vivo tonsillar B cells, that the cellular uptake of HBoV1 occurs via the Fc receptor (FcγRII) through ADE (Xu et al. mBio 2021). We now show by RISH that B19V DNA persists mainly in the blood vessels and lymphoid follicles of the intestinal mucosa, and that the infected cells are vascular endothelial cells and lymphoid B cells. Transcriptomic studies of ileum tissues with B19V DNA persistence, showed altered expression for genes involved in defence mechanisms against DNA-virus infections, cell viability, apoptosis, and mRNA splicing. The long-term persistence of parvovirus in tissues, with low viral load and infrequent mRNA transcription, may induce multiple cellular responses or lead to far-reaching disturbances. How these viruses establish and maintain persistence in the tissues will need further study.

440797

Unmasking Of Viral DNAs In Human Internal Organs: Distribution And Within-Host Sequence Diversities

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Many human DNA viruses are known to establish tissue persistence. However, the current knowledge is based mainly on the detection of viruses in particular tissue types, thus providing an incomplete picture of viral distribution among the organs of a given individual. Our objective was to assess the prevalence and distribution of viral DNAs in multiple organs of the human body. More specifically, we aimed to investigate the viral loads and the within-host viral sequence diversities. We collected in total 279 post-mortem samples of brain, blood, colon, hair follicles, heart, liver, lung, kidney, and skin from 31 recently deceased individuals. Viruses belonging to the families Anelloviridae, Hepadnaviridae, Herpesviridae, Papillomaviridae, and Parvoviridae were screened from the tissue samples by quantitative PCRs (14 viruses) and further analyzed by next-generation sequencing following targeted enrichment with virus-specific biotinylated RNA probes (38 viruses). The DNAs of Parvovirus B19 (B19V), Torque teno virus (TTV), Epstein-Barr virus (EBV), human herpesviruses 6B, and -7 (HHV-6B, HHV-7) were most frequently detected, each having a prevalence of over 80% in the studied individuals, followed by Merkel cell (52%) and JC (39%) polyomaviruses. The viral DNA distribution profiles and loads in the body were unique for each virus type. Using TRACESPipe, our customized bioinformatics pipeline, we reconstructed multiple (near)full-length genomes of B19V, HHV-6B, HHV-7, JCPyV, and MCPyV, thus allowing us to investigate intra-host variability of the viral sequences. In addition, we found good positive and negative agreement between our NGS-based

protocol and qPCRs (92 % and 96%, respectively). Our study revealed remarkably high prevalences of viral DNAs present throughout the human body in virus-specific spread and copy numbers, providing a framework for the tissue virome. Future studies should target alterations linked to clinical syndromes to uncover the significance of these findings in health and disease.

435682

Detection Of Unusual Rotavirus G5P[4], G6P[6], G6P[10], G10P[6], G10P[8], G10P[10], G10P[14] AND G11P[6] Etiologies Of Severe Diarrhea Among Nigerian Children

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Information is scanty about diarrhea in Nigerian children caused by group A rotavirus (RVA) genotypes G5, G6, G10, G11 and P[14] strains which are frequently found in animals but rarely in humans. Hence the gene sequences data from Nigeria, of these genotypes are unavailable in the Institutional Nucleotide Sequences Collaboration databases. We aimed to specifically capture any emergent unusual RVAs among hospitalized children for severe diarrhea from October 2012 to September 2014 in Ondo state, Nigeria, with a view to providing the genotypes, phylogeny and the sequences data.
Stool specimens were obtained by convenience sampling from accented under-5 children and tested for rotavirus antigen using Enzyme Immunoassay (EIA). Viral genomic RNA was extracted from EIA positive samples and the VP7 and VP4 genes were amplified in a one-step RT-PCR. The products were used as templates in semi- nested multiplex PCR for identification of the genotypes. Conveniently selected amplicons were purified and sequenced using the Big Dye method. Sequences were edited and queried to GenBank by BLAST, to retrieve reference strains and aligned using clustalW for phylogenetic analysis and reconstruction by Neighbor-joining at 1000 bootstrap replicates in MEGA6.
Unusual VP7 and VP4 genotypes existing as G5P[4], G6P[6], G6P[10], G10P[6], G10P[8], G10P[10], G10P[14], and G11P[6] were detected. The G6P[6] strain had the highest occurrence in female children, followed by the G10P[8] but generally, there was no dependent significant relationship between gender and infection by any of the rare strains. The VP7 and VP4 gene sequences of the isolates were submitted to GenBank and accession numbers obtained.
Emergent unusual rotavirus strain combinations that were rarely found in human infections were detected in severe diarrhea among Nigerian children. The strains were potentially from animals, phylogenetically indicative of exotic origin and trans- boundary. These findings underscore the genetic diversity of rotaviruses and may challenge the efficacy of rotavirus vaccines over prevailing serotypes in Africa. The absence of the G5, G6, G11 and P[14] sequences data from Nigeria in recognized DNA database libraries suggests that these isolates are the first verified G5, G6 and G11 genotypes from Nigeria.

440342

Modular Vaccine Platform Based On The Norovirus-Like Particle

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Virus-like particle (VLP) vaccines have recently emerged as a safe and effective alternative to conventional vaccine technologies. In addition to using VLPs as vaccines against the viruses they derive from, their strong immunogenic effects can be harnessed for making vaccines against other

pathogens too by decorating VLP surface with antigens from the pathogen. Here we covalently decorated the robust norovirus-like particle with two conserved influenza antigens using SpyCatcher/SpyTag conjugation technology (PMID: 22366317), and tested for the immunogenicity of the resulting vaccine candidates in BALB/c mice.
SpyTagged noro-VLP was expressed with high efficiency in insect cells and purified using industrially scalable methods. Like the native noro-VLP, SpyTagged noro-VLP is stable for months when refrigerated in a physiological buffer. The conserved influenza antigens were produced separately as SpyCatcher fusions in E. coli before covalent conjugation on the surface of noro-VLP. Producing the antigenic pathogen fragments and the VLP platform separately makes vaccine development rapid and convenient. The noro-VLP had a high adjuvant effect, inducing high titers of antibody production against the protein antigens presented on its surface.
The modular noro-VLP vaccine platform presented here offers a rapid, convenient and safe method to present various soluble protein antigens to the immune system for vaccination and antibody production purposes.

440673

Platinum Complexes Shield Cells From Virus Infection

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Multiple viral pathogens are known to latch on to host cell heparan sulfate (HS) proteoglycans as primary receptors for cell recognition. Being negatively charged, HS has been shown to be a target for positively charged Polynuclear Platinum Complexes (PPCs) such as TriplatinNC, which contributes to their anti-angiogenic properties. Here, we provide the first detailed mechanism of PPCs as antiviral agents. We discovered that TriplatinNC blocks infection of both enveloped and non-enveloped viruses such as human metapneumovirus, responsible for pneumonia in children and the elderly, and enterovirus 71, responsible for hand, foot and mouth disease in children, respectively. Importantly, the antiviral effect of TriplatinNC occurrs at the binding stage of virus infection, by shielding cells from virus entry. Using saturation- transfer difference NMR spectroscopy with whole virus, we could show that TriplatinNC and a HS surrogate, fondaparinux, form a ternary complex with virus. While this confirms the HS-binding properties of TriplatinNC, this supports the hypothesis that not only can PPCs shield cells from infection, they are also anticipated to further block virus propagation by sequestering virus at the cell surface. Our multidisciplinary study shows the potential of polynuclear metal complexes as broad-spectrum antiviral compounds which are expected to be active on other viruses depending on HS for binding. It also provides exciting opportunities for the development of new metal-based antivirals with improved biocompatibility.

441774 3min Student

Real-Time Detection Of Norovirus Capsid Protein Using Nanopore-Based Sensing Technologies

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Human noroviruses are the leading causes of foodborne illness and death globally, and induce a considerable public health burden. The properties of noroviruses make them difficult to control such as low infectious dose and high diversity. Nanopore based detection has the advantages of portable, rapid, sensitive, quantitative, resistant to matrix-associated inhibitors, capable of sensitively subtyping or fingerprinting in real-time, and capable of discriminating against active/infectious forms of an analyte. In this research we used Outer membrane protein G (OmpG). OmpG based sensing has been demonstrated for these purposes for clinical biomarkers but has not been investigated for

foodborne pathogens. We engineered an OmpG construct containing a 12 amino acid peptide sequence that specifically binds noroviruses and tested the ability of the sensor. The partially opened current state was observed more frequently for a longer period of time when capsid protein was added. The lowest target concentration generating this current signal was 100nM and dose dependent gating pattern was observed in the range of 0nM to 200nM. The generated construct was capable of binding and detecting norovirus capsid protein in less than 5 minutes. These data suggest that norovirus capsid protein detection using biological nanopore may help control foodborne outbreaks through rapid detection and subtyping. This work also provides the foundation for further research on virus detection in complex matrices.

441336 3min Student

Epidemiology Of Group A Rotaviruses Among Under-Five Children In Imphal, Manipur, India

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Rotavirus infection is one of the leading causes of acute gastroenteritis among children under five years globally. This hospital-based surveillance study was undertaken at the Regional Institute of Medical Sciences (RIMS), Imphal, from December 2015 to April 2019 to understand group A rotavirus gastroenteritis among under-five children and to identify the prevalent strains of rotavirus circulating in Manipur, northeast India. Of the total 527 stool samples collected, 378 (71.73%) samples were tested positive for group A rotavirus using VP6 antigen ELISA. Rotavirus dsRNA was extracted from ELISA positive fecal specimens and genotyping was carried out using sequencing of VP4 and VP7 genes. Samples that were untypable for VP7 and VP4 were further screened by VP6, NSP4, and NSP5 gene amplification followed by sequencing. A peak increase in the occurrence of rotavirus-led diarrhea was observed mainly in the cooler months during the study period. Children in the age group of 6 to 23 months were commonly infected with rotavirus (78.02%) as compared to other age groups. The findings of this study revealed the presence of commonly detected G genotypes such as G3, G1, G2, G9, and G12. The most common P genotypes found in the clinical samples were P[6], P[8], and P[4]. Strains with VP6 genetic drift and unusual rotavirus isolates have been identified in this study. Further co-infection of rotaviruses with other enteric viruses; Adenovirus, Astrovirus, and Norovirus was found to be 17.46% and the most common co-infection was rotavirus and astrovirus with 5.89%. In conclusion, the present study shows a high prevalence of rotavirus-led diarrhea among children under five years visiting RIMS, Imphal. This hospital-based surveillance study highlights the emergence of G12 strains in this region and the need for continuous monitoring of rotavirus strains evolution during post-rotavirus vaccination.

441152 3min Student

Multiplexed Serological Assay For Human Enteroviruses

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Multiplexed diagnosticsImmunological assays detecting antibodies against enteroviruses typically use a single enterovirus serotype as antigen. This limits the ability of such assays to detect antibodies

against different enterovirus types and to detect possible type-specific variation in antibody responses. We set out to develop a multiplexed assay for simultaneous detection of antibodies against multiple enterovirus and rhinovirus types encompassing all human infecting species. Seven recombinant VP1 proteins from enteroviruses EV-A to EV-D and rhinoviruses RV-A to RV-C species were produced. Using Meso Scale Diagnostics U-PLEX platform we were able to study antibody reactions against these proteins as well as non-structural enterovirus proteins in a single well with 140 human serum samples. Adult subjects had on average 33-fold stronger antibody responses to these antigens (p (Saarinen NVV et al., Microorganisms, 2020, PMID: 32604930)

441096 3min Student

Human Bocavirus 1 Respiratory Tract Re-activations Or Re-Infections In Two Adults, Contributing To Neurologic Deficits And Death

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Human bocavirus 1 (HBoV1), a DNA virus in the *Parvoviridae* family, causes mild to life-threatening respiratory tract infection (RTI) in children, but is infrequent in adults. HBoV1 re-infections or re-activations leading to casualties are rare, but might be underdiagnosed. Here, we report two young adults with rare diagnostic patterns of HBoV1 respiratory-tract re-infections, contributing to neurologic deficits and death.

Case 1: A young adult had an allogeneic T-cell depleted hematopoietic stem-cell transplantation (alloHSCT) for an inborn hemoglobinopathy. A year later the patient was hospitalized for a worsening RTI, with subsequent severe neurologic symptoms. The patient died 4 weeks later due to severe acute GvHD caused by reduction of immunosuppression for HBoV1 control. Blood, stool, pharyngeal and bronchial samples were HBoV1-qPCR positive (up to 10^9 copies/ml), but CSF was PCR and IgG negative. No other pathogens were found in any sample types, except for transient reactivations of BKV and HHV-6 in blood 4 months before RTI. Sequential serum samples were positive for HBoV1 IgG, with low-to-borderline IgM but high IgG avidity, already 9 months before RTI, confirming prior immunity.

Case 2: A healthy young adult had an upper RTI. The condition of the patient worsened with rapid cognitive decline until coma and death within 3 weeks. The patient's tracheal secretion exhibited only HBoV1 DNA (10^9 copies/ml) and rhinovirus (RV) RNA. CSFs were negative for 15 viruses, including HBoV1 and RV, but disclosed a seroconversion of HBoV1 IgG with low IgM. Paired serum samples showed low HBoV1 IgM and both HBoV1 and HBoV2 IgG of high avidity in competition EIA, but with considerable cross-blocking, suggesting an immunological phenomenon called original antigenic sin.
Conclusion: The molecular and serological results, together with their ages, suggest that both patients exhibited unusual re-infection or re-activation of HBoV1, directly or indirectly contributing to neurologic deficits and death.

437601 3min Student

Assessment Of Cardiac Inflammation In Viral Myocarditis

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An optimally functioning heart is an imperative aspect of our lives. Heart diseases are responsible for 1 in every 4 deaths in the USA. Being a part of the human body also means that the heart is susceptible to infections by microorganisms. Myocarditis is the inflammation of cardiac muscle usually caused by a viral infection, and it is one of the leading causes of sudden death in children and young adults. If left untreated, it can give rise to arrhythmias, and can also greatly increase the chances of a stroke, heart failure, and sudden cardiac death. Viruses, such as adenoviruses, parvovirus, coxsackieviruses, and more recently the SARS-CoV-2 virus, are causative agents that can be contracted easily, hence predisposing any healthy individual to myocarditis. The link between the aforementioned viruses and myocarditis is well established, however, the exact mechanism of inflammation still remains unclear. In this study, we analyzed human heart tissue samples derived from patients diagnosed with myocarditis or dilated cardiomyopathy to identify viral genomes. Briefly, a Zymo™ Research Kit was used to extract nucleic acids followed by a PCR to screen for the presence of viruses. Up to date, we have identified the following: Coxsackievirus B3, Herpesvirus 5, Herpesvirus 7, Epstein Barr virus, Respiratory syncytial virus, and Varicella virus in the analyzed samples. Concurrently, we are in the process of screening heart tissue samples from individuals without any history of heart diseases, to establish a baseline for the presence of these viruses amongst a general population. In addition, an analysis of the immune system markers associated with inflammation will show the extent of cardiac damage in the infected samples. In summary, this research will show the degree of cardiac inflammation in the context of virus-induced myocarditis.

445168 3min Student

In Vitro Studies And Clinical Observations Imply A Synergistic Effect Between Epstein-Barr Virus And Dengue Virus Infection

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Dengue virus (DENV) infection can lead to a complex spectrum of clinical outcomes, ranging from asymptomatic infection to life-threatening severe dengue. The reasons for thus drastically varying manifestations of disease remain an enigma. Herein, we reported an original discovery of the synergistic effect between pre-existing EpsteinBarr virus (EBV) infection and DENV superinfection in vitro and of a strong correlation of these two viruses in the clinical samples from dengue patients. We showed that I) DENV-2 infection of an EBV-positive cell line (EBV+ Akata cell) reactivated EBV through the PI3K pathway. II) examination of human peripheral blood mononuclear cell (PBMC) samples from dengue patients revealed significantly elevated cell associated EBV DNA copy number at the time of hospitalization versus at the time of disease recovery in most individuals. III) EBV infection promoted DENV propagation in both EBV hosting B cells and indirectly in THP-1 cells, supported by the following evidence: A) EBV+ Akata cells were more permissive to DENV-2 infection compared with Akata cells harboring no EBV virus (EBV- Akata cells). B) Low molecular weight fraction secreted from EBV+ Akata cells could enhance DENV2 propagation in monocytic THP-1 cells. C) While reactivation of EBV in EBV+ Akata cells further increased DENV-2 yield from this cell line, pharmacological inhibition of EBV replication by acyclovir had the opposite effect. To our knowledge, this is the first investigation demonstrating a positive correlation between EBV and DENV in vitro and in human biospecimens.

440287 3min Student

Immunoinformatics Approach To Design B- And T-cell Candidate Multi-Epitope Based Subunit Vaccine Against Zika Virus Infection

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Globally, the recent outbreak of Zika virus (ZIKV) in Brazil, Asia Pacific, and other countries, causing a spectrum of congenital diseases including microcephaly in newborn and Guillain-Barré syndrome (GBS) in adults. Currently, there are neither effective vaccines nor therapeutics available to prevent or treat ZIKV infection. To tackle this issue, we aimed to design B- and T-cell candidate multi-epitope based subunits against ZIKV using an in silico approach. We targeted the capsid (C), envelope (E) proteins, and non-structural proteins including NS5, NS3 and NS1. Then the energy minimization was performed with the molecular docking. Notably, we designed a multi-epitope peptide vaccine based on the potent major histocompatibility complex (MHC-I), and (MHC-II) epitopes of the three proteins (C, E, and NS1). Moreover, we predicted the Beta-Defensin as an adjuvant. Furthermore, the complex between the final vaccine and immune receptors (toll-like receptors (TLR-2 and TLR-4)) were evaluated by molecular docking.
Our data require experimental validation to determine its safety and efficacy to prevent ZIKV infections.

445657 3min Student

Development Of Potential Anti-influenza Agents By Rational Drug Design, Synthesis And Biological Evaluation Exploring Alternate Mechanism

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The high rate of mutability of Influenza A virus is a constant threat. The emergence of drug resistance to the current competitive inhibitors of neuraminidase (NA), such as oseltamivir and zanamivir, compels to overcome structural “me too” approach and highlights looking for an alternative approach. In search of new scaffold, series of scaffolds namely chalcones, cinnamic acids, linkages of cinnamic acid, aurones, indolones, quinolones, pyrimidines, benzimidazoles and piperazine derivatives were designed against Influenza A/H1N1pdm09 virus. Molecular docking studies revealed that the designed molecules occupied 430-cavity of NA, which is adjacent to the active site. Also, docking of sialic acid in the active site preoccupied with the docked molecules, i.e. in 430-cavity, resulted in displacement of sialic acid from its native pose in the catalytic cavity. The designed molecules were then synthesized and screened by evaluating their cytotoxicity and antiviral activity against Influenza A/H1N1pdm09 virus. Molecules with negligible cytotoxicity and good EC50 values were assayed for NA enzyme inhibition to determine their IC50 values. The mechanism of inhibition of the tested molecules was determined by enzyme kinetics studies which showed non-competitive inhibition when compared with competitive inhibitor viz. oseltamivir. Our findings support the hypothesis that our designed molecules possess an alternate mechanism of inhibition in-vitro in line with the in-silico studies. The molecules can be further developed to be used either alone or in combination of current NA inhibitors for better management of Influenza virus.

445734 3min Student

Enhanced Inactivation Of Foodborne Viruses By Cinnamaldehyde Nanoemulsions Require A Lipid Envelope

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Enhanced Inactivation of Foodborne Viruses by Cinnamaldehyde Nanoemulsions Require a Lipid Envelope Pragathi Kamarasu, University of Massachusetts Amherst, Amherst, MA and Matthew D. Moore, University of Massachusetts Amherst, Amherst, MA Introduction: Human noroviruses are the leading cause of foodborne illness globally. Many challenges exist in control of these viruses as many disinfectants show modest inactivation. Previous work has demonstrated that restructuring disinfectants into charged nanoemulsions can enhance inactivation of bacteria and fungi, but their effect on viruses is unknown. Purpose: The purpose of this study was to conduct comparative inactivation studies of cationic cinnamaldehyde nanoemulsions on norovirus surrogate phage MS2 and Escherichia coli to see if cationic nanoemulsions could enhance efficacy of cinnamaldehyde. Methods: MS2 bacteriophage, a norovirus surrogate, and E. coli strain C3000 were treated with different concentrations of cationic cinnamaldehyde nanoemulsion and the cinnamaldehyde essential oil (5.55 µg/mL - 27.7 µg/mL cinnamaldehyde) for 5-60 minutes at 37°C by suspension assay. Results: Overall, significantly more reduction for MS2 treated for 1 hour with cinnamaldehyde alone was observed compared to cationic cinnamaldehyde nanoemulsion. For instance, 4.02 ± 0.102 PFU/mL and 2.78 ± 0.34 PFU/mL log reductions were observed when treated with 27.7 µg/mL and 5.55 µg/mL of cinnamaldehyde alone, respectively. Whereas 1.54 ± 0.08 PFU/mL log reduction with 27.7 µg/mL and no reduction with 5.55µg/mL of cinnamaldehyde in nanoemulsion was observed. Alternatively, significant reduction of E. coli was observed with treatment of cinnamaldehyde nanoemulsions. For instance, >7-log reduction was observed with 16.6 µg/mL cinnamaldehyde nanoemulsion for 5 mins. Shelf-life study of nanoemulsion showed stability over 2 weeks when stored at 20°C and 4°C. Significance: These data suggest the hydrophobic protein contacts that maintain nonenveloped virus structure are not easily targeted by cationic nanoemulsions generated with low energy, high surfactant formulation, and suggests such a formulation requires targets with a lipid envelope for efficacy. This work informs future formulation to improve disinfectant efficacy against foodborne pathogens.

454217 3min student

Human Parvovirus B19 Infection in Bhopal Region of Central India: A Hospital Based Study

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Introduction: Human Parvovirus B19 (B19V) is a single-stranded DNA virus belonging to the family Parvoviridae. Clinical manifestations vary with the immunologic and hematologic status of the host. In healthy immunocompetent individuals, B19V is the cause of erythema infectiosum and acute symmetric polyarthropathy. In individuals with an underlying hemolytic disorder causes transient aplastic crisis. In the immunocompromised host, persistent B19V infection is manifested as pure red cell aplasia and chronic anemia. The congenital infection leads to fetal death in utero, hydrops fetalis, or the development of congenital anemia. Aim and Objective: To study the clinical and demographic characteristics of Human Parvovirus B19 infection. Material and Methods: An observational data-based study was conducted in the Regional virology lab, AIIMS Bhopal. The records were accessed for B19V samples received from January 2018 to March 2021. The clinical and demographic details were recorded for all the patients reported positive for B19V by IgM ELISA

(NovaTec Immundiagnostica GmbH, Germany). Results: A total of 263 samples from suspected B19V infection were received during the study period. 32 samples (12.16%) were reported positive with almost equal distribution among males and females. 22 (68.75%) positive patients were Conclusions: Significant infection rate has been reported for B19V in our region especially in the pediatric population. Since the symptoms may vary in B19V infection, a high index of clinical suspicion is required for any patient presented with fever of unknown etiologies for early diagnosis and better management. Acknowledgment: Authors are thankful to DHR-ICMR for their support

HIV & Hepatitis Viruses

441342

HIV-exposed Seronegative Women Express High Levels Of Systemic Regulatory T Cells And Low Immune Activation In The Cervical Mucosa

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Immunological correlates of natural resistance to HIV have been identified in HIVexposed seronegative (HESN) individuals and include a low-inflammatory genital mucosal status. The cervicovaginal epithelium has not been studied for such correlates despite constituting an important barrier against sexual HIV transmission. To fill this gap in knowledge, we collected samples of blood, cervical mononuclear cells, cervicovaginal lavage and ectocervical tissue from Kenyan HESN sex workers and controls. The samples were analyzed by flow cytometry, in situ image analysis, soluble cytokine levels and tissue-based RNA sequencing. A significantly higher relative proportion of regulatory T cells in blood, and a significantly lower proportion of activated cervical CD4+ and CD8+ T cells, respectively, were found in the HESN group compared with the controls. In contrast, the ectocervical tissue samples representing the two study groups had comparable epithelial thickness, E-cadherin expression, HIV target cell expression and RNA transcriptional profiles. In conclusion, the identification of an increased proportion of regulatory T cells in blood, a lower proportion of activated cervical T cells, and an intact ectocervical microenvironment in HESN individuals add new data to current knowledge about natural resistance to sexual transmission of HIV

445563

Characterisation Of The HIV Polymerase Cytotoxic T Lymphocyte Epitopes During Early And Chronic HIV-1 Infection Using Illumina Deep Sequencing

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Background: Despite multiple attempts to develop vaccines, there is still no effective HIV-1 vaccine. The HIV polymerase (pol) gene is a highly conserved region that encodes for replication enzymes and

harbours cytotoxic T-lymphocyte (CTL) epitopes. The aim of the study was to characterise HIV pol CTL epitopes in sample pairs obtained during early and chronic HIV infection. Methods: Total nucleic acids were manually extracted from plasma samples followed by amplification of the complete HIV pol. Illumina deep sequencing was performed in sample pairs of participants with early or chronic HIV. Illumina consensus sequences were generated using the PASeq pipeline, and aligned using MAFFT. Epitopes under immune selection pressure were assessed in MEGA by computing nonsynonymous to synonymous mutation ratios. Minority CTL epitope variants occurring at <5% were detected using a low-frequency variant tool in CLC Genomics. The Los Alamos National Laboratory epitope database was used for mapping mutations to HIV pol CTL epitopes. Results: Fifty-two participants were enrolled. Their median age was 28 (interquartile range: 24-32) and majority (92.3%) were female. Illumina consensus sequences (Sanger equivalent) identified 12 pol CTL epitopes under selection pressure, compared to 65 pol CTL epitopes that were identified through minority variant analysis. Minority variant epitopes existed at proportions ranging from 5.5 – 19.0%, and were detected in many samples at both baseline and follow-up. Majority of pol CTL epitopes were located in RT and integrase regions, and some have never been reported for HIV subtype C. Fewer minority variants could not be mapped to reported CTL epitopes in Los Alamos database. Conclusion: Deep sequencing revealed many pol CTL epitopes, some of which have never been reported for HIV subtype C. Variants that were not mapped within CTL epitopes could represent new epitopes. These findings may have potential implications for future HIV vaccine development.

432970

Human Cosavirus Infection In HIV Subjects With Diarrhoea: Persistent Detection Associated With Fatal Outcome

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Human cosavirus (HCoSV) is a new member of the Picornaviridae family, geographically widespread among humans. It has been suggested as a causative agent of acute gastroenteritis, but its pathogenicity is not currently certain. In HIV-infected subjects, diarrhoea is one of the most frequent gastrointestinal manifestations, whose aetiology remains often unexplained. The aim of this work was identifying the cause of viral diarrhoea among HIV infected patients by molecular assays. A total of 143 stool samples from HIV subjects with and without diarrhoea, were screened for conventional enteric viruses (rotavirus, adenovirus, norovirus and astrovirus) by molecular assays. The presence of HCoSV genome was investigated by nested RT-PCR for the 5’UTR region. Positive samples were further characterized by sequencing and phylogenetic analysis. Enteric viruses were more frequently found in diarrhoea cases (9/82) than controls (0/61) (p=0.007). HCoSV was detected in five (3.5%) of the subjects affected by diarrhoea. Phylogenetic analysis revealed the predominance of the species D. One patient suffered a persistent cosavirus infection with the same strain and after eight months he had a fatal outcome. No other pathogens could be detected. The results suggest a role of non-conventional enteric viruses, as HCoSV, as a potential opportunistic agent causing persistent infection and deterioration of the clinical conditions in HIVinfected patients. Screening procedures and monitoring including such viruses would be helpful in the clinical management of such patients.

441553

The Prevalence Of Hepatitis C In HIV-Infected And Uninfected Men Who Have Sex With Men Residing In Townships North West Of Pretoria, South Africa

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Objectives: More than 185 million people are estimated to have been infected with hepatitis C virus (HCV) worldwide, resulting in 350,000 deaths each year (Chen et al, 2017). Although the burden of HCV is reported to be low in South Africa, it continues to circulate in the South African general population. Studies in high-risk groups like men who have sex with men (MSM) are needed to evaluate the HCV prevalence, as well as circulating genotypes, that are essential for prevention, control, and management efforts. This study aimed to investigate the HCV prevalence in MSM residing in the townships located north west of Pretoria.

Methods: This was a descriptive study of 154 stored sera collected during 2016 and 2019 from MSM. HCV RNA was evaluated using RT-PCR assay with primers targeting the 5' untranslated region (UTR). PCR products were sequenced, and a phylogenetic tree was constructed using MEGA Version 7 to determine the HCV genotype.
Results: The overall prevalence of HCV in MSM was 27.2% (42 of 154). The prevalence of HCV was 25% (14 of 55) in HCV mono-infected MSM and 28% (28 of 99) in HIV/HCV co-infected MSM. Genotype distribution was 63.6% (98/154) genotype 1, 0.6% (1/154) genotype 4, and 0.6% (1/154) genotype 5.

Conclusion: This study found a high prevalence of HCV infection in MSMs and confirmed recent studies conducted in other regions of South Africa showing that HCV prevalence is higher than previously reported in MSM, in particular amongst those co-infected with HIV. Furthermore, while genotype 5a remains the predominant genotype in South Africa, the current study indicates that the majority of MSMs are infected with genotype 1. Thus more studies are needed in the MSM population especially those co-infected with HIV to understand their risk behaviour for prevention and control measures .

440340 3min Student

Analysis Of Biomarkers Of Inflammation In Cohort Of Human Immunodeficiency Virus (HIV-1) In Moroccan Patients: Impact Of First-Line Antiretroviral

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Background: Chronic inflammation marked by elevated interleukin (IL)-6 and soluble tumor necrosis factor (TNF)-α levels may play a detrimental role in HIV infection.

This study aimed to evaluate the relationships of soluble inflammatory TNF-α and IL- 6 levels in a cohort of people living with HIV-1 (PLWH) before and after initiating long- term suppressive combined antiretroviral therapy (cART).

Methods: Single-centre, prospective cohort study was conducted from March 2018 to January 2021.We analysed TNF-α and IL-6 in a cohort of 71 HIV-infected patients compared with 30 matched uninfected healthy controls. TNF-α and IL-6 were measured using ELISA assays.

Results: The mean ages of PLWH and of uninfected healthy controls were 37.2 ± 12.3 and 33.4 ± 14 years, respectively (P>0.05). Baseline plasma level of TNF-α level was at higher concentrations in untreated PLWH compared with healthy individuals (P= 0.0003). However, plasma levels of IL6 showed no significant difference between untreated PLWH and healthy subjects (P=0.1171). Notably, we found significant decrease in plasma levels of TNF-α and IL6 in PLWH after long- term suppressive cART(P Conclusion: Together, these data highlighted that plasma concentrations of TNF-α and IL-6 and showed a significant decrease after cART. Thus, our findings support the beneficial effect of cART in attenuating the inflammation in chronic HIV infection.

445636 3min Student

Hepatitis C Virus Genotypes And Resistance Associate Substitutions In Colombian Patients

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The hepatitis C virus (HCV) infection and the resulting burden disease is a public health problem. The aims of this study were to characterize the viral genotypes and resistance associate substitutions (RAS) in individuals with history of blood transfusion before 1996, patients with diagnosis of end-liver disease submitted to liver transplantation and individuals injecting drugs. Total antibodies anti-HCV were determined in samples from the seroprevalence study in the transfused population using a commercial test. Furthermore, 5'UTR, NS5A and NS5B HCV genome regions were amplified and sequenced from serum samples and/or liver explant samples of the 3 study populations. Prototype HCV sequences were included in the analysis. Two hundred sixty individuals with a history of blood transfusion were recruited in Medellin, Santa Marta and Pereira cities. Seven samples from this study population were positive for antibodies anti-HCV (2,69%) and the viral genome was detected in four of these samples. Genotype 1, subgenotype 1b, was characterized in 3 samples and genotype 4, subgenotype 4d, was identified in one sample. From liver explant samples, the subgenotypes 1b and 1a were identified in 8 and 3 samples, respectively. Additionally, the genotype 2, subgenotype 2a, and subgenotype 4a, were characterized in two samples. Finally, the subgenotype 1a was identified in two individuals injecting drugs. Moreover, three RAS were identified, Q30R, C316N, Y93H, in samples from 2 DAA naïve individuals with history of blood transfusion and in 5 liver samples from patients with end-liver disease. In conclusion, genotype 1, subgenotype 1b, is still the most prevalent in Colombia, regardless of the risk factor. This is the first characterization of the subgenotypes 4a and 4d in Colombian patients, as well as the first report of RAS in samples from DAA naïve Colombian individuals. Further studies are necessary to describe the baseline RAS in patients with diagnosis of HCV infection.

440416 3min Student

Hepatitis B Virus And Hepatitis Delta Virus Infection In Colombian Indigenous Population

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The World Health Organization (WHO) estimates 257 million people with chronic Hepatitis B Virus (HBV) infection. Latin America has a low to intermediate HBV infection prevalence, with regions with high prevalence like the Amazon Basin inhabited mainly by indigenous. Despite the universal vaccine program in Colombia, HBV prevalence is high in indigenous probably due to geographic, socio-economic, and cultural factors. Moreover, coinfection and superinfection with Hepatitis Delta Virus (HDV) are frequent in populations with high HBV prevalence. This study aims to characterize the molecular HBV and HDV infection markers in indigenous communities from Amazonas, Guaviare, Antioquia, and La Guajira states in Colombia. Indigenous with hepatitis B diagnosis and controls from the same communities were invited to participate in the study. HBsAg and anti-HBc markers were determined using commercial kits. The S, preS1, and preS2, regions of the HBV genome and the HDV genome were amplified to identify escape variants and circulating genotypes. Up to date 38 cases and 72 controls have been recruited in three states. Among the cases, the mean age is 33.6 years old and 82.5% are female. The cases are mainly from Tikuna, Nükak, and Wayuu ethnic groups corresponding to Amazonas, Guaviare, and La Guajira, respectively. From the 16 cases analyzed, 31.25% were positive for the HBV genome, 50% were positive for the HDV genome, and 5/8 cases were positive for both HBV and HDV. These preliminary results show that HBV and HDV infections are still public health issues in indigenous communities, despite the national vaccination program. The prevalence found shows a possible lack of notification and underestimation of the cases. This study will allow us to demonstrate if there is a correlation between viral markers and risk factors to improve their health conditions, contributing to the WHO goal towards the elimination of hepatitis B worldwide.

441027 3min Student

Association Between TLR4 and TLR9 Polymorphisms And The Risk Of Hepatocellular Carcinoma Development In Moroccan Patients With Chronic Viral Hepatitis: A Case-control Study

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Background and Aims: Hepatocellular carcinoma (HCC) is one of the most common clinically malignant tumors of the digestive system. Globally, HCC is the sixth most common malignant tumor and the fourth in terms of cancer-related mortality. Toll-like receptors (TLRs) are responsible for the activation of the innate immune response and are suspected to play an important role in

hepatocarcinogenesis by inducing inflammation. Among TLRs, TLR4 recognizes lipopolysaccharide (LPS) and plays an essential role in cancer progression, while TLR9 recognizes unmethylated CpG DNA present in endosomal compartments. In this case-control study, we aim to investigate the association between TLR4 (rs11536889) and TLR9 (rs187084) polymorphisms and the risk of Hepatocellular carcinoma in a North African context.
Methods: A series of 190 Moroccan subjects, including 95 patients with hepatocellular carcinoma (HCC), and 95 healthy (HC) controls were genotyped using a TaqMan allelic discrimination assay.
Results: Our result showed that the frequency of the C allele of TLR4 (rs11536889) was higher in HCC patients than in HC (P= 0.0013). Notably, patients carrying the C allele had 1.96 –fold the risk to develop HCC [OR=1.96; 95% CI 1.30-2.97]. Additionally, the frequency of the CC genotype was higher in HCC patients (32.63%) than in HC (1.05%) (P = 0.00017). However, we found no significant relationship between the rs187084 polymorphism in TLR9 and susceptibility to HCC [OR=1.00; 95% CI 0.22-22.46; P = 1.000].
Conclusions: Our study suggests TLR4 rs11536889 SNP may increase the risk of HCC. While TLR9 rs187084 polymorphism may not contribute significantly to the risk for hepatocellular carcinoma. Further studies on larger cohorts are needed to understand the effect of TLR4 and TLR9 polymorphisms on risk of HCC development.

Human Viruses: Molecular Virology

440839

A Functional Rotavirus-derived Small RNA Identified By miRNA Profiling Of Infected Cells And Extracellular Vesicles

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Small RNA expression is altered by viral infection, with the potential for both proviral and antiviral effects. While most small RNAs are derived by the host, some viruses are known to produce their own small RNAs which in turn can influence gene expression for the virus's benefit. Rotavirus A can infect cells as free particles, or as multiple particles transported via extracellular vesicles, which can also carry functional non-coding RNAs. We profiled and analyzed the miRNA profiles of healthy HT-29 cells verses vesicles, and rotavirus-infected cells verses vesicles. In doing so, we discovered a candidate microRNA-like molecule derived from segment 9 (encoding for the VP7 gene) of rotavirus A. We predicted a hairpin structure, candidate target genes, and measured conservation across rotavirus A sequences. This candidate miRNA was found to be relatively conserved throughout rotavirus A, and predicted to target many cellular pathways involved in viral infection. We present a verification and characterization of this virally-derived miRNA using both bioinformatics and in vitro assays. We directly verify Calcium/Calmodulin Dependent Protein Kinase IV (CAMK4) as a target of the RVA-derived miRNA-like molecule, showing a function for regulating protein expression. This small RNA was abundant in extracellular vesicles during rotavirus infection, which could indicate rotaviruses infecting via vesicles may benefit from being transported into the cell along with a proviral small RNA. The existence and function of microRNAs derived from cytoplasmic RNA viruses is somewhat debated. Here we provide evidence that rotavirus A is encoding a functional miRNA-like molecule, and discuss possible hypotheses for how this molecule was able to evolve.

440799

In Situ Detection Of Cutavirus In FFPE Skin Biopsies Of Patients With Cutaneous T-cell Lymphoma

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In 2016, metagenomic studies revealed a new parvovirus in the *Protoparvovirus* genus; cutavirus (CuV), in diarrheal stools from children and in skin biopsies from patients with cutaneous T cell lymphoma (CTCL). In serological studies, strong IgG responses have been observed for CuV suggesting that it causes systemic infections similar to other human parvoviruses. Excitingly, CuV DNA has by our group been associated with CTCL, and detected also in a melanoma and four organ transplant patients of whom two had CuV-positive skin carcinomas (Väisänen et al. Clin Infect Dis 2019). We are now studying CTCL and pre-CTCL skin tissues to provide information on the target cells harboring CuV and to further assess its role in CTCL.
By RNAscope in-situ hybridization (RISH) we studied 13 skin samples from eight CTCL patients, four of which were CuV DNA positive and four negative. We used two antisense RISH probes targeting the VP2 (Probe-V-gp3) and NS1 regions (Probe-V-CuV-NS1), and one sense probe targeting NS1 (Probe-V-CuV-NS1-O1). In one patient (C-53), clear RISH signals were visualized with all three probes in two PCR-positive CTCL FFPE skin samples with high viral loads of 10^8 copies per million cells. We did not observe RISH signals in the other samples with lower CuV viral loads (<10^7 copies per million cells) with any of the three probes. Skin samples from patient C-53 were further studied with immunohistochemistry, using cell-specific marker antibodies (e.g., K10, CD3, CD68 and CD20) to identify the RISH-positive cells. Further, using an in-house RT-qPCR, we detected spliced mRNA in a fresh skin biopsy of patient C-53, indicating viral gene expression in the tissue – despite the presence of circulating CuV antibodies. Our results provide data on the cell types harboring CuV in skin and assess the viral activity (transcription and possible replication) in CTCL tissues.

445690

Re-targeting Parvovirus To The Tumor Vasculature By Replacing Capsid Functional Domains With VEGF-blocking Peptides

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Re-targeting the capsid of oncolytic virus to the tumour vasculature may enhance their anticancer potential and favour the delivery of exogenous therapeutic genes. The anti-tumour specificity of the oncolytic mouse parvovirus Minute Virus of Mice (MVM) is hampered by the use as a receptor of sialic acid types present on the surface of many physiological cells. Three functional domains of MVM capsid, including a dimple at the twofold axis binding the sialic acid receptor, were replaced by vascular endothelial growth factor (VEGF) blocking peptides aiming at directing MVM infection to the tumour vasculature. We found that most substitutions impaired virus assembly or maturation, and conversely capsid assembly may distort the VEGF-R1 binding capacity of the exogenous peptides. However, some chimeric virions were infectious, and showed increased specific infectivity for human glioblastoma cells that could be further enhanced by controlled removal of sialic acid components of human transformed cells. The study illustrates the possibility to alter the tropism of oncolytic viruses through engineered peptide replacement at functional domains of their icosahedral capsids. However, it does also uncover the structural constraints to manipulations exhibited by viral capsids that have evolved to adopt narrow grooves to allocate sugar components as receptors.

440828

Characterization Of An Enterovirus D68 Structural Protein VP4 Interprotomer Interaction Network

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Enterovirus D68 (EV-D68), a respiratory virus in the Picornaviridae family, has been suspected in recent outbreaks among young children of Acute Flaccid Myelitis (AFM), a neurologic flaccid paralysis like polio. EV-D68 is an icosahedral virus with a capsid consisting of 4 structural proteins, VP1-VP4. Structural proteins VP1-VP3 constitute the external capsid shell while VP4 is a small predominantly disordered protein residing on the internal capsid surface. The structural proteins are critical for capsid meta-stability, viral breathing, as well as host cell entry and uncoating. VP4 is the most conserved structural protein among enteroviruses yet little is known about VP4 interprotomer interactions between the other structural proteins, the dynamic properties of these interprotomer interactions, or how these interprotomer interactions influence EV-D68 infection. We hypothesize that VP4 residues involved in interprotomer interactions play critical roles in the dynamics and functions of EVD68 infection as well as potentially influence the effectiveness of antiviral compounds. VP4 in silico analysis has indicated the presence of a potential interaction network between Lysine 33 of VP4 on one protomer with residues of VP3 and VP4 on an adjacent protomer. Ongoing mutagenesis experiments suggest that replacement of residues within this interaction network reduce viral titer or are lethal to viral replication. The significance of this interprotomer interaction network will be discussed as part of ongoing research on viral entry and inhibition of EV-D68. Characterization of this interprotomer interaction network and the residues involved will further elucidate how EV-D68 capsid proteins interact with each other for cell entry and aid in more targeted drug therapies.

441053 3min News

Structural Comparison Of Human Enterovirus D94 At Three Stages Of Viral Entry

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Human enterovirus D94 (EV-D94) is a picornavirus that was first characterized in 2007. Infections of EV-D94 have been linked to acute flaccid paralysis, a central nervous system disease that is similar to poliomyelitis; EV-D94 is also a potential diabetogenic enterovirus due to its ability to damage human pancreatic islets. Interestingly, there is a high prevalence of this virus among the Finnish population. Here, we present the structures of 3 stages of EV-D94 morphogenesis, including the full native virion, an uncoating intermediate and the empty virion, at atomic resolution using cryo-electron microscopy. The resolutions reach 2.9 Å, 2.6 Å and 3.0 Å, respectively. The structure of the full native EV-D94 virion shows extended protruding loops around the five-fold axes and an unusual canyon, suggesting a novel receptor usage. The comparison between the 3 forms of virus particles may delineate the viral uncoating process, which provides valuable information for future vaccine and drug design against EV-D94 and related human enteroviruses.

446346 3min Student

A Mouse Parvovirus Infecting Human Glioblastoma Stem Cells With Patient-specific Cytotoxicity

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Glioblastoma multiforme (GBM) remains a major type of cancer with no current effective therapy. On the other hand, multiple studies support that the efficacy of cancer therapies must be primarily shown against the cancer stem cells driving tumorigenesis. We have therefore attempted to develop a GBM therapy using two strains of the Minute Virus of Mice (MVMp and MVMi), a non-pathogenic parvovirus with evidenced oncolytic capacity and well characterized tropism determinants. To this aim, primary glioblastoma stem cells (GSCs) isolated from GBM patients were challenged with the MVM strains and the system was comprehensively studied in neurospheres. GSC neurospheres accumulated assembled capsids. Inter- and intrapatient GSC heterogeneity was dissected by their diverse innate responses and the ratio between structural and non-structural viral gene expression. Further, viral infection triggered a comprehensive DNA-damage response involving cell cycle arrest and leading to neurosphere disorganization. Notably, the MVM infection preferably targeted those GSC subpopulations within patients showing weak innate responses. This study provides a molecular foundation for personalized viral oncolytic therapies against devastating glioblastoma and other cancers with weakened innate responses.

445122 3min

The Effect Of SP100-V4 Protein Subtype On Replication Of HSV-1

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SP100 is a constitutive component of the PML body and an early target of HSV-1 ICP0 protein through Ubc5h mediated proteasome dependent degradation. Previous studies show that HSV-1 needs to degrade SP100 for efficient lytic replication in HEp2 cells at a low multiplicity of infection and that disruption of SP100 gene by CRISPR technique allows for higher replication center initiation efficiency of HSV-1 and facilitated lytic replication of the virus. Herein, based on combinational studies using overexpression cell lines and recombinant viruses in vitro, we are reporting the following observations: I. Among four SP100 isoforms (V1,V2,V3,V4) testes in this study ,SP100-V3 and V4 showed inhibitory effects on HSV-1 lytic replication in overexpression HEp-2 cell lines. II. Recombinant HSV-1-SP100 V4 virus was attenuated in lytic replication in vitro. III. Cytosolic SP100-V4 was not degraded and was exported into extracellular spaces through an exosomal pathway during HSV-1 infection. IV. SP100-V4 in secreted exosomes of both mock infected and HSV-1 infected cells could mount anti-viral effect against incoming HSV-1 infection.

440781 3min Student

Association Between The Peroxisome Proliferator-activated Receptor Gamma Coactivator 1 Alpha RS8192678 Polymorphism And Risk Of Hepatocellular Carcinoma In A Moroccan Population.

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Background & aims : Hepatocellular carcinoma (HCC) is the most common primary liver malignancy. It is the fourth cause of cancer-related mortality and morbidity worldwide. Hepatitis B and C viruses are the main risk factors of tumorigenesis. However, only a fraction of infected patients develop HCC during their lifetime suggesting that host genetic factors might modulate HCC development. Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PPARGC1A) plays a crucial role in regulating the biosynthesis of mitochondria, which is closely linked to the energy metabolism in various tumors. Recent study suggested that PPARGC1A acts as a tumor suppressor in HCC. There is still a lack of PPARGC1A gene single-nucleotide polymorphisms (SNPs) in HCC susceptibility. We aimed to assess the association between PPARGC1A rs8192678 polymorphism and HCC risk in a Moroccan population. Methods : In this case-control study of 123 HCC patients and 123 controls, were performed genotyping of PPARGC1A rs8192678 polymorphism and assessed its association with HCC risk. Results : We found that individuals carrying the GA/AA genotypes of PPARGC1A rs8192678 polymorphism were significantly associated with an increased risk of HCC compared with those carrying the GG genotype (adjusted odds ratio (OR) = 8.43, 95% confidence interval (CI) = 4.31-16,49, P = 2.072.10-11, and OR= 4.80, 95% CI 1.61-13.70, respectively). Moreover, the A allele was over-represented in patients with HCC (33%) in comparison with healthy controls (10%, P= 1.135.10-10). Stratification analysis according to gender showed an increased risk in the females with AA genotypes (OR = 6.48, 95% CI = 1.77-23.67), P = 0.00196) compared with males with the same genotype (OR = 4.64, 0.49-43,78, P = 0.154). Our results suggested that PPARGC1A polymorphism rs8192678 was associated with HCC susceptibility and may contribute to hepatocarcinogenesis. Our findings require validation in further studies with larger sample sizes.

440568 3min Student

Polymorphism Of Peroxisome Proliferator-Activated Receptor ɣ Coactivator-1 Alpha Influences Hepatitis B Virus Clearance In Moroccan Population

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Background : The peroxisome proliferator activated receptor gamma coactivator 1 (PPARGC1APGC1) family regulates hepatitis B virus (HBV) biosynthesis. Recently, several studies described that PPARGC1A variants are associated with different aspects of liver disease. However, the roles of these variants in the persistence of HBV infection have not been investigated in the HBV-infected population. We aimed to investigate whether the rs8192678 SNP (Gly482Ser) in PPARGC1A was associated with HBV spontaneous clearance in Moroccan patients.

Methods: The study included 181 subjects with spontaneous clearance, and 292 patients with chronic hepatitis B (CHB). The rs8192678 SNP was genotyped using a TaqMan allelic discrimination assay and we explored their association with HBV spontaneous clearance.
Results : Subjects carrying CT and TT genotypes were more likely to achieve spontaneous clearance compared with CHB (OR= 0.48, 95% CI (0.32-0.73), P= 0.00047 ; OR= 0.28, 95% CI (0.15-0.53), P= 0.00005, respectively). In addition, patients carrying the mutant allele T at rs8192678 were more likely to achieve spontaneous clearance compared with CHB (OR= 0.51, 95% CI (0.38-0.67), P =2.688.10-6). However, according to rs8192678genotypes, we found no significant associations between HBV viral load and alanine aminotransferase levels in CHB patients (P>0.05).
Conclusions : These results suggest the rs8192678 SNP (Gly482Ser) in PPARGC1A modulates HBV infection, which may be a predictive marker of HBV in the Moroccan population.

441443 3min Student

Changes Of M6A Modification In mRNAs Of Microglia Infected With HIV-1 Could Modulate Functions Of Nervous And Immunologic Systems

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Antiretroviral therapy (ART) is the main form to combat HIV/SIDA. Unfortunately, the therapy does not cure it due to the viral ability to integrate a viral DNA into a chromosome of the host staying as latency reservoirs in T lymphocytes, macrophages, and microglia. It knows that 30% of HIV-infected individuals present HIV-associated neurocognitive disorders (HAND). In this pathology, the microglia HIV infected result overactivated and, it can induce a neuronal injury through the release of proinflammatory cytokines and cytotoxic factors. Recently it has been described that the N-6 methyladenosine (m6A) may be responsible for epi transcriptomic modification of mRNA and produce inflammatory disorders by modulation of expression of molecules associated with the immune response. In this work, we proposed to analyze the epitranscriptomic profile of microglia infected by HIV-1. This profile was obtained by an RNA-seq analysis of mRNA with m6A modification isolated by immunoprecipitation. The conditions evaluated were microglia infected with HIV-1, non-stimulated microglia (mock), and microglia treated with TNF-α (control of stimulation). The sequencing results were analyzed bioinformatically to obtain the differential expression analysis, functional enrichment, and interactome analysis. The epitranscriptomic profiles obtained from microglia infected by HIV-1 show changes in the transcript expression of genes related to chemical synaptic transmission, maintenance of synapse structure, IL-6 pathway, complement activation, T cell activation, regulation of monocyte differentiation, and positive regulation of viral entry into the host cell. These results suggest a putative relationship between the exposition of microglia to HIV-1 and changes from m6A modification, which could impact nervous and immunological systems. When we analyze the immune response, we observe changes in the expression of interferon stimulated genes, such as APOBEC, MX2, TRIM55, and IRF7. Together, these results suggest that in HIV-1 infected microglia, the antiviral response may be associated with the host immune modulation mechanism to ensure the viral infection.

440765 3min Student

Association Of MBL2 Gene Polymorphisms With HIV-1 Infection And AIDS Development In Moroccan Subjects

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Background: Human Mannose-binding lectin (MBL) is a serum protein secreted by the liver. It is encoded by the MBL2 gene and participates to activate the innate immune defence through the lectin complement pathway. Several studies showed that the deficiency of the MBL2 gene is correlated to several infectious and autoimmune diseases susceptibility, including AIDS.
Aim: The present study aims to investigate the impact of the MBL2 gene polymorphisms rs503037, rs1800450 and rs1800451 located in exon 1 on HIV-1 infection outcome in Moroccan subjects.
Methods: This case-control study involves 407 consenting individuals, including 205 HIV-1 infected patients and 202 healthy controls. The genotyping of polymorphisms was performed by gene amplification using conventional PCR followed by DNA sequencing.
Results: The statistical analysis showed that rs503037 (C/T) and rs1800451 (G/A) polymorphisms in exon 1 are associated with an increased risk of HIV-1 infection susceptibility with p-values of 0.047 and 0.001, respectively. Furthermore, the allele A of rs1800451 had a protective effect against AIDS development with a p-value of 0.014. Moreover, the CGA haplotype has a protected effect of HIV-1 susceptibility (p-value = 0.003).
Conclusions: Our findings highlight the importance of the polymorphisms in the first exon of the MBL2 gene in the modulation of HIV-1 infection, and the contribution in the progression of the disease to AIDS stage in Moroccan subjects.

441158 3min Student

Molecular Characterisation Of Human Adenoviruses From Environmental Samples In Gauteng, South Africa Using Next Generation Sequencing

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Human adenoviruses (HAdV) are non-enveloped viruses with linear, double-stranded DNA genomes and are grouped in seven species (A-G) with over 88 genotypes. These viruses are associated with keratoconjunctivitis, hepatitis, respiratory, urinary and gastrointestinal infections. Transmission is via the faecal-oral route, inhalation of respiratory droplets and direct contact with contaminated environments. There is limited information on the presence of HAdV in water environments in Tshwane, Gauteng. Therefore, the aim of the study was to quantify and determine genotypes of human adenovirus in environmental samples, namely raw sewage and treated effluent, using molecular methods. A total of 150 environmental samples (75 raw sewage of 1 L each and 75 effluent of 10 L each) were collected from two wastewater treatment plants (WWTPs) in Tshwane over 18 months. Viruses were recovered by skimmed milk flocculation or glass wool adsorption-elution, followed by polyethylene glycol/sodium chloride precipitation. Mengovirus (MV) was used as a nucleic acid extraction control. Standard curves prepared from known dilutions of HAdV and MV were used for quantification purposes, using quantitative polymerase chain reaction (qPCR). Genotyping was done by conventional nested PCR, followed by next generation sequencing. Human adenovirus was

detected in 140/150 (93%) of the samples comprising 69/75 (92%) raw sewage and 71/75 (95%) effluent samples. The HAdV concentrations ranged from 6.84x104 gc/L to 1.69x1012 gc/L in raw sewage and from 5.08x103 gc/l to 4.30x108 gc/L in effluent. Next generation sequencing identified 77 genotypes. The HAdV-D and HAdV-B were the most predominant species, followed by HAdV-F, HAdV-A, HAdV-E and HAdV-C. Testing WWTP samples allows for the detection of HAdV types, causing symptomatic and asymptomatic infections, circulating in the surrounding communities. The detection of viruses in WWTP samples is a public health concern as the treated effluent is discharged into rivers, which may be used for domestic and recreational purposes.

445123 3min Student

Optimizing Recovery Of Non-enveloped Virus From Aqueous Solution Using Magnetic Ionic Liquids

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Isolation and identification of pathogens from food samples is critical for controlling foodborne illness. However, foodborne viruses are difficult to isolate due to low concentration and uneven distribution. Magnetic ionic liquids (MILs) are a diverse class of hydrophobic solvents that can be easily separated from aqueous solutions and have previously proven effective for capture of bacterial pathogens. In this study, we demonstrated and optimized the capture of bacteriophage MS2, a human norovirus surrogate, from aqueous solution using MILs. MS2 was diluted into 0.1% peptone water to 105 PFU/mL and extracted using cobalt- , manganese-, or nickel-based MILs. The MILs were added to the MS2 suspension, vortexed for 30 seconds to disperse, and separated using a magnet. The supernatant was then removed, and captured MS2 was eluted into fresh Luria broth by vortexing for 120 seconds. MS2 RNA was then purified from each fraction via phenol-chloroform extraction and quantified via RT-qPCR. Each condition was tested in triplicate. Cobalt- and manganese-based MILs showed recovery efficiencies of 12.5±4.65% and 13.8±3.81% respectively, significantly (p Our results indicate MILs have potential as a capture reagent for foodborne virus detection, with room for further optimization. This informs future work on preanalytical sample processing, one of the major hurdles to sensitive detection of viruses in foods.

445205 3min Student

Oct-1 Plays An Essential Role In Herpes Simplex Virus-1 Infection

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Herpes simplex virus-1 (HSV-1) is a highly prevelant pathogen, transmitted through droplets or direct skin-skin contact. The clinical manifestations of HSV-1 infection varied from self-healing symptoms such as cold sores and gingivostomatitis to life threatening diseases such as herpesviral meningitis and herpesviral encephalitis. Immediately after HSV-1 invades the body, it replicates vigorously in the epithelial cells and then enters the nucleus of sensory neurons through retrograde transportation to establish latent infection. To initiate lytic infection in the epithelial cells, the viral tegument protein VP16 is released into the cytoplasm immediately post membrane fusion of viral envelope and cell membrane, binds to host cell factor C1(HCF-1) and then enters the nucleus. It binds to the Octamer transcription factor (Oct-1) on the “TAATGARAT” motif in the IE gene promoter to form VP16 complex, activating the transcription of IE gene. In order to explore the role of Oct-1 in the replication process of HSV-1, we designed sgRNA sequences targeting different exon regions of Oct-1 and the corresponding homologous arm sequences, constructed a monoclonal Oct-1 KO cell line from HEp2

by CRISPR technique. In this study, we are reporting the following observations: 1. The replication of HSV-1 in Oct-1 knock-out cell lines was severely affected. 2. The transcript levels of the three stages of the HSV-1 life cycle, represented by ICP27, TK, VP16 are down-regulated in the absence of Oct-1 protein. 3. Infection of HSV-1 promoted extracellular secretion of Oct-1, HCF-1 and VP16 via exosomes, which could be well received by recipient cells. 4. Exosomes secreted by HSV-1 infected cells had a general anti-viral effect against HSV-1 infection. 5. Oct-1 in exosomes promoted replication of HSV-1 in neurons. These results indicated that Oct-1, in complex with HCF-1 and HSV-1 viral protein VP16, could be transported by exosomes and facilitated the next round HSV-1 infection.

Zoonotic Viruses: Clinical Virology

441109

Camel Pox Virus (CMLV) Infection And Zoonotic Importance

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Camelpox is a highly contagious skin disease of camelids caused by camelpox virus (CMLV), a member of the genus Orthopoxvirus within the family Poxviridae. The disease is often manifested as a mild local skin infection and sometimes in the severe form with systemic involvement. The disease is enzootic in the camel-rearing areas of arid and semiarid regions of the world and causes economic loss in terms of morbidity, mortality, loss of weight, and reduction in milk and wool production. The CMLV infection is transmitted mostly by direct contact and aerosol route. The disease gained attention globally in the recent past due to its close similarity with the causative agent of smallpox (variola virus) and irrefutable incidences of few zoonotic infections in humans. Like many other poxviruses, the CMLV has a large DNA genome, capable of encoding genes responsible for replication, host range, immunomodulation, virulence, and other functions. Despite the presence of a myriad of host range genes, the host tropism of camelpox virus is very limited. Both live attenuated and inactivated vaccines are available to combat the disease in camels; however, no vaccine has been developed until the date for use in humans. Few antiviral agents have been shown to be effective against CMLV; however, their use is very limited in field outbreaks. The research on CMLV is gaining global interest due to CMLV zoonosis especially in the context of naive human population to poxvirus immunity. The present seminar gives a brief enlightens of the background, zoonotic impact, incidence, and prevalence of the disease, immunobiology, diagnostics, risk factors, transmission, besides prevention and control of camelpox.

445377

Small Molecule-based Inhibitors Of Emerging RNA Viruses

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Emerging RNA viruses, such as vector-borne flaviviruses and coronaviruses, represent currently serious global health risks leading to severe health problems in many people or numerous deaths. Therefore, new and highly efficient antiviral drugs are urgently needed to treat diseases caused by emerging viral pathogens. In this study, we describe several types of small molecule-based inhibitors differing in chemical structures, as well as in mechanisms of action. Nucleoside analogues are the most promising group of drugs, which directly inhibit viral RNA-dependent RNApolymerases or methyltransferases. We discovered that 2´-C-methylated and 4´-Cazidated nucleosides are strong

inhibitors of tick- and mosquito-borne flaviviruses, whose antiviral potency is characterized by low-micromolar EC50 values. Fluoromodified nucleosides, such as 3´-deoxy-3´-fluoroadenosine or Gemcitabine, and also nucleosides with a flexible structure („fleximers") also strongly suppress flaviviral replication but show considerably worse cytotoxicity profiles. A C-nucleoside Galidesivir (BCX-4430), shows excellent anti-flaviviral properties and low toxicity for most tested cell lines. Many of these nucleoside analogues are active not only in vitro, but also in a mice model of viral infection. A very interesting group of viral inhibitors are natural or synthetic (metallo)porphyrins, which can be incorporated into viral membranes to block a viral-cell fusion process even in nanomolar concentrations. A similar mode of action was observed in synthetic „rigid amphipathic fusion inhibitors'', molecules composed of highly hydrophobic perylene core decorated by various polar/charged substituents. These unusual compounds, acting as lipid analogues, suppress SARS-CoV-2 in nanomolar levels via virus-cells fusion inhibition mechanism. Replication of SARS-CoV-2 in vitro was also efficiently inhibited with diphyllin, a natural inhibitor of vacuolar ATPases. Although, many of these structures are not directly applicable for antiviral drug development, they provide valuable information about viral replication strategies and allow us to better understand viral pathogenesis and virus-host interactions.

444962

Knowledge Of Chikungunya And Mosquito Avoidance And Control Practices Among Newly Admitted Students At An Offshore Medical University In The Caribbean

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Background: Chikungunya is a re-emerging viral disease transmitted by mosquitoes. Since there is no specific drug treatment or licensed vaccine, knowledge of this disease among people is very important for its prevention and control. This study aims to assess knowledge of chikungunya and pattern of implementation of mosquito avoidance and control practices among new students admitted to an offshore medical University in the Caribbean

Methods: A cross sectional study was conducted, where a pilot tested questionnaire was surveyed among all newly admitted students for a period of one year. The participants who were aware of the disease were included in the knowledge score analysis. Knowledge level of each participant was determined to be good (score > 70%), fair (score 50.1–69.9%) or poor (score < 50%). The study also evaluated WHO recommended mosquito avoidance and control practices among all the participants.
Results: Among the 129 students surveyed, 102 participants responded (response rate: 79.06%). Of 102 participants, only 39 (38.2%) were aware of the disease with 30.77% having good knowledge, 28.21% fair knowledge and 41.03% poor knowledge of the disease. Of the eight mosquito avoidance and control practices evaluated, none were implemented by 2 (1.96%) of the participants whereas one or more, but not all, were used by the remaining 100 (98.04%) participants. The association of these practices with awareness of the disease as well as levels of knowledge was statistically non-significant (P>0.05).
Conclusion: A large percentage of newly admitted students did not have adequate knowledge of chikungunya. However, implementation patterns of mosquito avoidance and control practices varied among them. The new students need early education about the disease and should be encouraged to avoid mosquito bites and control of mosquito vectors.

448276

Evidence Of West Nile Virus Circulation In Lebanon

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West Nile virus (WNV) has never been reported from Lebanon. Yet, this country is located on the flyway of migratory birds in the Middle East region. Serological screening was conducted to assess the potential circulation of this virus. Human, horse, and chicken sera were collected from the Bekaa and North districts. Specific IgG and IgY were first screened by ELISA. Then, positive samples were confirmed by plaque reduction neutralization test (PRNT). Moreover, adult mosquitoes were collected and tested for the presence of WNV RNA using conventional RT-PCR. Sera screening revealed a seroprevalence rate reaching 1.86% among humans and 2.47% among horses. Cross-reactions revealed by ELISA suggested the circulation of other flaviviruses including Dengue virus. None of the tested mosquitoes was positive for WNV. The observed results constitute strong evidence of local exposure of the Lebanese population to this virus and the first report of equine WNV in Lebanon

444332

Towards The Development Of New Surveillance Approach Of Tick Borne Encephalitis Virus

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Tick-borne encephalitis (TBE) is recognized as the most important zoonotic tickborne viral disease in Europe. Causative agent of the disease is typically transmitted to human beings through bites of TBEV-infected ticks. However, alimentary infection after consumption of unpasteurized milk from domestic ruminants has previously been reported.
In the present study we investigated a total of 2087 ruminant milk samples collected in 2018-2019 from systematically selected farms in various municipalities of Lithuania. All milk samples were collected once every four days from bulk tank throughout lactation period.
Samples were analyzed by PCR using primers targeting NS5, NCR5 and E regions. Virus was isolated in Neuro-2a, Vero and MARC-145 cell lines and serial passages were performed to assess its viability to infect cells and obtain sufficient number of viral copies necessary for detection by molecular methods. Positive samples were confirmed by genome sequencing. Overall 4.54% [95% CI 3.54 – 5.53] of samples were positive for TBE virus. Spatio-temporal analysis showed majority of farms being affected by TBE causative agent, indicating widespread geographical distribution covering all major districts of the country. Farm and animal-level factors influencing the occurrence of the virus have also been identified. Simultaneously, ticks were collected in aforementioned municipalities by traditional flagging method, resulting in 0.37% [95% CI 0,14-0,76] of positive pools, which indicates ticks as potential source of animal infection. However, positive tick samples were located only in few endemic regions, which indicate milk sample usage as potential strategy for large scale monitoring of TBE virus.

447588

Cache Valley Virus And Jamestown Canyon Virus Surveillance, Diversity, And Vector Competence

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Cache Valley virus (CVV) and Jamestown Canyon virus (JCV) are mosquito-borne pathogens belonging to the genus Orthobunyavirus (Family Bunyaviridae). Although the majority of exposures to these viruses result in asymptomatic or mild infections both JCV and CVV can cause neurological diseases. Both viruses are widely distributed across North America and infect a number of vertebrate hosts and mosquito. We report mosquito's surveillance results of CVV and JCV from 2000- 2020 in New York State. Cache Valley virus was isolated from 18 mosquitoes belonging to 7 genera while JCV was isolated from 12 mosquito species belonging to 5 genera. Infection rates by the maximum likelihood estimation method were calculated by year, mosquito species and region. The highest MLE's were from Anopheles and Aedes mosquitoes for CVV and Ochlerotatus and Anopheles mosquitoes for JCV. In addition, we showed that An. quadrimaculatus are competent vector CVV but not for JCV; however Ae. albopictus mosquitoes are competent for both viruses.

447551

Zika Virus Infection Results In Biochemical Changes Associated With RNA Editing, Inflammatory And Antiviral Responses In Aedes albopictus

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Rapid and significant range expansion of both the Zika virus (ZIKV) and its Aedes vector species has resulted in the declaration of ZIKV as a global health threat. Successful transmission of ZIKV by its vector requires a complex series of interactions between these entities including the establishment, replication and dissemination of the virus within the mosquito. The metabolic conditions within the mosquito tissues play a critical role in mediating the crucial processes of viral infection and replication and represent targets for prevention of virus transmission. In this study, we carried out a comprehensive metabolomic phenotyping of ZIKV infected and uninfected Ae. albopictus by untargeted analysis of primary metabolites, lipids and biogenic amines. We performed a comparative metabolomic study of infection state with the aim of understanding the biochemical changes resulting from the interaction between the ZIKV and its vector. We have demonstrated that ZIKV infection results in changes to the cellular metabolic environment including a significant enrichment of inosine and pseudouridine (Ψ) levels which may be associated with RNA editing activity. In addition, infected mosquitoes demonstrate a hypoglycemic phenotype and show significant increases in the abundance of metabolites such as prostaglandin H2, leukotriene D4 and protoporphyrinogen IX which are associated with antiviral activity. These provide a basis for understanding the biochemical response to ZIKV infection and pathology in the vector. Future mechanistic studies targeting these ZIKV infection responsive metabolites and their associated biosynthetic pathways can provide inroads to identification of mosquito antiviral responses with infection blocking potential.

440278 3min Student

Incidence Of Sindbis Virus In Hospitalised Patients With Acute Fevers Of Unknown Cause In South Africa From 2019 To 2020

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Background: Sindbis virus (SINV) is a mosquito borne alphavirus that is widely distributed worldwide. Little is known about the disease burden due to SINV in Africa, despite mild cases annually reported in South Africa.
Patients and methods: Clinical samples of patients with acute fevers of unknown cause with or without neurological signs (AFDUC) were collected through the African Network for Improved Diagnostics, Epidemiology and Management of Common Infectious Agents (ANDEMIA) at 3 sentinel surveillance sites at Kalafong hospital, Gauteng Province and Matikwane and Mapulaneng hospitals in Mpumalanga, South Africa.
In total 506 specimens (2019) and 374 specimens (2020) from 639 patients were submitted and screened using a PCR based macroarray (fever chip) that can simultaneously detect nucleic acids of 30 pathogens, including SINV. A commercial indirect immunofluorescence assay (Euroimmun, Germany) was used to detect SINV IgM in serum or plasma specimens, (115/223 patients in 2019 and 82/155 patients for 2020) and 40 CSF specimens, randomly selected from January-June each year. Micro-neutralization assays (micro-VNT) were performed on all IFA-positive samples and the incidence calculated.
Results: No specimens tested positive by PCR for SINV over the period. Overall, 38/197 (19.0%) samples were positive for SINV IgM. A total of 25/38 (65.8%) IgM positive samples tested positive for SINV neutralizing antibodies, giving an overall incidence of 12.7%. A total of 3/40 (7.5%) CSF specimens tested positive by IFA but tested negative for neutralizing antibodies. Multi-variables analyses indicated a significant association in March OR = 0.33 and April OR = 2.68 (p Conclusion: SINV contributed to 12.7% of AFDUC cases in hospitalised patients during late summer and autumn months in South Africa. Further investigation of the association with neurological cases are needed.

445747 3min Student

Chikungunya Virus Time Course Infection Of Human Macrophages

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Chikungunya virus (CHIKV) is an Alphavirus spread by Aedes spp. mosquitoes and is responsible for infecting 1.1 million people per year worldwide, including a large epidemic in the western hemisphere in 2014-2015. CHIKV infection generally causes acute disease with clinical symptoms including fever, joint pain, headache, muscle pain, joint swelling, and rash resolving after approximately one week. However, a subset of patients can develop chronic arthralgia and myalgia causing debilitating pain for weeks, months, or years, affecting the livelihood of the patient. During the body's immune response to CHIKV, human macrophages become infected after phagocytosis of CHIKV and undergo induced apoptosis, catalyzing the virus spread in the body. It is presently unclear what macrophage genes, functions, and intracellular signaling pathways are impacted during the early, intermediate, and late stages of CHIKV infection. Therefore we quantified the transcriptional response of human macrophage cells infected with CHIKV at two different timepoints. We collected RNA samples from either mock-infected or infected monocyte-derived macrophages at 24 or 48 hours after infection. Later RNA-sequencing was performed on an Illumina NovaSeq instrument for the three sets of duplicate samples. We then subjected the RNA-sequencing data to statistical analyses to identify differentially expressed genes, enriched functional annotations, and modulated intracellular signaling

pathways. Our results indicated three pathways that are impacted at the 24h timepoint and 235 pathways that are impacted at the 48h timepoint. Afterward, we then cross-referenced the proteins within each pathway against the OpenTargets database of known drug targets. Our ranked results indicated that sunitinib modulates the cytokine-cytokine receptor interaction at 24h and the Ras signaling pathway at 48h, while Telmisartan modulates the AGE-RAGE signaling pathway at 48h. These potential therapeutic drugs will require validation experiments to augment ongoing efforts to develop an effective prophylactic or therapeutic treatment for CHIKV.

445249 3min Student

First Detection Of Lymphocytic Choriomeningitis Virus From Iraq

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Abstract Zoonotic diseases are infectious diseases that are naturally transmitted from vertebrate animals to humans and vice versa. They represent a global threat to public health. Rodents are the most significant reservoirs for zoonotic pathogens that might be transmitted to humans, of which arenaviruses and hantaviruses are the most common. In this study, we attempted to estimate the disease burden of rodentborne viral infections in Iraq, in which rodent-borne pathogens poorly studies. Lymphocytic choriomeningitis virus (LCMV) can cause acute fatal disease in all continents but was never detected in Iraq. In this study, 261 serum samples and 40 CSF samples were collected from the Nasiriyah region, Dhi Qar Governorate, southern Iraq. The samples were screened by immunofluorescence assay (IFA) for LCMV IgG and IgM antibodies. The viral RNAs were extracted from acute infection samples (serum and CSF) (140 µL/sample) using a QIAamp Viral RNA Mini kit. Then, a pan-arena reverse transcription PCR (RT-PCR) using SuperScript II OneStep RT-PCR system with Platinum Taq High Fidelity. RT-PCR products were sequenced using next-generation sequencing (NGS). Here, we reported the first detection of LCMV seroprevalence (8.8%) and RNA in acute febrile patient samples in Nasiriyah city in Iraq. LCMV IgM was found in 2 serum samples (2/171) derived from patients with acute febrile illness; both serum samples were negative for LCMV IgG and LCMV RNA. Notably, we detected two unique LCMV variants (GenBank accession nos. MT093202 for CSF_sample_11_Iraq_2012 and MT093203 for CSF_sample_64_Iraq_2012) in cerebrospinal fluid samples of Iraqi patients with neurological symptoms. Collectively, the seroprevalence and detection of acute infection provide evidence that LCMV circulates in southern Iraq, and it is causing infections leading to acute neurologic manifestations in the population. More sequence data are needed to extend the knowledge on the molecular epidemiology and evolution of LCMV.

Zoonotic Viruses: Molecular Virology

440400

Filovirus VP24 Proteins Differentially Regulate Rig-I And MDA5-dependent Type I And III Interferon Promoter Activation

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Filovirus family consists of highly pathogenic members that have caused fatal outbreaks especially in many African countries. Previously, research focus has been on ebolaviruses (EBOV) and marburgvirus (MARV) leaving other filoviruses less well studied. Filoviruses, in general, pose a significant global threat since they are highly virulent and potentially transmissible between humans causing sporadic infections and local or widespread epidemics. Filoviruses have the ability to downregulate innate immunity, and especially viral protein 24 (VP24) and VP35 have variably been shown to interfere with interferon (IFN) gene expression and signaling. Here we systematically analyzed the ability of VP24 proteins of nine mammalian filovirus family members to interfere with retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated antigen 5 (MDA5) induced IFN-β and IFN-λ1 promoter activation. VP24 proteins of Zaire and Sudan EBOV, Reston and Llovio viruses (ZEBOV, SUDV, RESTV and LLOV, respectively) were found to inhibit both RIG-I and MDA5 stimulated IFN-λ1 promoter activation. The inhibition takes place downstream of interferon regulatory factor 3 (IRF3) phosphorylation suggesting the inhibition to occur in the nucleus. VP24 proteins of Bombali, Bundibugio, Thai forest, Marburgvirus and Mengla dianlovirus (BOMV, BDBV, TAFV, MARV, and MLAV, respectively) did not inhibit IFN-λ1 promoter activation. Interestingly, the ability of VP24 proteins to inhibit RIG-I/MDA5 signaling did not fully correlate with the nuclear localization of VP24s. Six ebolavirus family VP24s and Llovio VP24 tightly bound to importin α5, the subtype that regulates the nuclear import of IFN activated STAT1- STAT2 complexes. Our data provides new information on the innate immune inhibitory mechanisms of filovirus VP24 proteins, which may contribute to the pathogenesis of filovirus infections.

441377

The Diverse Structural Landscape Of The Flavivirus Antibody Repertoire

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Flaviviruses are emerging arthropod-borne, lipid-enveloped, single-stranded RNA viruses, causing a broad spectrum of life-threatening disease symptoms such as encephalitis and hemorrhagic fever. The most important disease-causing human flaviviruses are yellow fever virus, dengue virus, West Nile Virus, Japanese encephalitis virus, Tick-borne encephalitis virus and Zika virus. During the last 70 years, flaviviruses caused several epidemics, which include dengue virus, West Nile virus, and most recently the explosive epidemic of Zika virus in the Americas. According to CDC, up to 400 million people get infected with dengue virus annually. The potential of flaviviruses to sustain epidemic transmission is poorly understood and the global threat is of significant concern. Successful vaccines exist against YFV, JEV and TBEV. However, vaccine development against other flaviviruses like dengue virus is not straightforward. This is partly because of the high sequence conservation and immunological cross-reactivity among flavivirus envelope glycoproteins leading to antibody mediated enhancement of disease (ADE). Thus, understanding the immune response in consecutive flavivirus infections and virus neutralization mechanisms by various classes of neutralizing antibodies (nAbs)

may help to prevent disease severities leading to ADE, which is a major risk factor for vaccine development. The three dimensional structures of the mature and the immature flaviviruses and their complexes with nAbs, unfolded the structural components important for infection and neutralization mechanisms. These nAbs might inhibit several processes during infection including receptor binding and blocking conformational changes required for fusion of virus-host membranes. A comprehensive analyses of the structural landscape of humoral immune response against flaviviruses is crucial for antigen design. Here, we compare the available structural data of several flavivirus antibody complexes with a major focus on Zika virus and dengue virus and discuss the mapped epitopes, the stoichiometry of antibody binding and mechanisms of neutralization.

440960

Identification Of Flavivirus Nucleocapsid Core-envelope Glycoprotein Interactions

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Although flaviviruses (a family of over 90 known viruses, including Zika (ZIKV) and dengue) have been widely studied for several decades, the mechanisms by which the core and envelope of these viruses assemble to form infectious virions remain poorly understood. Virus assembly is an essential step in the life cycle of flaviviruses and represents a key target for antiviral therapeutics. Single particle Cryo-EM reconstruction has shown that the nucleocapsid core (NC) of immature Zika virus is found in proximity with the envelope glycoproteins on the inner side of the virus's lipid bilayer. We hypothesized that the NC interacts with the envelope glycoproteins during virus assembly via interactions between the capsid proteins (CP) of the NC and transmembrane helices of the precursor membrane (prM) protein or the envelope (E) protein. Two strategies to identify NC-prM/E interactions are being explored using infectious dengue virus serotype 2 (DENV2) and ZIKV molecular clones, and pseudo-infectious ZIKV reporter virus particles. Firstly, amino acids within the prM/E transmembrane helices posited to be key in the viral assembly process were mutated in all three virus systems to investigate their role in promoting particle assembly. The ability of these mutants to form infectious particles was experimentally determined using viral plaque assays, ELISA assays, flow cytometry, and electron microscopy. Secondly, the ability of the prM and E transmembrane helices to interact with CP is being examined using reconstituted prM and E proteins within styrene-maleic acid lipid nanoparticles. Our results identify mutations within the prM and E protein transmembrane helices that do indeed disrupt infectious flavivirus particle formation. Due to the high structural similarity among flaviviruses, the techniques, results and mechanisms identified in this study have the potential to be applied to other flavivirus infections.

440834

The Proprotein Convertases Of Aedes Aegypti: Characterization And Role During Flavivirus And Alphavirus Infection

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Mosquitoes have been attributed as being the most dangerous animal on earth because they transmit viruses and other pathogens that are medically relevant. For instance, the flaviviruses, which include dengue, pose a threat to millions of people annually. Flaviviruses are initially assembled as immature viral particles in the endoplasmic reticulum, and then enter the secretory pathway where they undergo a maturation process facilitated by the host protease furin. Furin, which belongs to the family of

proprotein convertases, cleaves at a poly-basic residue in the pr-M junction that renders the virus infectious. In contrast, alphaviruses such as Sindbis, furin processes p62 into E3-E2 prior to assembly, rendering the virus more labile to pHmediated fusion and entry. The protease(s) involved in this cleavage have not been characterized in mosquitoes. Here, we identified three genes in the AagL5.0 genome of Aedes aegypti: AaFurin1, AaFurin2 and AaNC2. These proteases displayed canonical features of proprotein convertases: Pro-segment, Catalytic domain and Pdomain. We produced recombinant protein(s) in Drosophila S2 cells and performed cleavage activity assays, which showed that AaNC2 is not active. However, AaFurin1 exhibited activity comparable to human furin but significantly higher than AaFurin2. Further, CRISPr knockouts in Aag2 cells revealed that AaFurin1 but not AaFurin2 is required for efficient cleavage of pr-M in dengue-2 and Zika, as well as p62 in Sindbis virus. Reduced proteolytic cleavage is also reflected in reduced viral titer. Combined, these experiments suggest that AaFurin1 has a role in the proteolytic cleavage of different families of arboviruses in mosquitoes and is associated with their maturation and infectivity.

441396

High Resolution Structures Of A Mature Flavivirus Render New Insights For Flavivirus Biology

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In 2002, the 24 Å structure of the mature dengue virus first revealed the arrangement of the structural proteins, the envelope glycoprotein (E) and the membrane protein (M) embedded in the lipid bilayer in the flavivirus particle. Similar organization of the
structural proteins was subsequently described in other flaviviruses, such as Zika, Dengue and Japanese encephalitis virus, at resolutions between 4.5-3.1 Å; however, structural details of the amino acid residues and their interactions with lipids remained elusive till recently. Our research on understanding the pathogenesis of the Usutu virus (USUV), a flavivirus belonging to the JEV serogroup, led to the determination of two structures of the mature USUV at a resolution of 2.4 Å; these are the highest resolution structures of a mature flavivirus solved to-date using singleparticle cyro-electron microscopy. Together, these USUV structures uncover new details in two areas of discovery: the high-resolution details of a mature flavivirus particle as well as the unique features of USUV. We identified three lipid-interaction sites in USUV, two in the E-stem region and one in the transmembrane region. The former reveal electron potential density for the lipid headgroup, with one site showing continuous density between the E protein and the lipid moiety; this site was not observed in the recently published structure of the mature Spondweni virus (2.6 Å resolution). The third site, present distinctly in one USUV structure and monomer, reflects both the lipid flexibility and asymmetric features between the structures. A second major finding is the asymmetric presence of a disulfide bond in the fusion loop, present in one of the structures but not the other, suggesting the presence of two subpopulations of the virus in solution. Since these features are relevant for flavivirus membrane fusion and therapeutics, the USUV structures provide new information for flavivirus biology.

445696

Molecular Mechanisms Of Inhibition Of Tick-borne Encephalitis Virus By Monoclonal Antibodies

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Tick-borne encephalitis (TBE) is a potentially lethal neuroinfection in humans, caused by TBE virus (TBEV), a member of genus Flavivirus, family Flaviviridae. The disease is prevalent in forested areas of Europe and northeastern Asia. Antibodies play an important role in control of TBEV infection, but the mechanisms of antibodymediated TBEV neutralization remains largely unknown. We determined cryo-EM structures of the native TBEV virion (strain Hypr, European TBEV subtype) and its complex with Fab fragments of a neutralizing antibody at nearatomic resolution. Unlike most of the previously studied flavivirus-neutralizing antibodies, the Fab fragments did not lock the E-proteins in the native-like arrangement, but prevented the virus proteins from inducing membrane fusion in the endosome and releasing the viral nucleocapsid into the cytoplasm. Analysis of human antibody response to TBEV infection or vaccination revealed that expanded clones of memory B cells expressed closely related anti-envelope domain III (EDIII) antibodies in both cohorts, but the most potent neutralizing antibodies were found only in individuals who recovered from natural infection. These antibodies also neutralized other tick-borne flaviviruses. Structural analysis revealed a conserved epitope near the lateral ridge of EDIII adjoining the EDI-EDIII hinge region. Prophylactic or early therapeutic antibody administration was effective at low doses in mice that were lethally infected with TBEV. Antibody-resistant TBEV mutants were generated and characterized. The mutants had amino acid substitutions in EDIII, were replication impaired, showed reduced growth and a small plaque size in mammalian cell culture and reduced levels of neuroinvasiveness in rodent models compared to the wild-type TBEV. The results demonstrate the importance of critical sites within the EDIII as determinants of virus virulence.

439557 3min News

Genetic And In Vitro Biological Characterization Of Tick-borne Tribec Virus (Orbivirus)

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TribeC virus (TRBV) is a zoonotic tick-borne orbivirus transmitted mainly by Ixodes spp. ticks. The genome of TRBV is encoded in 10 segments of dsRNA. TRBV is serologically relative to Kemerovo virus (KEMV) and Lipovník virus (LIPV) and is considered as serotypes of the Great Island virus. Despite the presence of TRBV antibodies in neurological human patients, its role in the pathogenesis is unknown. Here, the genetic characterization of a novel TRBV strain (16.C/16/Dúbrava/PO/SVK) and its replication kinetics in cell culture model was studied. The virus was isolated in Vero E6 cells from an engorged Ixodes ricinus collected from a goat. Each segment was sequence analysed and the proteins of the novel TRBV isolate were compared with other tick-borne orbiviruses. The closest viruses were the original TRBV and LIPV strains isolated in the 1960s in Slovakia and the recently isolated Ukrainian TRBV strains, where the overall similarity of the genome segments ranged 73.6-99.7%. KEMV strains were identical in a range of 59.6-91.0%.
The novel isolate was serially passaged (n=27) in Vero E6 cells. The replication kinetics of low (3.p) and high (27.p) passage viruses was studied by the 1-step growth curve in monkey Vero E6 and human A549 cell lines. Cells were infected with MOI=1 for 1 hour then 3-times washed with medium. Intra- and extracellular virus amount was measured by plaque-formation test in Vero E6 cells 2, 4, 6, 8, 12, 24 and 32 hours post adsorption (hpa). Differences in the time of intra- and extracellular infectious virus progeny production and its amount was noted in both mammalian cell lines. Infectious virus progeny appeared 6-8 hpa in the 27.p TRBV and the 8-12 hpa in 3.p TRBV. The amount of overall virus progeny of the 27.p TRBV reached 2.97×105 PFU/ml and 8.18×102 PFU/ml of the 3.p TRBV.

441257 3min Student

Role Of Filovirus VP24 Proteins On Interferon Induced Pathway In Innate Immunity

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Innate immunity is a non-specific yet powerful mechanism of counteracting pathogenic organisms in the bodies of both vertebrate and non-vertebrate organisms. One of the most important aspect of innate immunity is the production of cytokines which regulate anti-viral responses of both the infected as well as uninfected cells. Interferons are cytokines which play a major role in fighting viral infections. Filoviruses belong to the Filoviridae family and are responsible for a variety of diseases in humans. Filovirus encoded proteins can interfere with the interferon induced pathway (JAK/STAT pathway) in cells through several mechanisms. To analyse how the VP24 proteins of nine different mammalian filovirus members interfere with IFN signalling, expressed all nine VP24 proteins and monitored their effect on IFN-induced activation of MxA and IFITM3-promotors. Our results show that despite differential sub-cellular distribution, all nine VP24 proteins interfere with IFN-induced pathway and downregulate MxA and IFITM3 promoter activation. Our study provides new information on filovirus – host cell interactions on host innate immune responses on IFN-induced pathway

445526 3min Student

Genomic And Structural Characterization Of Dobrava-belgrade Orthohantavirus Isolate From Kirklareli Province, Turkey

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ABSTRACT Orthohantaviruses are transmitted to humans mostly through small mammals which are also the reservoirs of these viruses. Because orthohantaviruses show great genetic variability through regions, it is of importance to characterize their full genomes in different parts of the world. The aim of this study was the whole genome sequencing of DOBV Igneada strain from Turkey, which was performed by Illumina MiSeq directly from the rodent tissues. Furthermore, phylogenetic analysis was performed with the sequences of different DOBV strains to determine the differences of DOBV Igneada strain. Finally, viral proteins' structures of DOBV Igneada strain were modelled in order to see the effect of the amino acid changes identified in the sequence. As a result of this study, the whole genome sequence of DOBV Igneada strain was obtained from Turkey, from where no full genomes have been previously reported for DOBV. The complete sequences of all three segments were obtained directly from the lung tissue. The phylogenetic analysis showed that there is a close relation between the DOBV Igneada strain and the Ano-Poroia strain. SimPlot analysis of three segments also showed the similarities between DOBV Igneada strain and other DOBV strains from the Balkans such as Greece, Croatia, and Slovenia. Furthermore, the protein modelling of the structural proteins revealed that, even though there are some amino acid changes between two strains' models, there are not any conformational change on the proteins. In conclusion, while full orthohantavirus genomes

now can be successfully amplified and sequenced directly from the rodent tissues, which will enhance the understanding of the variability of orthohantaviruses in different regions and reservoir hosts, there are only a few available full genomes from the tissues for orthohantaviruses. Therefore, studies like this one will guide the further research to increase the number of available full orthohantavirus genomes from rodent tissues.

455179 3min student

Assessment of Anti-Chikungunya Potency of Thymoquinone, a Natural Drug Compound Targeting the Hydrophobic Pocket of Capsid

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Chikungunya virus (CHIKV) is a re-emerging alphavirus and a potential threat to global public health. Capsid protein (CP) of alphaviruses is a multifunctional protein with a conserved hydrophobic pocket that plays a crucial role in the viral life cycle, particularly in capsid assembly and virus budding process. CPs are emerging and promising antiviral targets against various viral diseases for developing new potent inhibitors in recent years. The present study demonstrates the antiviral activity of thymoquinone (TQ), a natural compound targeting the hydrophobic pocket of CP. Structure-based molecular docking of TQ to the conserved hydrophobic pocket indicates its high binding affinity towards CP. TQ binding to the hydrophobic pocket of purified CHIKV CP was validated by isothermal titration calorimetry and fluorescence spectroscopy experiments. The binding constant KD value obtained by ITC was 27 μM. Additionally, cell-based antiviral studies revealed that TQ diminished CHIKV replication with an EC50 value of 4.478 μM. Diminution in viral RNA copy number and viral replication as assessed by qRT-PCR and immunofluorescence assay, respectively, confirmed the antiviral activity of TQ against CHIKV. Furthermore, the time of addition and the time of elimination experiments indicated that TQ reduced the viral titer at the late stage of CHIKV life cycle. Our study demonstrates that TQ is a potential antiviral that targets the hydrophobic pocket of CHIKV CP and may serve as the basis for the development of a broad-spectrum antiviral therapy against emerging alphaviral diseases.

455357 3min student

Targeting Stress Response Pathway for Antiviral Therapy Against Chikungunya

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The periodic recurrence of viral infections and emergence of challenging variants has created an urgent need of alternative therapeutic approaches to control spread of viral infectious diseases, failing to which may pose a greater risk to mankind in future. Many viruses infect human body but our immune system controls majority of these viruses by generating antiviral innate immunity against viral infections. Innate responses work through various host factors and cellular mechanisms such as lipid/glucose/nucleotide metabolism, inflammatory &signalling cascades, stress response pathway etc which collectively cease the viral infection. Stress response proteins and formation of stress granules is an active defense pathway in response to + ss RNA viral infection. However, RNA viruses modulate

stress pathway at different levels of SG formation. Human G3BP protein (Ras GTPase-activating protein-binding protein) can initiate SG formation and its active role has been shown in ss various + ss RNA viruses such as SFV, SINV, CHIKV, SARS-CoV-2 etc .Therefore, viruses sequester major players and utilize these pathways for their own replication and progression of disease. This study focuses on targeting G3BP1 protein which plays important role in alphavirus infectivity including Chikungunya virus. Non-structural proteins of alphaviruses act as host defence modulator that functions by disrupting stress granules formation. The disruption mechanism is through nsP3-mediated recruitment of human G3BP1 protein via two tandem FGDF motifs. Small molecules targeting molecular G3BP-nsP3 interactions are expected to block G3BP interaction with the viral partner resulting in viral attenuation. In this study, virtual screening, docking and simulation has identified potential antiviral molecules, Binding kinetics uisng biophysical techniques verified binding of these to target molecule. Further, the in vitro cell-based antiviral testing of these compounds shows effective antiviral activity against CHIKV.

Animal Viruses: Molecular Virology

441042

The Capsid Structure Of Acheta Domestica Segmented Densovirus, A Novel Parvovirus With A Bipartite Genome, Reveals A Unique Surface Morphology And Potential DNA Packaging Strategy

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Parvoviruses (family *Parvoviridae*) are united by their small, monopartite ssDNA genomes, which are flanked by hairpin-like DNA secondary structural elements. Genome segmentation is uncommon among DNA viruses, as opposed to their RNA packaging counterparts. Recently, we discovered a novel parvovirus infecting the common house cricket (*Acheta domestica*), which possessed a bipartite genome of 3.3-kb-long segments, encoding either non-structural proteins (NS segment) or structural proteins (VP segment). Acheta domestica segmented densovirus (AdSDV) is a potential new member of genus *Brevihamaparvovirus* (subfamily *Hamaparvovirinae*), yet capable of expressing a protein (VP ORF3) with a phospholipase A2 (PLA2) domain, possibly due to horizontal gene transfer from genus *Scindoambidensovirus* of subfamily *Densovirinae*. The PLA2 is responsible for lysosomal egress during intracellular trafficking of most parvoviruses. Here, we show that AdSDV assembles two different capsids during its natural course of infection, differing in buoyancy and genome packaging capability, as well as in the incorporation ratio of its two capsid proteins (VP1 and VP2). Neither of them, however, incorporates copies of the PLA2-including VP ORF3. We report the nearatomic 3D capsid structure, determined by cryo-electron microscopy and image reconstruction, of both AdSDV capsids at 2.1 and 2.3 Å, respectively, along with the structure of a recombinantly-expressed virus like particle (3.3 Å), assembled exclusively from products of VP ORF1, a homologue of Brevihamaparvovirus structural proteins. AdSDV displays a surface morphology unique among parvoviruses and possesses multimer interactions, which suggests a previously unanticipated diversity in structural aspects of the Parvoviridae, previously believed to be conserved. Our findings

suggest a formerly undescribed genome packaging model for AdSDV, linked to the threefold symmetry axis instead of the channel and pore at the fivefold axis. AdSDV is the first parvovirus with a segmented genome, hence characterization of its capsids provides important structural insights into the evolution of DNA virus genome segmentation.

445733

Activation Of AKT During Murine Norovirus Infection Is Important During Late Viral Stages

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Akt (Protein kinase B) is a key signaling protein in eukaryotic cells that controls many cellular processes such as glucose metabolism, cell proliferation, and survival. As obligate intracellular pathogens, viruses modulate host cellular processes, including Akt signaling, for optimal replication. The mechanisms by which viruses modulate Akt and its effect on the infectious cycle differ widely depending on the virus. In this study, we explored the effect of Akt phosphorylation (p-Akt) on serine 473 residue during murine norovirus (MNV) infection. Akt phosphorylation increased during infection of murine macrophages with acute MNV-1 and persistent CR3 and CR6 strains. Inhibition of Akt with MK2206, an inhibitor of all three isoforms of Akt1/2/3, reduced infectious virus progeny with all virus strains. This reduction was due to defects in virus assembly (MNV-1) or egress (CR3 and CR6) in a virus straindependent manner. Collectively, our data demonstrate that MNV activates Akt to promote late steps in the viral infectious cycle. These data for the first time indicate a role for Akt signaling in virus assembly and highlight additional phenotypic differences between closely related MNV strains.

445435

Expression Profile Of Toll-like Receptors In Koala (Phascolarctos cinereus) Peripheral Blood Mononuclear Cells Infected With Multiple KoRV Subtypes

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Toll-like receptors (TLRs) are evolutionarily conserved pattern recognition receptors that play an important role in innate immunity by recognizing microbial pathogenassociated molecular patterns. Koala retrovirus (KoRV), a major koala pathogen exists in both endogenous (KoRV-A) and exogenous forms (KoRV-B to J). However, the expression profile of TLRs in koalas infected with KoRV-A and other subtypes is yet to be characterized. Here, we investigated TLR expression profiles in KoRV-A only and KoRV-A with KoRV-B and/or -C infected koalas. To do this, we cloned partial sequences for TLRs (TLR2–10 and TLR13), developed real-time PCR assays, and determined the expression patterns of TLRs mRNA in koala PBMCs and/or tissues. All the reported TLRs for koala were expressed in PBMCs, and variations in TLR expression were observed in koalas infected with exogenous subtypes (KoRV-B and KoRV-C) compared to the endogenous subtype (KoRV-A) only suggesting the implications of TLRs in KoRV infection. TLRs were also found to be differentially expressed in koala tissues. This is the first report of TLR expression profiles in koala, providing insights into koala's immune response to KoRV infection that could be utilized for the future exploitation of TLR modulators in the vaccine study.

441263

Molecular Characterization Of Lumpy Skin Disease Virus Isolates From 2019 LSD Outbreaks In Cattle In India

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Background and Aim: Lumpy skin disease (LSD) is an economically important transboundary viral disease of mainly cattle and occasionally buffalo. LSD is caused by lumpy skin disease virus (LSDV), belonging to the genus Capripoxvirus (CaPV) in the family Poxviridae. LSD has recently expanded its geographical range into South, East and South East Asia. LSD emerged in cattle in India in August 2019, first in the Odisha State, and then spread to other regions. To gain insights into LSDV introduction and molecular epidemiology in India, we determined phylogenetic relationships of LSDV isolates (n=12) from 2019 outbreaks in Odisha and West Bengal States, based on the Gprotein coupled chemokine receptor (GPCR) gene sequences.
Materials and Methods: Twenty two skin biopsies from cattle in 7 districts, tested positive for LSDV by realtime PCR, were subjected to virus isolation using primary lamb testis cells. Twelve LSDV isolates obtained were then characterized genetically. The full-length GPCR gene (1146 bp) was amplified, sequenced and the genetic and phylogenetic analyses were performed.
Results: The phylogenetic analysis revealed that the Indian LSDV isolates from 2019 outbreaks are very closely related to the historical Kenyan NI-2490/Kenya/KSGP-like strains and field strains (2019) from Bangladesh at both nucleotide (99.7-100%) and amino acid (99.2-100%) level indicating a common source of introduction of LSDV. In contrast, other field strains circulating in Africa, Middle East and Europe and vaccinelike LSDV from China and Russia clustered separately. Importantly, absence of 12- nucleotide deletion was evident in LSDV strains from India and Bangladesh has critical implications in GPCR based DIVA diagnostic assays.
Conclusion: The LSDV strains from 2019 outbreaks in India and Bangladesh are very similar and closely related with the historical Kenyan NI-2490/Kenya/KSGP-like field strains in GPCR. The findings here demonstrate change in nature of globally circulating LSDV field strains, highlighting the importance of molecular characterization of LSDV strains for developing effective diagnostic and control strategies against LSD.

445226 3min Student

The Multiple Roles Of Canine Parvovirus Nonstructural Protein 2

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Autonomous parvoviruses encode two nonstructural proteins, NS1 and NS2. While NS1 is linked to critical nuclear processes required for viral replication, much less is known about the role of NS2. Specifically, the function of canine parvovirus (CPV) NS2 has remained undefined. Here, we have used mass spectrometry-based proximity-dependent biotin identification (BioID) to identify proteins associated with nuclear CPV NS2. Most of the identified proteins were seen in both noninfected and infected cells. However, the location of interacting proteins shifted from nuclear envelope proteins to chromatin-associated proteins in infected cells. BioID highconfidence interactions revealed a potential role for NS2 in DNA remodeling and damage response. NS2 was also found to be associated with essential proteins that control the splicing, binding, and nuclear pore complex docking of mRNA. Further protein-protein interaction analysis by proximity ligation assay confirmed the nuclear interactions of NS2 with selected key proteins identified by BioID analysis. Mutations to the NS2 splice

donor and acceptor sites affected chromatin organization and the amount and distribution of intact capsids and DDR-related proteins in infection. Additionally, mutation of the NS2 splice donor site led to the insufficient formation of autonomous parvovirus-associated replication (APAR) bodies. Our findings suggest that CPV NS2 might have previously undiscovered roles in the modification and egress of mRNA and the interplay with cellular machinery regulating chromatin functions, autonomous parvovirus-associated replication body formation, and DNA damage.

Veterinary Virology

445710

Bluetongue, A Journey From Virus To Vaccine: Indian Perspective

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More than a century ago, the disease Bluetongue (BT) was initially reported in South Africa, continues to be an economically important disease affecting susceptible domestic and wild ruminants in tropical, semi-tropical and temperate regions around the globe to date. The disease has been listed under 'notifiable diseases' by office OIE Global. Recently in 2011, BT has been categorized as multispecies disease by OIE. Because of the OIE status of the disease, mandatory restrictions are imposed on movement of ruminant animals, their germplasm, embryos and other animal products from BT endemic regions to BT free zones. The economic losses due to bluetongue virus (BTV) infection is mainly attributed to high morbidity, mortality, abortions, still-births, foetal abnormalities, meat and fleece loss. The first outbreak of BT in sheep and goats in the country was recorded in 1964 in Maharashtra State. Since then, several outbreaks of BT have been reported in sheep in almost all states of India. A serological survey has indicated the presence of bluetongue virus (BTV) antibodies in Indian cattle, buffalos, goats, camels and some wild ruminants. However, clinical BT has not been observed in cattle or buffalo to date, whereas in goats, there are sporadic reports of occurrence of clinical BT either alone or as coinfection with peste des pestis ruminants. . First time virus isolation was done in 1984, and claimed to have isolated serotypes 8 and 18 from affected sheep. Subsequently, serotype 1 was isolated from Rambouillet sheep from Central Sheep Breeding Farm (CSBF), in 1986 in Hisar, Haryana. Later on many other workers reported isolation of different BT virus serotypes (BTV2, 3, 9, 10, 16, 18, 21 and 23) from different parts of country. High prevalence of BT virus antibodies by AGID test in sheep, goats, cattle, buffalo and in camel sera but not in horse have been reported through epizootiological survey carried out in northern states of the country including Haryana, Punjab, Rajasthan, Himachal Pradesh and Jammu and Kashmir. Prevalence of bluetongue group specific antibodies was reported in elephants, black bucks and spotted deer in Tamil Nadu and in domestic and wild ruminants in and around Sariska Tiger Reserve, Rajasthan. In 1997 Culicoides oxystoma was identified as the potential vector for BT virus in the northern part of India. Recently, in a study carried out under Indo-UK collaborative project, still C. Oxystoma, has been reported as the predominant species followed by C. reconditus, C. imicola, C. pseudopalidipennis, C. Schultzei, C. peregrines and C. kubenesis. Bluetongue virus serotypes BTV1, BTV 2, BTV16, BTV23) have been isolated from cattle, sheep, goat and culicoides vectors confirmed by migration pattern of dsRNA in PAGE, RT-PCR and whole genome sequencing. The studies revealed that the Bluetongue virus isolates reported from vectors were similar to those isolated from hosts in the same vicinity. The viruses have the ability to undergo rapid evolution due to the segmented nature of the genome leading to frequent mutations. The sequence analysis of these serotypes revealed presence of both eastern as well as western topotypes and were of reassortant nature. Based on the surveillance of more than 15 years under ICAR funded 'All India Network Programme on Bluetongue (AINP-BT);

India's first ever inactivated pentavalent vaccine has been developed and the technology has been transferred for commercialization

442815

A Highly Effective Low Dose Live Attenuated Vaccine For Pandemic Strains Of African Swine Fever Virus Adapted To Grow Cell Cultures

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Animal; Clinical/applied virology,Epidemiology/evolution/taxonomy
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African swine fever virus (ASFV) causes a virulent, deadly infection in wild and domestic swine, and is currently causing a pandemic covering a contiguous geographical area from Central and Eastern Europe to Asia. No commercial vaccines are available to prevent African swine fever (ASF), resulting in devastating economic losses to the swine industry. The most advanced vaccine candidates are live attenuated strains developed using genetically modified virulent parental viruses. Recently we developed a vaccine candidate, ASFV-G-ΔI177L, by deleting the I177L gene from the genome of the highly virulent pandemic ASFV strain Georgia (ASFVG). ASFV-G-ΔI177L is safe and highly efficacious in challenge studies using parental ASFV-G. Large-scale production of ASFV-G-ΔI177L has been limited because it can only efficiently replicate in primary swine macrophages. Here we present the development of an ASFV-G-ΔI177L derivative strain, ASFV-G-ΔI177L/ΔLVR, that efficiently replicates in a stable porcine cell line. In challenge studies, ASFV-G-ΔI177L/ΔLVR maintained the same level of attenuation, immunogenic characteristics and protective efficacy as ASFV-G-ΔI177L. ASFV-G-ΔI177L/ΔLVR is the first rationally designed ASF vaccine candidate that can be used for large-scale commercial vaccine manufacturing.

445705

Tracing Immunogenic Changes During Piscine Novirhabdovirus Evolution In The Great Lakes

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Genogroup -IVb of the generalist Piscine novirhabdovirus, viral haemorrhagic septicaemia virus (VHSV), resulted in fatalities of >32 fish species in the Great Lakes since its appearance in 2003. The largest mortality outbreaks occurred in 2005 and 2006, followed by periods of apparent dormancy and punctuated smaller and more geographically-restricted outbreaks in 2007, 2008, and then in 2017. Many new haplotypes evolved, approximating a "quasi-species" evolutionary pattern. Whole genome sequence analysis evaluated changes in 48 VHSV-IVb isolates, showing 253 single nucleotide substitutions (2.3% of VHSV genome), with 85 being nonsynonymous. The greatest number of substitutions occurred in the non-coding region (4.3%), followed by the NonVirion (3.8%), and Matrix (2.8%) genes. A 2016 VHSVIVb isolate from Gizzard Shad (Dorosoma cepedianum) retrieved from Lake Erie was the most divergent (38nt/15aa). The in vitro pathogenicity and immunogenicity of three different 2016 Lake Erie isolates, including one from Gizzard Shad and two from Largemouth Bass (Micropterus salmoides), were compared to the original VHSV-IVb isolate, from Lake St. Clair Muskellunge (Esox masquinongy) in 2003 (C03MU). Isolates from 2016 exhibited milder cytopathogenicity when passaged in EPC cells, yielding less viral RNA at 48-72 hours post infection. Their phenotypic effects were studied in relation to profiles of host antiviral gene transcription, tracking

Type I Interferon (IFN) expression. All 2016 isolates tested produced an earlier and higher IFN transcription, compared to C03MU. Consistent with this transcriptional response, the 2016 Gizzard Shad isolate induced more IFN activity, in comparison to C03MU. Results suggest continued sequence change and lower virulence over the evolution of VHSV-IVb, which may facilitate its long-term persistence in the Great Lakes ecosystem. Nevertheless, VHSV may continue to comprise a threat for aquaculture and immunologically naïve wild fish populations in the Great Lakes region and other waterways, as it continues to mutate and evolve.

445449

Emerging Viral Diseases In Tilapia Farming Industry

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Tilapia aquaculture became one of the fastest-growing animal productions during the last decades. However, extensive aquaculture production and rapid change of environment have been associated with the emergence of viral diseases that cause serious economic impact to tilapia farmers. Such emerging viral diseases include Tilapia tilapinevirus (TiLV) and the more recently discovered Tilapia parvovirus (TiPV). Outbreak investigations of unusual mortalities of tilapia in Thailand since 2015 revealed that TiLV and TiPV are associated during co-infection patterns. High mortality between 50-90% are commonly observed in farmed Nile tilapia (Oreochromis niloticus) and red hybrid tilapia (Oreochromis spp.) associated with TiLV and TiPV infection. These newly identified viruses are very contagious, making them a great threat to the tilapia culture worldwide and raising global awareness. Given that tilapia is a common freshwater fish species culture throughout Asia and the rest of the world, further studies are needed to elucidate TiLV and TiPV genomic variance, pathobiology, development of efficient diagnostic assays, and to design adequate control measures aimed at reducing pathogen transmission, including vaccine against these emerging viruses.

442090 3min Student

A Study On In-Vitro Cross-neutralisation Assay For Identifying Antigenic Relationship Of Canine Parvovirus Types

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Canine Parvovirus (CPV) causing hemorrhagic gastroenteritis in dogs is much prevalent throughout India and worldwide. The virus comprises structural proteins (VP-1, VP-2 and VP-3) forming the capsid and two non-structural proteins (NS-1 and NS-2). VP2 is the immunodominant protein of the virus and major capsid protein responsible for various antigenic types of CPV including CPV 2, CPV 2a, CPV 2b and CPV 2c. Commercial vaccines for CPV are available but despite vaccination dogs are suffering from the disease. One reason for this is attributed to the difference in the antigenic types in vaccines and field strains. Therefore, it is utmost important to identify and understand the prevailing antigenic type/s of CPV in various geographical regions of India and to study the cross protectivity of the various antigenic types prevailing using cross-neutralisation assay. Methodology, results and discussions: The prevailing antigenic type/s of CPV in the northern region of India was already identified using real-time PCR (RT PCR). The samples available in the department were collected from Punjab (n=119), Assam (n=36), Delhi (n=11), Jammu (n=6) and Chandigarh (n=6). Cross-neutralisation assay: For this study CPV 2a and CPV 2b were produced in bulk in MDCK cell line, cytopathic changes were observed and virus was harvested from the inoculated cell line. The bulk virus was purified by ultra-centrifugation and the protein concentration of the purified virus was estimated by nanodrop method and found to be 1198bp and 548bp for CPV 2a and CPV 2b

respectively. The cell culture supernatant was also confirmed by PCR and nested PCR and the band for both CPV 2a and CPV 2b isolate was observed at 1198bp and 548bp respectively. The purified virus of both the CPV types was used to raise hyper immune serum in rabbits which was finally collected by intracardiac route. The antibody titre estimated by Indirect ELISA was found to be 99606.24 and 86940.95 for CPV 2a and CPV 2b respectively. The TCID50 of the purified virus was calculated by Reed and Muench formula. Four sets of serum-virus mixture were made for cross-neutralisation tests in which two fold serial dilution of serum and 100 TCID50 of each virus was used. TCID50 of the virus was found to be 103/ml for CPV 2a and 102.53/ml for CPV 2b. The maximum dilution at which serum against CPV 2a could neutralize CPV 2b virus was 1:4096 and the maximum dilution at which serum against CPV 2b could neutralize CPV 2a was 1:2048. The titre of serum 2a at which it can neutralize virus CPV 2b was 4096 and the titre of the serum 2b at which it could neutralize virus CPV 2a was 2048. The titre of both the serum for the homologous virus type was found to be higher than the former i.e. 8192.

445223 3min Student

Expression Of Major Immunogenic Glycoprotein GP5 Of Betaarterivirus SUID 2 (PRRSV2) in Prokaryotic System and Detection by SDS-PAGE and Western Blotting

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Betaarterivirussuid 2 is responsible for causing porcine reproductive and respiratory syndrome (PRRS), one of the most important swine diseases resulting in huge economic losses worldwide. The most immunogenic glycoprotein of this virus is GP5 which is an envelope glycoprotein. The present study was conducted with the aim to express the major immunogenic glycoprotein in the prokaryotic system using the pRham N-His SUMO Kan vector, which can be used for developing a diagnostic strategy. To amplify the whole GP5 gene from an isolate in the 2016 outbreak in Mizoram was selected and amplified the full-length GP5 gene. The amplified gene was gel purified and performed co-transformation and plated along with kanamycin antibiotic marker. After 16 hours of incubation, colony PCR was performed to confirm the positive clones. After the orientation study, one of the clones was selected for autoinduction in the presence of sugar glucose and rhamnose for 4 hrs and 8 hrs. Cell lysates were prepared by ultrasonication and expression was detected by SDS-PAGE. SDSPAGE showed a positive expression at ~44kDa as the GP5 is tagged with SUMO protein. Protein was purified by nickel column and the purified expressed protein was further confirmed by western blotting method in a nitrocellulose membrane (NCM) and the membrane was developed using a commercial PRRSV-GP5 polyclonal antibody (Cat. No. bs-4504R Bioss Antibodies). Further protease cleavage was performed to release the GP5 from SUMO and detected by SDS-PAGE where a positive band of 25kDa could be detected. Purified protein-coated @ 1 microgram to ELISA plate could detect positive serum samples correctly as compared with known negative samples.

Animal Viruses: Epidemiology

440288

Serological, Entomological, And Molecular Data On Bluetongue In Morocco

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Bluetongue is an arthropod-borne viral disease transmitted by Culicoides biting midges, affecting domestic and wild ruminants. It is considered worldwide as a threat to ruminant livestock and wildlife. Since 2004, Bluetongue has emerged as an enzootic disease in Morocco. The country has been exposed to numerous incursions of Bluetongue viruses (BTVs), involving serotypes 1 and 4. Since then, Morocco has lost its status as an officially BTV disease-free country, which has led to significant restrictions on the animal and animal semen trade. A vaccination program has been started to control circulating BTV strains. Nevertheless, multiple serotypes of BTV in Morocco complicate this preventive action.
The aim of this study is the confirmation of the active circulation of the Bluetongue virus in Morocco. For this, serological, molecular, and entomological surveys in different districts in Morocco have occurred. The samples were collected from small ruminants. Sera were screened by competitive ELISA, and seropositive samples were tested by real-time RT-PCR. Culicoides collections were carried out using light OVI traps on different sites during different periods. Collected Culicoides were morphologically identified. Pools of parous non-fed females were analyzed using real- time RT-PCR for detecting possible vectors involved in BTV transmission.
The results showed a seroprevalence of 41.12 % with a percentage of positivity of 44.35 %. The proportions also varied according to risk factors (species, sex, age, geographical region, season). Statistical analysis was performed by the chi-square test using OpenEpi software with a significance level of 5 %.
For the entomological results, a total of 3643 Culicoides belonging to at least 11 species were captured. C. imicola was the most abundant species (74 %), followed by Culicoides paolae (9.41 %) and Culicoides newsteadi (8.62 %). Viral RNA was detected in 24 of the 31 pools tested of C. imicola, C. newsteadi, and C. paolae. That suggests their implication in the transmission of BTV in Morocco.
From these results, it is concluded that the virus is still endemic at the national level. Keywords: Bluetongue, Culicoides, Seroprevalence, RT-PCR, Morocco.

440926

Low Pathogenic Avian Influenza Virus, H9N2, Morocco, Sequencing Full Genome

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Background: Low pathogenic avian influenza is considered one of the most important pathologies affecting birds (broiler, laying hens, breeding chickens and turkeys). This infection causes considerable economic losses. The objective of this work is to monitor and assess the prevalence of H9N2 in eight different regions of Morocco using real-time RT-PCR, and to assess the phylogenetic and molecular evolution of the H9N2 virus between 2016 and 2019.
Methods: Field samples were collected from 137 farms suspected of being infected with LPAI H9N2 virus. Samples were analysed using H9N2-specific real-time RTPCR. 11 highly positive samples were isolated and 7 of them were fully sequenced.

Results: The survey resulted in a disease prevalence of 56.2% and a different distribution of this disease among the 8 regions studied. The relative prevalence of H9N2-positive farms was estimated at 36.62% in the vaccinated group, while it reached 75.75% in the non-vaccinated group. Phylogenetic and molecular analyses showed that our Moroccan viruses were closely related to 2016 Moroccan viruses and grouped in the G1 lineage. Nevertheless, several mutations were detected in the HA and NA proteins of our Moroccan viruses compared to Moroccan viruses isolated in 2016 and to reference strains.
Conclusion: Specific amino acid substitutions were identified in Moroccan H9N2 viruses that are believed to lead to increased transmissibility in mammals, as well as resistance to antiviral drugs.

440130

Avian Influenza Surveillance In Wild Birds In Peru, 2019-2020

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Avian Influenza virus (AIv) is a highly contagious agent that infect a wide range of species including wild birds and poultry. Waterfowl is considered to be the main natural reservoir for all subtypes representing an important source for transmission to other susceptible species. Peru has a crucial location within the migration route of wild birds in the Americas, and therefore of relevance for viral spreading. For this reason, our laboratory has implemented massive efforts and adopted strategies for AIv surveillance within the last 15 years, reporting multiple subtypes in waterfowl habitat in the Peruvian coast. Due to the relevance of AIv monitoring, the current study aimed the virus isolation in fecal samples from aquatic birds along the coast of Lima. Samples were taken from March 2019 to March 2020. Hence, a total of 421 samples were first analyzed by virus isolation in embryonated eggs and hemagglutination test. Viral detection was performed by RT-PCR and whole genome analysis by NGS. As a result, four low-pathogenicity AIv subtypes were detected, comprising H2N6, H6N2, H6N8 and H13N6. Among them, H2N6, H6N2 and H13N6 had never been found previously in Peru, representing the first report of these viral subtypes and their genome characterization in our country. Moreover, phylogenetic analysis revealed that these Peruvian isolates were closely related to North American AIvs, highlighting distinctive features that might confer them peculiar properties. In conclusion, our study contributes to the better understanding of AIv epidemiology and provides new insights of the viral evolution in waterfowl populations in Peru. This work was fully funded by FONDECYT-Peru and World Bank (02-2019-FONDECYT-BM-INC-INV)

439829 3min Student

Syndromic Surveillance Of West Nile Virus In Animals In South Africa, 2017-2020

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West Nile virus is a flavivirus that is transmitted by mosquitoes to susceptible human and animal hosts and has now been recognized as one of the most widespread flaviviruses, however, it still remains understudied in Africa. Previous South African studies up to 2015 identified West Nile virus as an agent of febrile and neurological disease in animals. Syndromic surveillance for West Nile virus in animals displaying neurological or febrile disease was continued in South Africa from 2017-2020. Samples from 1078 animal cases were submitted for molecular testing, whilst West Nile virus IgM

antibody was investigated in 657 equine specimens. A total of 18/1078 (1.7%) West Nile virus infections were identified through RT-PCR of blood and/or tissue specimens, specifically in 1.3% of equine, 3.0% of wildlife species, 1.8% livestock, 2.5% avian species and 8.3% non-equine domestic animal cases. Phylogenetic analysis identified all but one strain to be closely related to previously identified South African lineage 2 strains. A single lineage 1 infection was identified. Additionally, 68/657 (10.4%) equine infections were identified through IgM antibody detection. In 2017, 10.2% of all submissions tested positive for West Nile virus, 2.8% in 2018, 7.0% in 2019 and 7.2% in 2020. Neurological disease was observed in 91.0% of total West Nile virus positive cases, whilst 34.0% of infections were fatal. Future work will focus on confirming IgM positive results by virus neutralization tests. The results of this study confirm that 8.0% of total neurological animal cases submitted in 2017-2020 were associated with West Nile virus infection. Annual circulation of endemic lineage 2 strains between mosquitoes and susceptible animal hosts is associated with a significant incidence of neurological and fatal infections. The use of animals as sentinels for West Nile virus may predict areas of increased risk for human disease.

439798 3min Student

Neurological Alphavirus Infections In Horses And Wildlife In South Africa: A Survey (2019 – 2021)

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Introduction: Alphaviruses can be divided into the New World alphaviruses that are associated with encephalitis in animals and humans in the Americas, while the Old World alphaviruses, which are mostly believed to be associated with arthralgia in humans, with less data available as to their importance in animals. Recent reports from the Zoonotic Arbo and Respiratory Research group of neurological infections in animals and some human cases with Old World alphaviruses such as Middelburg virus (MIDV) and Sindbis virus (SINV) in South Africa have renewed interest in the potential of these viruses as animal pathogens.
Methodology: Blood and tissue samples of a total of 333 animal cases showing neurological signs were received from various veterinarians across South Africa during January 2019-March 2021. RNA was extracted under laboratory biosafety level 3 conditions. A genus specific nested PCR with published primers and probes specific to MIDV and SINV based on the alphavirus non-structural protein 4 (nsP4) were used for RT-PCR screening. PCR-positive cases were confirmed by sequencing of the nsP4 amplicons followed by maximum-likelihood analysis using MEGA version 7 and P-distance analysis. MIDV confirmed cases were further amplified using a MIDV specific PCR to obtain a larger fragment of the envelope protein.
Results: EDTA samples from a total of 10/333 animal cases tested positive for alphaviruses and included 4/39 wildlife and 6/275 horse cases. MIDV positives were identified in a wildebeest, 2 rhinoceros and 3 horses, whereas SINV positives were obtained in 3 horses. A buffalo tested positive for a virus that is closely related to the mosquito specific Eilat virus. Neurological symptoms were identified in 8/10 cases including fore-and hindleg paralysis, paresis, ataxia, paddling and recumbency, 2/10 animals only presented with a fever and 2/10 cases were fatal.
Conclusion: This study implicates MIDV and SINV, and possibly a novel alphavirus as causes of neurologic disease in wildlife and equine species in South Africa during the 2019-2021 arbovirus season.

440842 3min Student

Epidemiology And Molecular Characterization Of Canine Bufavirus And Cachavirus In Grey Wolves (Canis lupus) Of The Northwest Territories

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Canine bufavirus (CBuV) and cachavirus (CachaV-1) are parvoviruses in the subfamilies *Parvovirinae* and *Hamaparvovirinae*, respectively. CBuV has previously been found in free-ranging canids and domesticated dogs, whereas CachaV-1 has only been found in domesticated dogs. Although both viruses have been associated with gastrointestinal symptoms their ability to cause clinical disease is undetermined. The focus of this study was to test for the presence of CBuV and CachaV-1 among grey wolves (Canis lupus) from the Northwest Territories (NWT) and molecularly characterize identified strains. DNA isolated from the spleens of 305 wolves (West N=28, East N=188, South=12) previously analyzed for other common dog viruses, was screened for CBuV and CachaV-1 using nested-PCR. Of the 305 samples, 131 were CBuV-positive (42.95%), and 50 (38.9%) of these presented with a co-infection. Eight samples were CachaV-1-positive (2.62%) and 7 (87.5%) of these presented with a co-infection. CBuV was significantly more prevalent and the co-infection rate for CachaV-1-positive animals was significantly higher. Sex and age were not significant in infection for either virus. There were significantly less CBuV infections in the west region than in the east, and all CachaV-1-positive samples with known sampling locations came from the east region. A partial nucleotide sequence was obtained from 28 CBuV- and 4 CachaV-1-positive samples and full genome sequences were obtained for 5 CBuV and 1 CachaV-1 strain. Maximum-likelihood phylogenetic trees revealed the presence of two distinct strains for both viruses. CBuV strains were 90.2-100% identical to each other and 85.5-100% identical to sequences in public databases, while CachaV-1 strains were 95.9-100% identical to each other and 95.5-100% identical to sequences in public databases. Our results indicate different lineages of these recently discovered parvoviruses of dogs are present within the NWT wolf population and warrant further investigation of any potential clinical significance to free-ranging or domestic canids.

440297 3min Student

Molecular Investigation Of Canine Parvovirus-2 (CPV-2) Outbreak In Household Dogs On Nevis Island: First Report On Nearly Complete CPV-2 Genomes From The Caribbean Region

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Canine parvovirus-2 (CPV-2) is a major cause of hemorrhagic gastroenteritis in domestic dogs. To date, there is a single report on CPV-2 (25 sporadic cases from St. Kitts between Feb 2015-July 2016) from the Caribbean region. During Aug 1, 2020-Oct 17, 2020, the veterinary clinic on the Caribbean island of Nevis reported 64 household dogs with CPV-2-like clinical signs, of which 27 animals died. Rectal swabs/fecal samples were obtained from 39 dogs. All the samples tested positive for CPV-2 by IDEXX SNAP test (24/39) and/or PCR (32/32), whilst 15 untested dogs had CPV-2 SNAP/PCR positive litter mates. Analysis of the complete VP2 amino acid (aa) sequences (from 32 PCR positive

dogs) revealed new CPV-2a as the predominant genotype. The new CPV-2a strains from Nevis shared absolute VP2 aa identities between themselves and with those reported previously from the neighboring island of St. Kitts, except for Ala262Thr in 4 strains and Asp373Asn in 3 other strains. Analysis of the nearly full-length genomes of 4 new CPV-2a strains from St. Kitts and Nevis (including one from each of the VP2 mutants) revealed (i) >99% nucleotide sequence identities with several CPV-2a, -2b and -2c strains, (ii) presence of 5 nonsynonymous mutations that emerged during the CPV-2 to CPV-2a global sweep, (iii) a rare nonsynonymous mutation (VP2, Asp373Asn), and (iv) some unique synonymous mutations. Phylogenetically, the nearly complete CPV-2 sequences formed a distinct cluster near CPV-2b/1998 strains from USA, corroborating previous observations on a lack of monophyletic segregation of the CPV-2 VP2 variants, and multiple evolutionary origins for certain mutations. Our findings suggest that new CPV-2a might be endemic in the region, with the potential to cause severe outbreaks, warranting further studies in other Caribbean islands. This is the first report on analysis of nearly complete CPV-2 genomes from the Caribbean region.

433765 3min Student

Determination Of Risk Factors Associated With Bluetongue Virus Antibodies Detected From Camels In Central Sudan

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Background: Bluetongue (BT) virus is the causative agent of the arthropodtransmitted BT disease that affects many domestic and ruminant species. The virus can be transmitted by certain Culicoides biting midges, causing an illness that tends to be severe, especially in sheep, thus lead to significant economic losses due to the reduction in milk, body weight, and decrease reproductive performance. At the same time, cattle and camels may develop subclinical infections. We aimed by this study to determine the prevalence of the Bluetongue virus (BTV) in camels that reared in central Sudan and to identify the potentiality of any risk factors. Methods: 184 blood samples collected between 20 November 2014 and 23 May 2015 from six localities of Khartoum state, Sudan, by using multi-stage random sampling. The sampled farms' characteristics, including individual and management factors, were obtained using a semi-structured questionnaire. We employed the competitive enzyme-linked immunosorbent assay (cELISA) to detect the antibodies of BTV.
Results: Our findings indicated that the BTV is highly circulating in the tested camels, with an overall 66.8% prevalence. We identified that the source of camels (OR = 0.424, CI = 0.223-1.071, p-value = 0.040), as well as a presence of the other animals commingling with camels (Mixed raring) (OR = 0.176, CI = 0.43-0.771, p-value = 0.016), are potential risk factors for the emerging of BT disease.
Conclusion: Camels harbor the BTV infection with no clinical signs as appeared in the presence of BTV antibodies in our study's serum samples, which may play a role in spreading the disease. Control measurements are recommended to improve the prevention of vulnerable animal species from BTV infection.

Novel And Endogenous Animal Viruses

441087

A Systematic Investigation On Arthropod Associated Viruses Reveals The Central Role Of Arthropods In The Macroevolution Of RNA Viruses

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Many viruses of arthropods also infect other organisms, including humans, sometimes with devastating consequences. Yet, for the vast diversity of arthropods, their associated viruses remain unexplored. Here, we mined metatranscriptomic data from 711 arthropod species, including insects, arachnids, myriapods, and crustaceans, and uncovered more than 1300 previously unknown RNA viruses, representing 822 novel evolutionary groups at a level between species and genus. These newly found viral groups fill major evolutionary gaps within the five branches of RNA viruses, bridging the evolution of viruses infecting early and later diverging eukaryotes. Additionally, co-phylogenetic analysis suggests that RNA viruses of arthropods have commonly co-evolved with the hosts. Our analyses indicate that arthropods have played a central role in the macroevolution of RNA virus by serving as virus reservoirs where the viruses have co-evolved with arthropods and meanwhile being exchanged with a vast diversity of organisms.

435351

Newlavirus, A Novel Highly Prevalent And Highly Diverse Protoparvovirus Of Foxes

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The genus Protoparvovirus (*Parvoviridae*) includes several viruses of carnivores. In this study, we describe a novel fox protoparvovirus, which we named Newlavirus as it was discovered in samples from Newfoundland and Labrador, Canada. Although genome walking is still in progress, analysis of the near-complete non-structural protein (NS1) sequence indicates that this virus is a previously

uncharacterized species, as it is approximately 50% identical to the NS1 of its closest relative (California sea lion parvovirus). Viral prevalence was investigated by nested-PCR performed on DNA isolated from tissues (10mg for spleen, 20mg for lymph nodes), fecal suspensions (0.5cm3 stool dissolved in 1ml of universal transport media, UTM), or rectal swabs (submerged in 3ml UTM). The virus showed high prevalence in foxes both from the mainland (Labrador, 54/137, 39.4%) and from the island of Newfoundland (22/50, 44%) but was not detected in samples from other carnivore species, including coyotes (N=85 spleen; N=39, fecal), lynx (N=58 spleen), marten (N=146, spleen), mink (N=47, spleen), weasels (N=17, spleen) dogs (N=48, fecal), and seals (N=18, fecal). Newlavirus was found at similar rates in stool and spleen (24/80, 30% vs. 59/152, 38.8%, p=0.2) but at lower rates in lymph nodes (N=2/37, 5.4%, p

445151

Transmission Of Koala Retrovirus From Parent Koalas To A Joey In A Japanese Zoo

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Background: Koala retrovirus (KoRV) is endemic in both the wild and captive populations, and represents a major threat to koala health. KoRV is of interest to virologists due to its currently active endogenization into the koala (Phascolarctos cinereus) genome. Although KoRV has frequently been isolated in wild and captive koala populations, its pathogenesis and transmission remain to be fully characterized, and most previous research has concentrated on adult koalas rather than jeoys.
Objectives: We aimed to improve understanding of KoRV transmission mode and pathogenesis in joeys by investigating KoRV status for the parents and their joey and by characterizing KoRV in blood and tissues from the joey.
Methods: Genomic DNA and viral RNA were isolated from EDTA-treated whole blood samples, frozen tissue samples and plasma. Isolated genomic DNA and RNA were quantified by real time PCR.
Results: We sequenced the KoRV LTR and envelope genes isolated from the joey and its parents, and found KoRV-A and KoRV-C—the endogenous strains—in genomic DNA from both the parents and joey. Notably, both parents were also positive for KoRV-B, whereas the joey was KoRV-B negative; further confirming that KoRV-B is an exogenous strain. The KoRV LTR sequence of joey was considerably closer to that of its sire than its dam. For further characterization, total KoRV proviral, KoRV-A, -B and -C proviral loads were quantified in peripheral blood mononuclear cells from the parents and blood samples from the joey. Total KoRV proviral, KoRVA and -C loads were also quantified for different tissues (bone, liver, kidney, lung, spleen, heart, and muscle) from the joey, revealing differences suggestive of a distinct tissue tropism (highest total KoRV proviral load in the spleen, and lowest in bone). KoRV-C amount in parents were less than that in the joey. Our findings contribute to an improved understanding of KoRV pathogenesis and transmission mode, and highlight useful areas for future research.

441226 3min News

Atypical Porcine Pestivirus – A Widespread And Highly Abundant Virus In The Swedish Wild Boar Population

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The recently identified causative agent of congenital tremor in domestic piglets, atypical porcine pestivirus (APPV), has been detected in Swedish wild boar. A previous study from Sweden has described APPV in piglets suffering from congenital tremor, but the APPV situation in the wild boar population has till now been unknown.
In the present study, 595 serum samples from wild boar originating from 13 counties in the south and central parts of Sweden collected in the years between 2000 – 2018 have been investigated for APPV-specific antibodies against the APPV-glycoprotein Erns and the APPV-genome. The results revealed that APPV is highly abundant in the Swedish wild boar population; 72% (433/595) of the tested wild boars had APPV specific antibodies and 12% (73/595) were APPV-genome positive in serum. The present study also shows that APPV has been present in the Swedish wild boar population since at least the year 2000.
The viral sequences obtained from the wild boars are highly similar to APPV sequences obtained from Swedish domestic pigs suffering from congenital tremor suggesting a viral exchange between wild boars and domestic pigs. The high proportion of viraemic and seropositive wild boar is indicative of wild boar being an important reservoir for APPV. It also raises the question of APPV causing chronic or persistent infections in wild boar.

440223 3min News

A Complete Genome Sequence Of A Bagaza Virus From South Africa And Its Phylogenetic Characterisation Within The Flavivirus Ntaya Serogroup

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Bagaza virus (BAGV), a member of the Flavivirus genus of the Flaviviridae, was isolated from the brain tissue of several Himalayan monal pheasants (*Lophophoius impejanus*) that died following neurological signs in Pretoria, South Africa in 2016/2017. Next-generation sequencing was carried out on one of these isolates resulting in a full genome sequence of 10811nt. The genome sequence of this isolate, designated ZRU96-16, shared 97% nucleotide identity with a Spanish BAGV sequence isolated from an infected partridge. In total 13 nucleotide and 8 amino acid variations were unique to ZRU96-16 after alignment with other BAGV genomes. The most distinctive feature of the new South African sequence is a truncation of 132nt on the terminal end of the 3' untranslated region (UTR). Phylogenetically, the South African isolate clustered closely with isolates from Spain as well as with Israel Turkey Meningoencephalomyelitis viruses (ITMV). Sequences which were isolated from infected birds also all shared the truncation at the 3'end. The phylogenetic analysis also revealed that the time at which the isolate was sequenced was more predictive of ancestry than the geographical location. This therefore could support the hypothesis that migratory birds travelling between Europe

and Africa contribute to the spread of this virus. All together this work shows that Bagaza virus circulates in South Africa and spilled over into monal pheasants likely causing the outbreak of fatal neurological disease. Furthermore, it shows further sequence similarity between BAGV and ITMV isolates and calls into question whether these viruses should truly be considered as different species.

440896 3min Student

The Identification Of A Novel Iridovirus Within The Deep-sea Carnivorous Sponges Chondrocladia grandis And Cladorhiza oxeata From The Gulf Of Maine And Baffin Bay (Canada)

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Sponges are the oldest extant animals on the planet and are conspicuous in all ocean basins. While most sponges have an aquiferous system lined with flagellated cells that enable them to suspension-feed as water is pumped through the sponge's body, members of the family *Cladorhizidae* either lack or have a reduced aquiferous system and capture small crustaceans or larvae on Velcro-like spicules, giving them the name "carnivorous sponges". Suspension-feeding sponges have diverse microbiomes and viromes, partly resulting from exposure to the large volumes of water processed by these animals. While there have been some efforts to elucidate the microbiome of carnivorous sponges, their viromes remain unexplored. Here, we report on the presence and identity of a novel member of the family Iridoviridae found within the deep-sea cladorhizid sponges *Chondrocladia grandis* and *Cladorhiza oxeata* sampled from the Gulf of Maine and the Canadian Arctic. Based on sequencing of a fragment of the conserved major capsid protein (MCP), which is commonly used for phylogenetic analysis of iridoviruses, the virus present in the cladorhizid samples shows the highest identity with the recently identified but partially sequenced Acetes erythraeus iridovirus. We screened for the presence of this virus in samples of extracted DNA from morphologically distinct body regions of the two carnivorous sponge species using PCR. Positive samples were sequenced and a scaffold genome sequence was obtained from one C. grandis sample via Illumina sequencing. The novel iridovirus was present in different body regions of both C. grandis and C. oxeata, with identical sequences found spanning considerable geographic distances (from Gulf of Maine to Baffin Bay). Preliminary genomic data indicate that members of the genus *Decapodiridovirus* (subfamily *Betairidovirinae*) are the closest relative to the sponge-associated iridovirus. Potential linkages between the new iridovirus, the carnivorous sponges, their prey and environment will be discussed.

440768 3min Student

Novel Bat Vesiculovirus In The Mediterranean Region

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Bats are the natural reservoirs of various emerging or re-emerging viruses. Among them, lyssaviruses (family *Rhabdoviridae*) are ones of the most iconic viruses described in these animals, but other bat rhabdoviruses (Vesiculovirus and Ledantevirus) exist. These results suggest that bats are playing an important role in rhabdovirus diffusion and highlight the importance to perform an active surveillance of this reservoir. In this study, we developed and validated a combined nested RT-qPCR technique dedicated to the broad detection of animal rhabdovirus, which was applied to largescale screening of 1962 bat samples, including blood (n=816), brain (n=723) and saliva (n=423) samples, collected from various bat species between 2007 and 2019 in 9 different countries in Europe (Czech Republic,

France, Spain) and Africa (Algeria, Central African Republic, Egypt, Guinea, Ivory Coast, Morocco). A total of 23 bat samples (1.2%) was found positive, with 17 (2.1%) blood and 6 (1.4%) saliva samples. All positive samples were obtained from bats in the Mediterranean region. Next generation sequencing was performed on the positive samples and phylogenetic analysis based on the nearly complete genome sequences demonstrated closely related to other bat vesiculoviruses previously described in China and in North America. In addition, all of them were strongly clustered together, indicating that they belong to the same putative new species, tentatively named Mediterranean bat virus (MBV). Interestingly, MBV was detected in different bat species, including Miniopterus schreibersii, Rhinolophus euryale and Rhinolophus ferrumequinum. Our results suggest that MBV is widely distributed in the West part of the Mediterranean region, where it can circulate in multiple bat species. These findings expand the host range and the viral diversity of bat vesiculoviruses, paving the way to determine their route of transmission and their dynamic of diffusion into bat colonies, as well as to evaluate their potential hazard for public health.

439975 3min Student

First Report Of Non Primate Hepacivirus In Horses And Dogs, Morocco

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A numerous homolog of hepatitis C virus (HCV) have been identified this past few years from different animals. Non Primate Hepacivirus (NPHV), isolated from horses and dogs, has been stated as the most genetically close to HCV, and has been identified in many different parts of the world except in African continent, in which such information is lacking and rare.
This present study represents the first investigation of NPHV prevalence in horses and dogs in North Africa. One hundred seventy-two horses’ and 36 dogs’ blood samples were collected from different locations in Morocco. Samples were screened for NPHV RNA by nested PCR targeting both 5’UTR and NS3 regions and analyzed for anti-NPHV NS3 antibody using a Gaussia luciferase immunoprecipitation system to determine seroprevalence. A total of eight sequences of the NS3 region isolated from positive serum samples were subjected to phylogenetic analysis.
NPHV RNA positivity rates in horses and dogs were respectively 10.5% and 5.5% and seroprevalences about 50.0% and 5.5%. Seropositivity was more important among mares than stallions (60.9% vs. 32.4%, P=0.0001). Also, NPHV RNA positivity rate was essentially observed in Juvenile horses (25.3%). Phylogenetic analysis of NPHV NS3 genes isolated from horses and dogs are clustered all together, and no correlation was observed between the different NPHV strains and geographic location within Morocco.
In conclusion, there are evidences that Moroccan horses are subjected to previous and/or current NPHV infection, with young age and female sex to be risk factors. We noted that NPHV is not only circulating in horses but also in dogs, proving that maybe it has crossed species barriers and both animals (horses and dogs) could be potential vectors by which an ancestor to hepatitis C virus was presented to human populations.

Plant Viruses: Molecular Virology

445248

Identification Of Chemical Inhibitors For Viral RNase III, An RNA Silencing Suppressor

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Sweet potato chlorotic stunt virus (SPCSV) can synergistically interact with several plant viruses from different genera causing substantial yield losses worldwide. There are currently no effective measures to control the synergistic disease induce by SPCSV. As its infection mechanism, SPCSV encodes an RNase III (CSR3) that suppresses antiviral RNA silencing defense of plants. Therefore, a chemotherapy strategy to identify CSR3 inhibitors to control SPCSV-associated viral diseases is indispensable. Here, we screened 136,353 compounds and identified four belonging to two structural classes, using Glide-docking, a novel kinetic-based high-throughput screening method, two complementary binding affinity assays utilizing surface plasmon resonance and microscale thermophoresis, and verification assays in plants grown in medium and soil. As results, we identified five compounds belonging to two structural classes that inhibited CSR3 activity and reduced viral accumulation in plants. These results provide the foundation for developing antiviral agents targeting CSR3 to provide new strategies for controlling sweetpotato virus diseases.

444777

Fraternizing With The Enemy: A Novel Pro-viral Role For Plant ARGONAUTE1

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Global food security is increasingly threatened by plant virus infections that damage crops and decrease yields. Potyviruses are a large group of positive-sense single-stranded RNA viruses that infect economically important edible plants including potato and tomato. The ARGONAUTE protein family is deeply involved in plant defense mechanisms through their role as effectors of RNA-mediated gene silencing. Helper component Protease (HCPro) is the main potyviral silencing suppressor that is involved in many host-virus interactions. We have recently demonstrated that HCPro recruits host ARGONAUTE1 (AGO1) via a WG/GW motif and redirects it to pro-viral functions (Pollari et al. PLOSP 2020). Investigations with an HCPro mutant with impaired capacity to interact with AGO1 showed that HCPro engages AGO1 to promote the systemic spread of potato virus A and turnip mosaic virus infections. Moreover, the interaction was crucial for the accumulation of virus particles. We found that HCPro mediates the association of the viral coat protein and AGO1, a function which may be linked to particle stability. This study highlights that viral proteins are capable of imposing surprising role changes on host factors, exemplified by a novel pro-viral role for AGO1.

445372

Differential Expression Of Cell Wall-related Genes In Rice Are Determined By Levels Of Rice Tungro Bacilliform Virus

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The symptoms which appear in virus-infected plants, including leaf curl, mosaic, stripes, stunting and other deformities, are all believed to arise due to the perturbation of the normal growth and developmental processes of the host by viral components. We have used the rice-rice tungro virus system to investigate this aspect in some detail. Rice tungro disease (RTD), a viral disease of rice prevalent in South and Southeast Asia, causes stunting and yellow-orange foliar discolouration in its host. Two viruses, rice tungro bacilliform virus (RTBV), family Caulimoviridae and rice tungro spherical virus (RTSV), family Secoviridae cause RTD, the symptoms develop primarily due to RTBV. Earlier, we have reported the development of transgenic rice lines, which show tolerance against RTBV, showing 500- to 1000-fold less accumulation, compared to the non-transgenic controls, the RTSV levels showing no change. To correlate stunting, the most prominent symptom of tungro with RTBV levels, we have looked at the expression levels of 23 genes important in cell wall dynamics, between the genetically altered rice line described above and its untransformed controls, following challenge with RTBV and RTSV. In parallel, we also studied the changes in gene expression levels and cell wall components after challenge with only RTBV. There was downregulation in expression levels of genes encoding cellulose synthases, expansins, glycosyl hydrolases, exostosins, and xyloglucan galactosyl transferase, whereas there was upregulation in genes encoding defensin or defensin-like proteins with increasing titers of RTBV. Cellulose and pectin contents showed a downfall, whereas the lignin contents increased with RTBV titers. On examining the promoter regions, potential binding sites of transcription factors, affected by RTBV could be identified in some genes. Our results suggest a possible mechanism for the differential regulation of a network of genes, which may result in stunting, the principal symptom of RTD.

445659

Identification Of Silencing Suppressors In Croton Yellow Vein Mosaic Virus

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Croton yellow vein mosaic virus (CYVMV; genus *Begomovirus*; family *Geminiviridae*) is a prolific begomovirus in the Indian sub-continent. Begomoviruses cause severe diseases in crop plants resulting in yield loss. The CYVMV DNA genome is 2.7 kb in size with six partially overlapping open reading frames (ORFs). The viral sense strand encodes a coat protein (CP, AV1) and a pre-coat protein (AV2). Its complementary strand encodes a replication initiator protein (Rep, AC1), a transcriptional activator protein (TrAP, AC2), a replication enhancer protein (REn, AC3) and an AC4 protein. At least three begomovirus-encoded proteins, AV2/V2, AC2/C2 and AC4/C4, have been shown to act as RNA silencing suppressors to overcome plant defense for efficient infection, replication and systemic spread in the host. The majority of monopartite begomoviruses are associated with a betasatellite, which encodes a C1 protein that exhibits suppressor activity. In multiple reports, begomovirus silencing suppressors act as symptom determinants when expressed alone. Here, we report that four CYVMV-encoded proteins, V2, C2, C4 and βC1, all have silencing suppressor activity. When expressed alone, they all can move in the plant with V2, C2 and C4 capable of inducing disease symptoms. V2 exhibits selfinteraction in yeast two-hybrid and bimolecular fluorescence complementation assays. In addition, V2 and AV1/V1 are also involved in CYVMV movement and physically interact with each other. Taken together, our work demonstrates the silencing suppressor and virulence functions of CYVMV V2, C2, C4 and βC1, and the V2's protein binding characteristics and its additional role in virus movement.

445547

Advancements in RNA-based Technologies for Management of Plant Viral Diseases

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Gene silencing is one of the most important mechanisms of gene regulation in plants, which is mediated by 19–24 nt sized small RNAs. In plants, the small RNAs can be grouped into different classes, microRNAs (miRNAs), small interfering RNAs (siRNAs), Trans-acting siRNAs (tasiRNAs) etc. Transgenically induced RNA interference (RNAi), has been employed to control diverse plant viruses (Patil et al., 2011, Mol. Plant Pathol. doi: 10.1111/j.1364-3703.2010.00650.x.). The tasiRNAs are a recently identified class of small RNAs, which are derived from TAS gene-derived transcripts after being acted upon by a miRNA. The miRNA173 directs the cleavage of TAS1 & TAS2 leading to the generation of tasiRNAs from the sequences located downstream of miRNA173 recognition site. The cleavage mediated by miR173 is sufficient to initiate transitivity, and targeting of a given gene by miR173 results in the production of secondary siRNAs originating from the target nucleotide sequence. The above studies lead to the emergence of a gene regulation technique termed as “miRNA-Induced Gene Silencing” (MIGS), that is essentially based on the unique feature of the miR173 to trigger the generation of secondary siRNAs (tasiRNAs) from its target sequences. MIGS can also be used to simultaneously silence multiple genes by fusing multiple MIGS modules (miR173 target site plus sequence of interest) to generate a single MIGS construct, which subsequently can be cloned into a binary vector for plant transformation. This fusion MIGS construct is capable of simultaneously silencing different genes with the same efficiency. In collaboration with ICAR-IARI (India), Chemveda (India) and UC Davis (USA), we have explored the utility of MIGS technology for the combinatorial management of cotton leaf curl disease, whitefly and root knot nematode. The study demonstrated successful validation of the MIGS approach in the model host plant Nicotiana benthamiana (Hada et al., 2021, Pest Management Science, doi: 10.1002/ps.6384).

445700

Identification Of Potato Virus Y-interacting Host Proteins

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Potato virus Y (PVY) is one of the most economically important plant pathogens that affects staples such as potato and several other solanaceous plants and is listed among the top-five economically important viruses in the world. PVY affects the yield of three important crops that contribute to food and nutritional security - potato, pepper, and tomato. PVY exists as several biological distinct strains and most of the strains are the result of recombination. The PVY genome encodes a single polyprotein which is then post-translationally cleaved into P1-pro, HC-pro, P3, CI, VPg, NIa-pro, NIb, and coat protein (CP). In this study, to dissect the PVY-host interactome, we used PVYNTN-Nicotiana benthamiana plant as a model system. We carried out Yeast-2-Hybrid (Y2H) library screening using N. benthamiana Y2H cDNA library and pGBKT7-PVY-CP as bait. One of the proteins that was found to interact with PVY-CP was cytosolic Phosphoglucomutase (cPGM). cPGM is an enzyme that catalyzes the reversible conversion of glucose-6-phosphate to glucose-1-phosphate and is involved in controlling the partitioning of both sugar-phosphate into respiratory pathway, cell wall synthesis and sucrose synthesis pathways. This interaction between the CP and cPGM was further confirmed by Y2H approach. Yeast transformants carrying pGBKT7-CP and pGADT7-cPGM plasmids were able to grow on SD-Leu-Trp-His selection plates supplemented with 1 mM 3-amino-1,2,4-triazole, whereas the yeast transformants carrying negative control combination plasmids pGADT7 and pGBKT7, pGADT7-cPGM and pGBKT7 or pGADT7 and pGBKT7-CP were unable to grow. Similarly, yeast transformants carrying positive control plasmids pGADT7-TAg and pGBKT7-P53 grew on selection plates suggesting the interaction between TAg and P53 protein. Ongoing studies include investigation into the biology and importance of CP-PGM interaction in PVY infection of potato\

445701 3min student

Advances in Tobacco Rattle Virus (TRV)-Based Virus-Induced Gene Silencing (VIGS) in Tomato Plants

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Plant genome sequence projects have provided huge genomic information but decoding of information in the form of functional annotation is the next step to achieve goal of projects. Among the widely used functional genomic approaches, virus-induced gene silencing (VIGS) has emerged as convenient and most extensively applied tool for functional characterization of target genes. VIGS is a highly efficient tool to analyze gene function through reverse genetic approach which utilizes post transcriptional gene silencing mechanism to downregulate the target gene. Tomato is a model crop plant to study the plant virus interaction and unveiling of its complete genome sequence demands significant advances of tomato functional genomics. Although Tobacco rattle virus (TRV)-based VIGS is optimized in model plants like Nicotiana benthamiana but optimization of highly efficient, reliable and reproducible VIGS protocol was urgently needed for model crop plants tomato. In the present study we optimized TRV-based VIGS protocol in tomato. Analysis of potentially affecting parameters in tomato VIGS has been carried out. These parameters included pre-incubation of Agrobacterium, concentration of acetosyringone, concentration of agro-inacula, plant growth stage and agro infection method (Agro infiltration/inoculation). Efficiency of TRV-based VIGS was also analyzed in through different Agrobacterium strains in different varieties of tomato. Optimized protocol for TRV-based targeted gene silencing in tomato plants will facilitate high-throughput functional analysis of genes in tomato.

445658 3min Student

Role of Non-coding Regions of DNA-B in Begomovirus Pathogenesis

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Begomoviruses are considered as one of the most devastating pathogens across the globe. These viruses can infect a wide range of hosts leading to billion dollar losses globally.Begomoviruses are predominantly monopartite, having a single genome. However, some of these have two genome components, DNA-A and DNA-B, and hence called as bipartite. In the Indian sub-continent, leaf curl disease of tomato is caused by both monopartite as well as bipartite begomoviruses. Tomato leaf curl Gujarat virus (ToLCGV) is one of predominant begomoviruses. Along with the coding regions of viral genome, non-coding regions (NCR) also play important role in pathogenesis. Although in case of several RNA viruses, NCRs like 5’ and 3’ UTR significantly influence viral replication, transcription and translation, such study in case of begomoviruses is lacking. In this study, we put our efforts to manipulate the 5’ and 3’ UTR of the DNA-B ORFs (BC1 and BV1) of ToLCGV by site directed mutagenesis. We found that manipulation of UTRs of BC1 ORF aggravates virus induced symptoms and also significantly influences viral replication and transcription. Our study delineates the putative regulatory roles of NCR in begomovirus pathogenesis.

445683 3min Student

Pathogen-triggered Metabolic Adjustments To Potato Virus Y Infection In Potato

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Plant viruses are among the most important phytopathogens worldwide, with nearly half of the emerging epidemics having viral etiology. In potato (Solanum tuberosum L.) – the fourth most important food crop in the world, several viral pathogens have a worldwide distribution, with the most economically damaging being potato virus Y (PVY). At least nine biologically distinct variants of PVY are known to attack potato. These include the relatively new recombinant types named PVYNTN and PVYNWilga, which induce tuber necrosis in susceptible cultivars. So far, the molecular plant-virus interactions underlying this pathogenecity have been studied primarily at the transcriptome level. On the other hand, the metabolic responses, which often correlates poorly with gene expression but directly mediates these phenotypic outcomes, are not well characterized. In this study, gas chromatography in tandem with mass spectrometry (GC-MS) was used to compare potato's metabolic response to infection with PVYNTN and PVYN-Wilga. A combination of univariate and multivariate methods showed PVYNTN and PVYN-Wilga infections elicited statistically distinguishable alterations for 25 metabolites. This included pools of amino acids, sugars and sugar derivatives, esters and lactones, organic acids, and fatty acids. From this metabolite set, a 10-feature biomarker model for discriminating host response to PVYNTN and PVYN-Wilga infection was identified that has predictive accuracy of 86.5%. This model may be useful for selection of potato for increased resistance, or reduced susceptibility, to these necrotic strains of PVY.

451191

Transmission of Tospoviruses by Thrips Palmi, a Predominant Vector in Asia

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Thrips palmi (Thysanoptera: Thripidae) is one of the key agricultural pests and predominant tospovirus vector in Asia. It feeds on more than 200 plant species belonging to family Cucurbitaceae, Solanaceae, Fabaceae, and Asteraceae. The distribution of T. palmi was thought to be restricted to southern Asia, but it has spread throughout Asia in recent decades. T. palmi has widely invaded the Pacific, Florida, the Caribbean, South America, Africa, and Australia. To date, seven tospoviruses (family Tospoviridae, order Bunyavirales) viz. groundnut bud necrosis virus (GBNV), melon yellow spot virus (MYSV), calla lily chlorotic spot virus (CCSV), watermelon silver mottle virus (WSMV), watermelon bud necrosis virus (WBNV), tomato necrotic ringspot virus (TNRV), tomato spotted wilt virus (TSWV), and capsicum chlorosis virus (CaCV) are reported to be transmitted by T. palmi. The present understanding of thrips-tospovirus relationship is largely based on the Frankliniella occidentalis (Thysanoptera: Thripidae) and TSWV. Less is known about the relationship of T. palmi with tospovirus. An exposure to GBNV and WBNV alters the biological traits of T. palmi. The survivability and oviposition potential of T. palmi decrease post GBNV and WBNV exposure. The virus exposure favors a female- biased ratio in the experimental population of T. palmi. Host plant also influences the life cycle, reproduction, and virus transmission efficiency of T. palmi. An increased oviposition potential of T. palmi has been noted in cotton and brinjal compared to other host plants. T. palmi acquires the tospoviruses at early larval instar and can only transmit the virus during adult stage. The anterior midgut is the first to be infected with WBNV in the first instar larvae. The midgut of

T. palmi is connected to the principal salivary glands (PSG) via ligaments and the tubular salivary glands (TSG). The infection progresses to the PSG primarily through the connecting ligaments during early larval instars. Infection of WBNV spread to the Malpighian tubules, hindgut, and posterior portion of the foregut during the adult stage. Maximum virus-specific signal in the anterior midgut and PSG indicates the primary sites for WBNV replication. GBNV-nucleoprotein (N) localizes in the nucleus of T. palmi cells in primary cell culture. Potential interactions of GBNV glycoproteins have been found with T. palmi enolase, cathepsin, C-type lectin, clathrin, and vacuolar ATP synthase subunit E. In silico analyses suggest that C-type lectin is the primary cellular receptor to interact with GBNV-GN. After receptor binding, virus particles probably enter vector cells by clathrin-mediated endocytosis. A total of 2,365 (1384 up- and 981 downregulated) genes of T. palmi are differentially expressed in response to GBNV infection. The key differentially regulated genes are involved in cellular transport, cell signaling, innate immunity, reproduction, and cellular metabolism

451479 3min student

Transcriptomic Response to Chilli Leaf Curl Virus Infection in Bemisia Tabaci Asia II 1

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Chilli leaf curl virus (ChiLCV; genus: Begomovirus), transmitted by whitefly (Bemisia tabaci, Hemiptera: Aleyrodidae) in a persistent-circulative manner, is a major constrain in chilli production worldwide. Control options are very limited as insecticides continue to lose their efficacy due to the emergence of resistant B. tabaci populations. Besides, insecticides adversely affect the environment and human health. Understanding the molecular interaction between ChiLCV and B. tabaci and interrupting the interrelationship can provide an alternative to pesticide- based management of the virus-vector complex. We observed significant changes in the transcripts of B. tabaci in response to ChiLCV infection. A total of 80 genes of B. tabaci were differentially expressed immediately after acquisition of ChiLCV. Among them, 29 and 51 genes are significantly up and down-regulated, respectively. The expression of twenty highly regulated genes was validated in RT qPCR. The majority of the differentially expressed genes were categorized under cellular components followed by biological processes and molecular functions. KEGG pathway analysis showed differential regulation of metabolic pathways, transforming growth factor (TGF) ? signaling pathway, regulation of actin cytoskeleton, cytokine-cytokine receptor interaction, cell adhering molecules (CAM), and adherens junction related pathways. The key genes that were highly regulated involved in receptor binding, antigen binding, epithelial cell differentiation, extracellular matrix organization, cell to cell and cell surface receptor signaling, purine metabolism, such as Toll receptor 3, Dynein heavy chain 17, Down syndrome cell adhesion molecule 1, isoform BD, Cytosolic carboxypeptidase 3, Tob1 protein, CG13607, Glutamyl-tRNA(Gln) amidotransferase subunit A, putative, Replication factor-a protein 1, GMP reductase, Dual specificity protein phosphatase, putative, T-box transcription factor TBX20, Major royal jelly protein, Inhibin beta chain, Neurobeachin-like protein 1, Protein phosphatase 1L, AT-rich interactive domain-containing protein, Homeobox protein Hox-A2, putative, Adenosine deaminase, Anther specific protein-like, NCAM.Differential regulations of genes involved in key pathways might favor the ChiLCV to get entry and circulate in B. tabaci system. The candidate genes involved in key physiological processes and ChiLCV transmission by B. tabaci would be novel targets for sustainable management of the whitefly-begomovirus complex.

Novel Plant Viruses

444910

Known And Novel Viruses Infecting Major Spices In India: Their Characterization And Management

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Spices have been considered important in culinary from time immemorial and are used for flavoring and imparting aroma in various cuisines. Though India is a leading country in the production of spices globally, viral diseases are the major yield-limiting factor in many spice crops such as stunted disease in black pepper, mosaic, chlorotic streak, and vein clearing diseases in cardamom and, chlorotic fleck disease in ginger. Though viral diseases were reported on these crops as early as the 1970s, the identity of the causal viruses remained unknown leading to the spread of the diseases to many growing regions. Research carried out at the ICAR-Indian Institute of Spices Research, India for the first time determined the etiology of these diseases. Viruses such as cucumber mosaic virus (CMV) (genus: *Cucumovirus*) and Piper yellow mottle virus (PYMoV) (genus: *Badnavirus*) are involved in the stunted disease of black pepper, cardamom mosaic virus (CdMV) (genus: *Macluravirus*), banana bract mosaic virus (BBrMV) (genus: *Potyvirus*) and cardamom vein clearing virus (CdVCV) (genus: Nucleorhabdovirus) are associated with mosaic, chlorotic streak and vein clearing diseases of cardamom respectively. Similarly, ginger chlorotic fleck associated virus 1 (GCFaV-1), (Fam: *Tombusviridae*), and GCFaV-2 (genus: *Ampelovirus*) are associated with chlorotic fleck disease of ginger. We have determined complete genome sequences of all the above viruses and diagnostics such as polymerase chain reaction (PCR), real-time PCR, loop-mediated isothermal amplification (LAMP), and recombinase polymerase amplification (RPA) assays have been developed and validated for the identification and production of virus-free planting materials. Besides, protocols for the elimination of viruses through somatic embryogenesis and meristem tip culture and production of transgenic plants have been developed in black pepper. Integration of various approaches such as resistant varieties, virus-free certified planting materials, vector management, and cultural practices are recommended for the management of viral diseases.

445665

Characterization of Virus and Virus-like Pathogens from North East India and Development of Robust Diagnostics

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The diseases caused by virus and virus-like pathogens are a major constraint in production and productivity of different crops in North East (NE) region of India. Characterization of virus and virus-like pathogens associated with these diseases and development of simplified diagnostics is a pre-requisite in designing long term durable management strategy. The virus and virus-like pathogens associated with viral complex of chilli, passion fruit and citrus were characterized. Chilli veinal mottle virus (ChiVMV), cucumber mosaic virus (CMV), capcicum chlorosis virus (CaCV) and large cardamom chirke virus (LCCV) were found associated predominantly with the viral complex of chilli in NE India with 28.4% of the tested samples showing mixed infection of ChiVMV and CMV. Association of a new potyvirus with yellow mottle and fruit deformation disease of passion fruit having a similarity of 66 to

70% for nucleotide sequences of coat protein (CP) gene with bean common mosaic virus (BCMV) group was identified. Genetically diverse isolates of Ca. Liberibacter asiaticus and citrus tristeza virus (CTV) were identified from citrus groves of NE India. Simplified template preparation and isothermal recombinase polymerase amplification (RPA) assays were developed for sensitive and robust detection of CMV, ChiVMV, passion fruit potyvirus and CTV. These assays could detect the respective pathogen up to 10-7 to 10-9 dilution of crude sap template and was as sensitive as bench mark PCR. After large scale validation using field samples, these assays are now used in routine virus indexing as standards.

441333

Wastewater Based Epidemiology: From Infectious Plant Virus Discovery, To Monitoring Of SARS-CoV-2

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Wastewater-based epidemiology enables us to study the trend of viral epidemics in larger populations and in specified geographic regions. Our established expertise in this field previously included studies of human pathogens in wastewater and studies of wastewater viromes, with special focus on plant viruses (Bačnik et al. 2020 Water Research). Our results revealed a high diversity of plant viruses and confirmed their infectivity even after their route through the tertiary wastewater treatment plant. For the purpose of studying viruses in water, we developed methods for efficient virus concentration, qPCR/ddPCR quantification, and shotgun high-throughput sequencing (HTS). With the onset of current COVID-19 pandemic, in March 2020, we have extended our experiences to determine the changing trends of SARS-CoV-2 concentrations in untreated wastewater. The presence of SARS-CoV-2 RNA in feces and subsequently in wastewater provides an opportunity to use wastewater as a complementary surveillance of the spread of SARS-CoV-2, the efficiency of adopted measures, and the detection of current and future SARS-CoV-2 variants of concern. We have assessed the stability of inactivated SARS-CoV-2 particles and patient derived SARS-CoV-2 RNA, compared different virus concentration approaches, validated the concentration using ultrafiltration and virus quantification by RT-qPCR and RT-ddPCR. We have used the obtained evaluation data to deploy an efficient approach for SARS-CoV-2 wastewater monitoring, where final normalization of obtained concentrations included quantification of naturally occurring pepper mild mottle virus as process control and spiked luciferase RNA for RNA extraction and PCR inhibition monitoring. Additionally, we used HTS, followed by bioinformatic analysis, to detect and quantify the presence of key mutations belonging to SARSCoV-2 variants of concern. Screening for B.1.1.7 variant and increasing frequencies of mutations linked to this variant in wastewater was in agreement with clinical data over the same period. The national wastewater monitoring including different Slovenian wastewater treatment plants, school and retirement home is currently helping us to follow the COVID-19 epidemics in Slovenia and support the governmental scientific advisory body.

445786 3min student

Virome Study For Identification Of Viruses Associated With Common Bean (Phaseolus vulgaris L.) In Kashmir, India

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Viruses have become major constraints in the production of Common bean (*Phaseolus vulgaris* L.). Common bean is infected by numerous viruses worldwide, mostly transmitted by aphids and some by whiteflies. To study the virus community affecting common beans in Jammu and Kashmir, India, a composite leaf samples with different symptoms representing different regions of the state were analyzed using next-generation sequencing (NGS) technology. NGS data was trimmed and the sequence reads assembled into contigs, and were analyzed using a publicly available virus sequence database. A total of three potyviruses were identified viz. Bean common mosaic virus (BCMV), Bean common mosaic necrotic virus (BCMNV), and Clover yellow vein virus (CYVV). Both BCMNV and CYVV are reported for the first time in India. Among different contigs, complete sequence representing almost full genome was used for designing primers for all three viruses to validate the NGS data. Multiple infections were found in several samples. A multiplex RT-PCR protocol was standardized for the detection of all three viruses. These findings will contribute to the development of molecular diagnostic tools and management strategies of virus diseases of common bean.

445912 3min student

Public Domain RNA Datasets As A Valuable Resource For The Discovery Of Putative Novel Viruses In Endangered Plant Species

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Transcriptome datasets available in public domain serve as a valuable resource for exploring the viral spectrum of endangered plant species. An attempt to unveil the virome of four endangered plant species native to India and China using the publicly available RNA datasets revealed seven putative novel phytoviruses. Two deltapartitiviruses- cinnamomum chago deltapartitivirus 1, 2 (CcDPV1, 2) were discovered in Cinnamomum chago while a deltapartitivirus (dactylorhiza hatagirea deltapartitivirus- DhDPV), an ophiovirus (dactylorhiza hatagirea ophiovirus- DhOV) and an unusual plant virus (dactylorhiza hatagirea benylike virus- DhBLV) were identified in Dactylorhiza hatagirea. Glehnia littoralis and Trillium govanianum contained a marafivirus (glehnia littoralis marafivirus- GlMV) and cheravirus (trillium govanianum cheravirus- TgCV), respectively. Each of the coding-complete genomic segments of deltapartitiviruses- CcDPV1, 2 and DhDPV coded for either RNAdependent RNA polymerse (RdRp) or coat protein (CP). DhOV and DhBLV genomic segments encoded CP and RdRp, respectively. Coding-complete genome of GlMV contained a large ORF coding for a polyprotein with viral methyltransferase, endopeptidase, RNA helicase, RdRp and tymovirus CP domains. A marafibox motif (CAACGCGAATTGCTTT) was also identified in the genome of GlMV. Two codingcomplete genomic segments of TgCV contained a single large polyprotein ORF similar to other cheraviruses. Phylogenetic analysis revealed the relatedness of CcDPVs and DhDPV to deltapartitiviruses of persimmon and beet, DhOV to lettuce ring necrosis virus, DhBLV to red clover RNA virus 1, GlMV and TgCV to marafiviruses of alfalfa and stocky prune virus, respectively. The viral genome sequences recovered in the study will facilitate the development of detection assays for the production of virus free plant propagules (V. Kavi Sidharthan, Gene, 2021).

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Comparative Genome Analysis And Characterization Of Banana Streak Viruses Infecting Banana Mats Of North East India: Evidence Of Recombination And Variation In Their Genetic Composition

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Banana streak disease caused by serologically and genetically heterogeneous cryptic Badnavirus species complex collectively known as “banana streak viruses” (BSV) is a major constraint in banana production worldwide. Interestingly some of the BSV species have a very unique characteristic of being integrated as endogenous forms known as endogenous BSV (eBSV) that get activated as episomal virus particles when prone to temperature stress, environmental stress, interspecific hybridization or in vitro propagation. Systematic studies on genomics of episomal BSV was lacking from Indian subcontinent, although being one of the leading region in banana cultivation and diversity. The sampling of episomal BSV in different banana cultivars from the unexplored area of North East Region (NER) of India that shares geographical boundaries to the China, Myanmar and considered as the center of origin to the Musa balbisiana (BB genotype) was done during the years 2017-19. Since, BSVs are highly complex and genetically diverse, in-depth analysis of their genetic structure and diversity are prerequisite to understand the mechanisms leading BSVs diversification. In the current study, using random-primed rolling circle amplification (RCA) we successfully sequenced nine full length episomal BSV genomes infecting natural banana hybrids in NER and characterized as banana streak MY viruses (BSMYV-IN4 to IN10), and banana streak IM virus (BSIMV-IN1 and IN2). The complete genome sequences of three BSMYV isolates viz., IN6, IN9, and IN10 were 7,650 nt in length with the predicted size of open reading frames (ORFs) similar to the reference genome of BSMYV (KR014107) while in case of BSMYV isolates IN7, IN8 and IN4, IN5; the genome varied in their sequence length from 7,466 to 7,562 nucleotide (nt), respectively, and also in their predicted size of ORFII (P2), ORFIII (P3) and intergenic region (IGR). In the phylogenetic analysis using complete genome sequences, the isolate BSMYV-IN4 and IN5 were found to be congruent in its phylogenetic placement and formed a separate group within same cluster of other BSMYV isolates. Additionally, both isolates exhibited unusual high genetic distance of 0.178 from other corresponding BSMYV isolates contrary to mean distance of 0.062 among intra BSMYV isolates. Pairwise sequence comparison of the RT/RNase H (equivalent to commelina yellow mottle virus: ComYMV K1429-M1838 ORFIII protein) region revealed that the four BSMYV isolates (IN7, IN8 and IN4, IN5) shared only 85% and 97% identity, respectively (at nucleotide level) to the reference genome (KR014107) while the corresponding sequences of BSMYV-IN6, IN9 and IN10 had 100% sequence similarity. Also, deletion of 61 residues within the RT/RNase H region of BSMYV-IN7 and IN8 resulted into shorter ORFIII. To investigate the role of the codon usage (CUB) in the evolution of the BSMYV and BSIMV isolates, a comprehensive study of relative synonymous codon usage (RSCU) pattern in the ORFIII polyprotein coding region (n=9) was performed. Two of the most frequent overrepresented codons (>1.5) such as AGA and AGG coding for arginine were found to be evolutionary conserved in the isolates of BSIMV and BSMYV and GGA coding glycine in BSMYV. Overrepresented synonymous codons coding for serine (UCU, UCA) and alanine (GCU) varied among BSMYV isolates; possibly due to variation in their genome composition. Overall, 14 recombination events were detected within 36 BSV genomes (including nine from the present study) in recombination analysis. Recombination study revealed that BSV isolates sharing high nucleotide differences in their genome sequences, took part in inter-BSV recombination. These findings provide evidence of the evolution of distinct isolates of BSMYV and BSIMV with variation in genetic compositions affecting banana hybrids in NER India.

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