VirusResearch News

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In this Issue

RESEARCH NEWS

Rapid, accurate and cost-effective peptide-recombinant VP6 protein based enzyme immunoassay for detecting group A rotaviruses

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Most of us have heard well about the rotaviruses (RVs) and their associated consequences in humans. The World Health Organization estimates that globally 2,15,000 child deaths occurred during 2013 due to rotavirus A infection, wherein India ranked on top of the list with 22% of total deaths. Epidemiographic data are available for humans, but what about animals? Such kind of comprehensive data in animals, especially in poor and middle income countries doesn't exist, in spite of critical role played by animals in the transmission of RVs to humans and generation of new reassortant RV strains with novel genomic constellations. The central reason behind this is probably non-availability of accurate and economic diagnostic tools. The high cost involved in acquiring commercially available diagnostic kits, created a major hurdle in RVs surveillance studies in animals.

To resolve this, we designed and developed a novel enzyme immunoassay for the detection of rotavirus A (RVA) antigen in faecal samples of multiple host species. The assay is based on the detection of conserved VP6 protein using anti-recombinant VP6 antibodies as capture antibodies and anti-multiple antigenic peptide (constructed from highly immunodominant epitopes within VP6 protein) antibodies as detector antibodies. For the detector antibodies, we first identified a highly immunodominant region (125-149aa) within rotavirus VP6 protein based on the high reactivity with polyclonal anti-rotavirus A sera. The solid phase peptide synthetic approach was used to synthesize this region. Four arm copies of this region/peptide sequence (multiple antigenic peptide- MAP) were constructed on lysine mosaic prepared on Wang resin. All the peptides and multiple antigenic peptides were purified by Reversed Phase-High Performance Liquid Chromatography (RP- HPLC) and characterized using Circular Dichroism (CD) spectroscopy. For capture antibodies, expression of full length VP6 protein of RVA was optimized to get high yield of recombinant protein and *E. coli* cells fulfilled this. The fusion recombinant protein (rVP6) was purified by affinity chromatography under denaturing conditions, and confirmed in the Western blot reaction. The hyper immune sera were raised against MAP and rVP6 protein in both rabbits and guinea pigs as per the standard protocol.

We used sandwich ELISA format consuming these antibodies to detect RVA antigen and compared this to Reverse Transcription- Polymerase Chain Reaction (RT- PCR). The clinical utility of the assay was evaluated using a panel of 914 diarrheic fecal samples of four host species (bovine, porcine, poultry and human) from different geographical locations of India. The diagnostic sensitivity (DSn) and specificity (DSp) of the assay in comparison to VP6 based diagnostic RT- PCR were found to be high [bovine (DSn= 94.2% & DSp= 100%); porcine (DSn= 94.6% & DSp= 93.3%; poultry (DSn= 74.2% & DSp= 97.7%) and human (DSn= 82.1% & DSp= 98.7%)]. This assay didn't show cross reactivity with other enteric (Rotavirus B, C, & Picobirnavirus) and non-enteric (Bluetongue virus) viruses and was repeatable with a low and acceptable variations. The enzyme immunoassay presented here displayed high concordance with diagnostic RT-PCR [weighted kappa (k) = 0.831–0.956 at 95% CI= 0.711–1.0] compared to RNA-Polyacrylamide gel electrophoresis (RNA-PAGE).

The performance characteristics of this assay were also comparable to commercially available ELISA kits. The prospective advantage of this assay is its cost effectiveness (Rs. 30 per sample) while maintaining high diagnostic sensitivity and specificity in multiple host species. Thus, this assay may serve as a preliminary assay for epidemiological surveillance of RVA antigen and for evaluation of vaccine effectiveness especially in low and middle income settings.

Racial factors might determine the fate of Epstein-Barr virus infections in Indian populations

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Epstein-Barr virus (EBV), a human gammaherpes virus, causes infectious mononucleosis (glandular fever), various types of

cancers, and a number of autoimmune diseases including systemic lupus erythematosus (SLE). EBVs are seen throughout the world and viral latency is seen in both epithelial and B-cells. However, the virus may enter lytic phase; after infection, the EBV nuclear antigens (EBNAs) are expressed. Thereafter, the EBV latent membrane proteins 1 and 2 (LMP1 and LMP2) are expressed; all these affect the host cellular events.

Burkitt's lymphoma (BL) was the first cancer reported with EBV infection and is the prevalent pediatric cancer in Africa. In India, EBV infection is common, but association of EBV with BL varies from 25% to 80%. Researchers have found altered expression of HLA class-I antigens, and chromosomal abnormalities like translocations in 2p, 22q, and translocation of telomeric bands between chromosomes 8q and 14q. In 2010, Vasudevan et al. have found the later type of translocation in an Indian boy with BL. Of note, all these translocations put the MYC oncogene to an enhancer of the immunoglobulin gene; thus, constitutive overexpression of MYC helps carcinogenicity. EBV infection can modulate the MYC pathway; this explains the molecular basis of BL and EBV association. Nasopharyngeal carcinoma (NPC) is a rare cancer showing prevalence in specific geographical locations with racial bias. In India also, NPC is rare except in mongoloid populations of Northeast (NE) India. The risk factors include genes, viral infection, environment, and diet. EBV infection in NPC is well reported and various viral genes are shown to contribute to carsinogenesis. The strong ethnic bias of NPC indicates specific dietary habits, like consumption of salt- or smoke-dried foodstuffs; but it is also reported that these foods contain nitrosamines, which accelerate carcinogenic characters of EBV, like alteration of host signaling pathways and HLA class-I genes. A multifactorial autoimmune disease, systemic lupus erythematosus (SLE), is also reported to be associated with EBV infection. Aberrant expressions of EBV lytic (BZLF1) and latency (LMP1 and 2A) genes, and alteration of HLA genes and T cell responses have been detected in SLE patients. Recently, Harley et al. have reported a cross-reactive antiviral antibody having identical epitopes which may bind either EBNA-1 or a self-antigen; thus explains genetic susceptibility towards SLE. In India, more than one million cases of SLE are reported per year. But EBV association with SLE, BL, or NPC is not established in Indian populations.

Interestingly, all these three diseases show higher incidences in non-European populations. They are mainly prevalent in specific races and are seen in low socioeconomic groups with poor hygiene. They are associated with EBV infections and involved HLA class-I antigens. We have established a highly efficient PCR system to detect EBV DNA in the blood sample of Indian patients with NPC. We have also found an association of p53 codon72 Arg to Pro polymorphism with susceptibility to NPC in NE patients; therefore the cellular immortalization is justified. At present, any correlation between HLA class-I alleles and EBV infection in cancers are being investigated in these populations. These might help find the molecular mechanisms involved in the EBV-mediated diseases; and procedures for early diagnosis and better treatment plans may emerge.

Chemical inhibitor of hepatitis B virus

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Plants defend themselves against the infecting viruses using their own host RNAi factors. It has been long conjectured that similar antiviral features of RNAi are also operative against the mammalian viruses. However, evidences supporting the latter hypothesis are rather meager but fast emerging. A few years ago, a group of ICGEB scientists at New Delhi in collaboration with researchers of ILBS, New Delhi, wanted to throw light on this subject by following a deadly human virus, namely, hepatitis B virus (HBV) which causes liver cirrhosis and cancer. HBV is also the cause of death of about eight lakh patients in a year worldwide. Using immunohistochemical approach on the human patients' liver samples, they showed that the regions harboring viruses are also deficient in RNAi factors, namely DICER. Down regulation of DICER correlate very well with enhanced titer of the virus. Hepatic cellline based studies also indicated that viral growth was associated with the downregulation of other RNAi factors, namely ARGONAUTE2. These findings indicated that human RNAi factors are antiviral in nature. All plant viruses encode suppressors of RNAi as a hallmark of viral counterstrategy. Presence of such suppressors of HBV should also provide firm evidence of the antiviral feature of human RNAi. Using a variety of in-house developed RNAi-sensor lines of plant, insect and mammalian cells, the group showed that the X protein of HBV, i.e., HBx, acts as a RNAI suppressor [Biochem J. (2014) 462, 347-358].

For prevention, Hepatitis-B vaccine is available and for antiviral treatment 'tenofovir' is used generally. However, there are still ample scopes for discovery of better antiviral treatment. The same group hypothesized that a small molecular drug, which can inactivate the HBx, should also act as an antiviral agent. Recently they have identified a small molecule IR-415, which was screened out of about fifty thousand compounds, derived mostly from Maybridge library that can efficiently block biochemical activities of HBx-mediated RNAi-suppression activity. The specificity of HBx mediated RNAi suppression by IR-415 (Fig. 1) was demonstrated

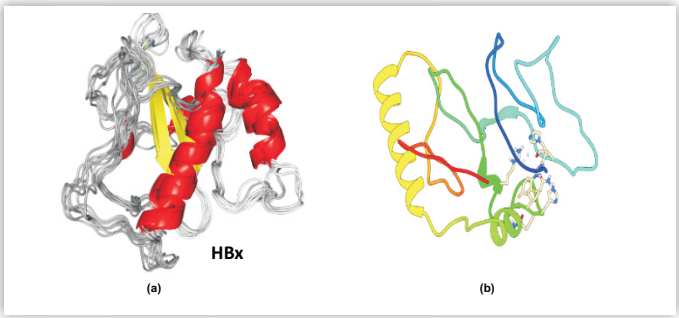

Fig. 1: (a) Ensemble of different HBx conformations during complete simulation period and; (b) The AutoDock predicted drug binding orientation of IR415 on the modeled structure HBx. Dashed lines represent Hydrogen bonds between the atoms involved.

in several ways. In cell-line based assays, IR-415 blocked suppression of HBx only and not of other suppressers, namely the B2 protein of the insect Flock house virus (FHV-B2). IR-R15 bound tightly with HBx in vitro with a K_d of 2nM. HBx represses the DICER function but IR-415 released the repression. Thus the drug IR-415 specifically blocked the RNAi suppression function of the multitasking protein HBx. The scientists predicted that IR-415 should also inhibit the viral growth. They tested the hypothesis in cell-line based studies. The transient viral replication and the production of viral proteins were inhibited by IR-415 in HepG2 cells. Thus their expectation bore fruits, proving the antiviral character of the drug IR-415 (J.Biol.Com online/doi/10.1074/jbc. M117. 775155). IR-415 was as good as tenofovir or even better as an antiviral compound depending on the cell lines used. The ICGEB group believes that similar approach could also be undertaken to inhibit the growth of other mammalian viruses like Dengue, Chikungunya, and HIV etc.

VIROLOGY NEWS

Nanoparticles as carriers of dsRNA to induce RNAi against Plant Viruses

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The discovery of Gene Silencing or RNA-interference (RNAi) has heralded a new revolution in the area of Biotechnology. RNAi-technology is successfully employed for developing Transgenic Crop Plants with desirable traits, in particular for the management of plant viral diseases. However, because of ethical concerns associated with the transgenic crops, their acceptance and cultivation may not be realized immediately. To alleviate the concerns of environment and food safety, plant biotechnologists are exploring alternative technologies that can induce robust viral resistance without having to genetically modify the crop plants. Hence, biotechnological interventions, which don't involve development of transgenic plants, are ideal alternatives for control of plant pests and pathogens.

Interestingly, in contrast to transgene derived viral resistance, the RNAi can be readily induced by topical/foliar spray application of dsRNA molecules. The dsRNA is central to the induction of the RNAi pathway in both transgenic and naturally infected plants, and thus the progress in the area of dsRNA expression systems and the application of dsRNA as a 'spray-on' technology for non-transgenic induction of virus resistance is very important and promising for the control of plant viral diseases. However there are major limitations in the topical delivery of dsRNA on the plant foliage and the stability of the dsRNA that is applied. A team lead by a plant virologist Dr. Neena Mitter and a nano-technologist Dr. Xu, from University of Queensland (Brisbane, Australia), were able to develop a nano-formulation for the topical delivery of the dsRNA and also enhance the stability and durability of dsRNA on plant foliage. This work was published in the January 2017 issue of *Nature Plants*. They identified naturally occurring non-toxic and degradable Layered Double Hydroxide (LDH) clay nanosheets. The dsRNA molecules can be firmly coated on the LDH nanosheets, which are not washed off and are gradually released for over a period of 30 days of its application. Further, the uptake of dsRNA by the plant cells and subsequent silencing of the homologous viral RNA, which ultimately results is protection from plant viruses was demonstrated. A single spray of LDH loaded with dsRNA called as "*BioClay*" protected the plants from viral infection for up to 20 days.

Although nanoparticles-based delivery of RNAi is successfully employed for human therapeutics and also for the control of viral diseases of aquatic organisms such as shrimp, the present innovation helps in the commercial exploitation of dsRNA based virus management strategy in crop plants. In addition to the control of plant viruses *BioClay* based technology could also be employed for effective delivery of RNAi to other plant pests and pathogens.

Recently Held IVS Conference

VIROCON-2017

VIROCON-2017, the 26th Annual Conference of the Indian Virological Society was hosted by Nitte University at Mangalore during December 7-9, 2017. The theme of the Conference was "**Viruses to viromes in health and disease**". The Conference was unique in bringing together virologists from medical, veterinary, agricultural and basic science disciplines at one platform. The Conference was addressed by keynote and lead speakers from India, USA, UK, Australia, St Kitts and Nevis. A total of 260 participants including 43 invited speakers and 30 senior scientists as Chair of various sessions attended the conference. Of the registered participants, there were 95 postgraduate and doctoral students from different parts of India and from Sudan and Nigeria. The Indian Council of Agricultural Research, Department of Science and Technology, Government of India, Indian Council of Medical Research and Karnataka Science and Technology Academy supported the Conference. The event was marked by the august presence of Dr. Andrew Davison, Chairman, International Committee of Taxonomy of Viruses (ICTV).

Upcoming Conference

VIROCON-2018

To be held during November 12-14, 2018 at Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh

Publications

A Century of Plant Virology in India

Mandal B, Rao, GP, Baranwal VK and Jain RK. (Editors). 2017. Springer Nature p 805. https://doi.org/10.1007/978-981-10-5672-7

The book showcased the research work on plant viruses carried out in India during past 100 years. The book provides comprehensive information on the biology, molecular biology, epidemics, crop losses, diagnosis and management of viruses and viroids occurring in India. Description of properties of the viruses are provided in the chapters comprising of different genera such as *Allexivirus, Begomovirus, Babuvirus,*

Badnavirus, Carlavirus, Carmovirus, Cucumovirus, Closterovirus, Ilavirus, Mandrivirus, Potyvirus, Tospovirus, Tungrovirus and *Sobemovirus.* Virus-vector research related to aphid, thrips and whitefly is discussed. The work on the management aspects of plant viral diseases has been described with reference to the conventional, antiviral and transgenic approaches. Further, the quarantine mechanism developed in India for the exclusion of viruses and vectors has also been included. The book also provides useful information about the capacity building on the research and education on Plant Virology in India. Overall, the book covers a wide range of accounts of research findings and innovations in Plant Virology in India during past 100 years. The book was released during the Virocon-17 at Nitte University, Mangalore.

IVS AWARDS 2017

Shyama Prasad Raychaudhuri Lifetime Achievement Award

Dr. A.C. Mishra; *Director, Interactive Research School For Health Affairs (IRSHA), Pune*

Prof. K.S. Bhargava Award

Dr. R.K. Jain; *Joint Director (Edn.) & Dean, IARI, New Delhi*

IVS Fellows

Medical Virology

Dr. Tapan Dhole; *SGPIMS, Lucknow*

Plant Virology

Dr. M. Krishna Reddy; *ICAR-IIHR, Bangaluru*

IVS-NIV Travel Bursary Awards

Gaurav Kumar; *University of Delhi (South Campus), New Delhi*

Shambhavi Rao; *AMITY University, Noida, UP*

IVS Young scientist awards

Animal Virology

Dr. S.K. Minhas, *Division of Virology, IVRI, Mukteswar, Uttarakhand*

Medical Virology

Dr. Kshitija Suhas Rane-Yadav, *MGM Institute of Health Sciences, Navi Mumbai*

Plant Virology

Dr. Gaurav Kumar, *Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi*

Poster awards

Animal Virology

First :Manu M, *ICAR-Indian Veterinary Research Institute, Izatanagar*

Saraswathy P V, *National institute of Animal Biotechnology, Hyderabad*

Second: Gnanavel Venkatesan, *ICAR- Indian Veterinary Research Institute - Mukteswar*

Subhankari Sircar, *ICAR-Indian Veterinary Research Institute, Izatanagar*

Medical Virology

First: Mini P Singh, *PGIMER, Chandigarh*

Sudheesh N., Manipal Centre for Virus Research, Manipal University

First: Barnali Nath, Indian Institute of Technology, Guwahati

Kannan Balakrishnan, School of Life Sciences, University of Hyderabad

Plant Virology

First: A I Bhat, ICAR- Indian institute of spices research, Kozhikode

Y S Shreenath, IARI, Pusa campus, New Delhi

Second: Namisha Sharma, National Institute of Plant Genome Research, New Delhi

Kamlesh Kumari, University of Delhi South campus

Award of Best Paper in VirusDisease

Springer Nature-IVS Award-2017

Animal Virology

Molecular typing and phylogenetic analysis of classical swine fever virus isolates from Kerala, India. Nimisha Bhaskar, Chintu Ravishankar*, R. Rajasekhar, K. Sumod, T. G. Sumithra, Koshy John, M. Mini, Reghu Ravindran, Shiju Shaji and J. Aishwarya

Aquatic Virology

Gene expression profiling in gill tissues of *White spot syndrome virus* infected black tiger shrimp *Penaeus monodon* by DNA microarray, M. S. Shekhar*, A. Gomathi, G. Gopikrishna, and A. G. Ponniah

Medical Virology

Awareness and acceptance of human papillomavirus vaccination among health sciences students in Malaysia. Kingston Rajiah*, Mari Kannan Maharajan, Nang Sue Chin and Kelly Sze Fang Num

Plant Virology

First genome analysis and molecular characterization of Chickpea chlorotic dwarf virus Egyptian isolate infecting squash. Inas Farouk Fahmy*, Omnia Taha and Abdel Nasser El-Ashry

Guidelines

Submit news article, which has some application prospect to any one of the editors. The article to be written in a popular format not exceeding 1000 words with a few simple table and or high quality figures. Article structure: Title, author(s), full address, email, telephone, self photo of corresponding author, running text.

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