VirusResearch News

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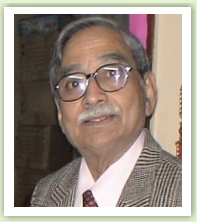
From IVS President Desk



It is always a pleasure to bring something latest to our valuable reader of the IVS Newsletter: VirusResearch News. The increased city pollution and conditions like a gas chamber being in process of development, the human sufferings are on increase with each day. As we have created the situation we have to find a solution too. The earlier, the better. This is an invitation to all our readers to discuss and suggest. Most of the classic discoveries are found with our observation and initially discarded as lunatic views.

The atmospheric pollution is due to our excess use of hydrocarbon. There is considerable increase in respiratory cases in young children. What to do and what not to do is a question before us? There are many more added to our misery and resultant increase of new diseases in human including animal as well as plant. Diagnostic and research tools are increasingly made available. I wish to appeal through this message that we should become more vigilant and observant to detect any abnormalities and report. The year 2015 is about to close and a new a NEW 2016 about to start, my best wishes to each one of you that the incoming year proves a year of correction for the world to live in a better health environment.

A.K. Prasad
President, Indian Virological Society

In this Issue

RESEARCH NEWS

Diagnostics, vaccines and drugs for dengue

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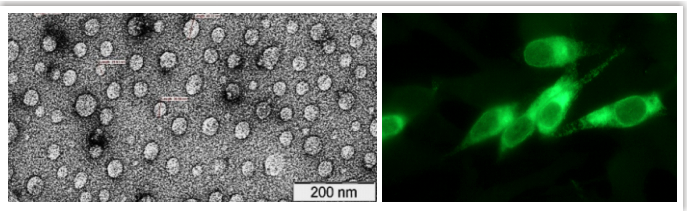
India is home to roughly half the world population estimated to be at risk of dengue. Clinical presentations of the disease range from mild dengue fever to severe and potentially fatal dengue haemorrhagic fever and dengue shock syndrome. Our group has been working in developing diagnostics, vaccines and drugs to address the problem of dengue in India. The group was born out of collaboration with my colleague and friend Dr. Navin Khanna way back in the late 1990s in the International Centre for Genetic Engineering and Biotechnology (ICGEB) in New Delhi. This collaboration has endured despite my relocation to Birla Institute of Technology-Pilani, Hyderabad Campus (BPHC) a little over two years ago.

We developed a technology to create designer antigens capable of detecting serum antibodies to dengue with a high degree of sensitivity and specificity. Essentially, this technology which we call the Multi Epitope Protein (MEP) technology, involves careful selection of linear, immunodominant epitopes from key viral proteins involved in the induction of anti-dengue immune response, and splicing them together in a tandem array, separated by flexible peptide linkers. This artificial antigen is produced in *E. coli.* The dengue MEP technology has been successfully transferred to two companies in India. A few years ago, we also developed a sensitive test for dengue antigen detection and incorporated it into a combo test that detects dengue NS1 antigen as well as anti-dengue IgM and IgG antibodies. This is currently available in the market as “Dengue Day 1 Test”. The MEP technology has proven to be a versatile diagnostic platform as we have successfully adapted it to the detection of HCV and HIV infections as well.

Our group has made significant progress in dengue vaccine development in recent years with impetus from the Indo-US Vaccine Action Program. We have been exploring various strategies based on the major envelope (E) protein of dengue viruses to develop vaccine candidates. The region of the E protein important in the infection process is known as EDIII. Our group discovered that linking EDIIIs of dengue virus type-2 and -4 resulted in a chimeric antigen capable of eliciting potent immune responses to both these dengue virus types. We then provided proof-of-concept for the first time that linking EDIIIs



Dengue VLPs induce antibodies which can recognize dengue virus in infected cells efficiently (left panel: electron micrograph of non-infectious dengue VLPs produced using *P. pastoris*; right panel: indirect immunofluorescence assay of using anti-VLP antiserum).

of all four dengue virus serotypes into single antigen molecule can elicit potent immune responses to all four dengue viruses. In parallel, we collaborated with Prof. U. Rinas in Germany to develop the yeast *Pichia pastoris* as a possible host system for the production of vaccine antigen in the form of virus-like particles (VLPs). Using hepatitis B surface antigen (HBsAg) as a model, this collaborative effort was successful in obtaining the highest reported yields of this antigen.

Together with Ranbaxy Research Laboratories, we have found that an alcoholic extract prepared from a plant, *Cissampelospareira* Linn, could function as a pan-dengue inhibitor in dengue virus-infected cells in culture and also conferred significant protection in mice against dengue infection. The extract did not did not have toxic effects in rats and did not affect platelets and red blood cells.

Passive protective efficacy of polyclonal anti-A27L anti-H3L antibody cocktail in mice model against buffalopox virus

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Buffalopox virus (BPXV) is responsible for emerging and reemerging zoonosis affecting buffaloes, cattle and humans in India and other countries. Buffalopox primarily affects buffaloes but recent outbreaks show trend of increased and concurrent incidence in cows and humans with unique report of generalized buffalopox in humans in Andhra Pradesh in which patient was suffering from Darier's disease, an immunodeficiency disease. Affected animals develop localized pock lesions on udder and hindquarters with secondary infections or rarely generalized lesions and transmit infection to in-contact humans which show local pock lesions on hands, arms accompanied with fever and axillary lymphadenopathy. Zoonotic nature of the virus doesn't permit live virus based diagnostic assays and prophylactic measures.

With an aim to develop suitable recombinant protein based prophylactic as well as immunodiagnostics, two immunogenic envelope proteins of BPXV viz. A27L (14kDa) and H3L (35kDa), which are involved in viral entry and maturation, were expressed and evaluated for their combined passive protective efficacy in mice model. We cloned mature A27L and partial H3L genes of BPXV-Vij/96 strain into pET32a vector and over-expressed in expression hosts, *E coli* BL-21 and Origami cells respectively as fusion proteins. After confirmation of specificity of proteins using SDS-PAGE and Western blot using anti-BPXV and anti-Camelpox virus sera as 30 and 50 kDa fusion proteins corresponding to A27L and H3L respectively, recombinant proteins were purified using Ni-NTA affinity chromatography under native conditions. Purified proteins were combined at varying doses and used for immunization of adult mice with or without adjuvants (Freund's adjuvant, alum and CpG) followed by passive-transfer experiments in suckling mice for evaluation of *in-vivo* protection by anti-A27L and anti-H3L serum. Following immunization with protein cocktail, indirect-ELISA and SNT showed revealed a gradual increase and higher specific IgG and neutralization antibody as compared to that elicited by individual proteins. Passive administration of combined anti-A27L and anti-H3L serum to suckling mice showed a complete 100% pre-exposure protection upon challenge with virulent BPXV as compared to 60% and 80% protection with individual anti-A27L and anti-H3L serum, respectively.

In the post-smallpox eradication phase where current population lacks antibodies to poxviruses, chances of genetic changes in zoonotic pox viruses leading to generalized disease and possible human-to-human transmission can't be ruled out. Therefore occurrence of buffalopox, camelpox and other zoonotic orthopoxviruses needs to be kept under surveillance.

Light intensity affects systemic spread of silencing signal in plants

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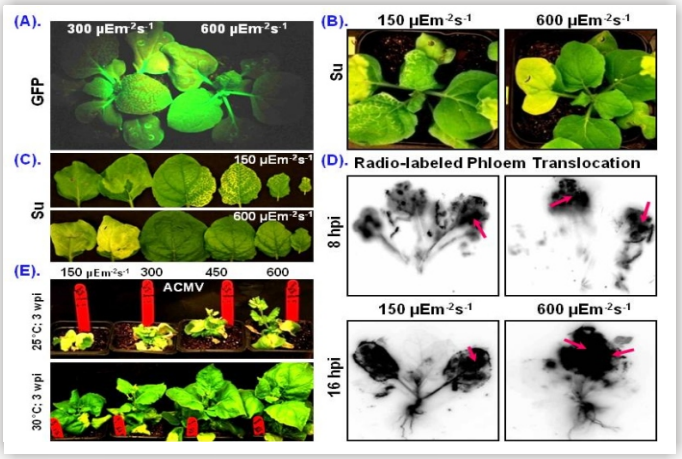
Environmental conditions have a critical role in determination of symptom severity of virus infected plants.RNA-interference (RNAi) is a natural antiviral defense mechanism present in the plants, which is part of the host innate immunity and to counteract this, viruses have evolved gene silencing suppressors. Past studies have shown that several factors manifest the movement of silencing signal. Our transient agro-infiltration studies show that, the light intensity has a significant impact on systemic movement of silencing signal in Nicotiana benthamiana. At higher light intensities ($\geq$450 $\mu Em^{-2}s^{-1}$), the gene silencing was localized to the leaf tissues. Interestingly, in this light condition ($\geq$450 $\mu Em^{-2}s^{-1}$) the *N. benthamiana* plants also showed recovery from the viral symptoms. However reduced systemic silencing and the reduced viral symptom severity at higher light intensities were due to the change in the sink-source status of the plant, ultimately affecting the phloem translocation of small RNAs or the viral genome. Whereas at lower light intensities (<300 $\mu Em^{-2}s^{-1}$) with a constant temperature of 25°C, there was strong systemic movement of silencing signal in the *N. benthamiana* plants and also there was reduced recovery from virus infections. This work has been recently published in the journal Molecular Plant Pathology (2015). There are several potential applications of these important findings in the field of functional genomics and virus control using RNAi. Of late most of the virus resistant transgenic plants are based on RNAi strategy and it is important that these transgenes express efficiently to effectively ward off the viruses. Such RNAi-based transgenic plants, like the vegetable or flower crops, which are grown in green house conditions, can be subjected to optimum environmental conditions for effective expression of small RNAs. Transient virus protections can also be taken up in favorable light and temperature conditions by spraying synthetic small RNAs on the plant foliage.



Different levels of systemic silencing of GFP and Su genes by RNAi through transient agro-infiltrations in Nicotiana benthamiana at different light intensities (150 and 600 $\mu Em^{-2}s^{-1}$). (Patil and Fauquet, 2015, Molecular Plant Pathology. 16: 484-494).

Epstein barr virus promote cancer metastasis

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Even with the recent advances in medical science, cancer metastasis is still an unsolved problem and is the major cause for cancer related deaths. One of the key process in cancer cells metastasis is epithelial to mesenchymal transition (EMT) when epithelial cells loses their epithelial properties and become progressively multiplying migratory cells. Recent advances in understanding of cancer stem cells suggest that trying to control and contain the cancer instead of trying to cure it may prove to be a better option. Therefore there is a need to develop new methods and strategies that can prevent cancer from metastasizing and keep it localized. Studying modulation of cellular pathways such as important for EMT in virus associated cancer might provide an opportunity to develop such strategies. One such virus is Epstein-Barr virus (EBV) that affects 90% of human population and is associated with many lymphoid and epithelial malignancies. In EBV latently affected cells only a small subset of genes is expressed constitutively including nuclear proteins EBNA1 and EBNA3C. We had previously reported that cancer cells expressing EBV nuclear antigen EBNA3C and/ or EBNA1 showed higher motility and migration potential and had a propensity for increased metastases when we tested in nude mice model. In our recently published study, seven EMT markers were investigated that include 3 transcription factors Slug, Snail, and TCF8/ZEB1 and 4 cell adhesion or cytoskeletal molecules vimentin, β-catenin, ZO-1, and Ecadherin. We showed that EBNA1 and EBNA 3C modulates different EMT markers indicating a clear shift toward mesenchymal phenotype. The detection of similar EMT marker expression patterns in mice primary tumor and lung metastases derived from EBV latent antigen-expressing cells strongly suggested the critical role of EBV latent antigens EBNA3C and EBNA1 inepithelial to mesenchymal transition leading to cancermetastasis. Any successful cancer treatment will have to take into account possibility of recurrence and metastasis. Our work suggests a key role for EBV latent antigens in this process. Understanding of molecular mechanism for modulation of cellular pathways by EBV latent antigens may provide us clues for intervention in patients diagnosed with EBV-mediated cancers and who are at greater risk of relapse and metastasis.

Human papillomavirus, an oncogenic Sumo wrestler!

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Human Papillomavirus (HPV) is a major causative factor of cervical cancer, a slow growing fatal disease. Cervical cancer is associated with a significant women death burden worldwide with a death toll of 16% women in India and 7.5% worldwide. Despite extensive research in this area, the precise mechanism that operates behind HPV induced cervical cancer remains undefined. However, it is known that the HPV oncoproteins E6 and E7 are the casuals for malignancies in most of the cases. E6 acts by interfering with the tumor suppressor functions of p53 whereas E7 degrades pRb leading to loss of cell proliferation regulation and thus, uncontrolled cell growth. Previous studies have suggested that there might be other cellular proteins that 16E6 may target in order to promote oncogenesis. One such novel target of high risk HPV16E6 is the human ADA3 protein which is a transcriptional activator adaptor by nature. Abolishment of the co-activator function of hADA3 by interaction with the oncogenic HPV16E6 has been shown to contribute to the risk of developing cervical cancer. However, the mechanism by which E6 brings about the degradation of hADA3 is elusive. We have attempted to answer some of the major questions of this area by using HPV-positive cervical cancer cell lines. We have demonstrated a reduction in the level of endogenous hADA3 as a result of E6 mediated proteolysis which was shown to be stimulated by ubiquitination of hADA3 by HPV16-E6 via active participation of E6AP ubiquitin ligase. The hADA3 was also shown to undergo extensive SUMOylation in the presence of HPV16E6. We provided evidence, for the first time, that E6 mediated ubiquitination and degradation of hADA3 may be driven by post-translational modification of hADA3 in the form of SUMOylation which makes it unstable. Also, we investigated the functional relevance of E6 mediated degradation of hADA3 in HPV cancer cell lines. Our study proposes a possible mechanistic basis for HPV pathogenesis by identifying HPV16E6 mediated SUMOylation of hADA3 as the most likely cause of its down regulation in cervical cancer cells and thus, leading to oncogenesis. This work also shows how E6 attacks the cellular SUMO machinery and wins the battle by manipulating key enzymes. These findings open up exciting perspectives in the study of this new posttranslational modifications of hADA3 as well.

A novel scorecard technique to assess the disease severity pattern of peste des petits ruminants in sheep and goats

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Peste des petits ruminants (PPR) that literally means “plague of small ruminants” and is associated with high morbidity and mortality in small ruminants like sheep and goats. The disease is endemic in India and affects the economy of the small ruminants rearing farmers. The DADF, GoI, had launched a National Control Programme for PPR during the year 2010-11 as first phase with a mass immunization vaccination programme using live attenuated PPR vaccine with an aim to control and eradicate this disease from India in a time bound manner on the lines of rinderpest eradication (http://dahd.nic.in). The disease incidence has been in decline over the past five years due to progressive mass vaccination in some states of India.

Currently, due to vaccination programme implementation, the disease epidemiology has altered and warrants the studies on the effect of vaccination; disease severity pattern in different geographical locations under both vaccination and non-vaccinated areas. In this direction, a methodology on PPR clinical scorecard was attempted by the authors to assess the clinical disease pattern in sheep and goats flocks using certain scientific inputs and rational assumptions.

The clinical score card was developed based on the international and national researchers' opinion and field veterinarians' assessment by Delphi technique. Score card was prepared taking into consideration of five major clinical signs of PPR viz. fever, nasal and ocular discharge, oral or mucosal lesions, respiratory signs and diarrhoea.

The scores assigned for different clinical signs were based on the severity of clinical signs. The weightage were given for different major clinical signs along with mortality and morbidity. Thus, based on score, Weighted Score Index was calculated to know the range of disease severity pattern from very mild to very severe.

The developed clinical scorecard was evaluated in the field during outbreaks to assess the disease pattern in sheep and goats flock. This scorecard would be useful in assessing the severity of the disease pattern during field investigation of PPR outbreaks and also useful in assessing disease pattern in vaccinated and non-vaccinated regions.

Recently held IVS Conferences

1. "VIROCON-2015"

The **XXIV National Conference** on "**Transboundary Viral Diseases under One Health: Perspective and Challenges"** organized by the **North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences (NEIGRIHMS)** at **Mawdiangdiang, Shillong**, **Meghalaya** from 8-10 October, 2015 was a great success. A flag of IVS design by Dr. Anil Phukan and his collegues was released.

Young Scientists Awards 2015
1. Mr. Abdul Kader Jailani, Plant Virology
2. Ms. Ankita Agarwal, Medical Virology
3. Dr. Lipsa Dash, Animal Virology

IVS-NIV Travel Bursary Awards 2015
1. Ms. R. Priyanka, TNAU, Coimbatore;
2. Ms. Archana Anokhe, ICAR-IARI, New Delhi
3. Mr. Pradeep Kumar, ICAR-IARI, New Delhi

2. One day Symposium

Challenges in Plant Virology and Our Preparedness was held at IARI, New Delhi on Dec 5, 2015. The 75th birth anniversary of Prof. Anupam Varma was celebrated in one day symposium.

Awardees of **"Best Poster Presentation"** at One Day Symposium:

1. Vipin Singh Rana, Dept. of Zoology, University of Delhi, Delhi
2. Nandita Shahana, UBKV, Cooch Behar, West Bengal
3. Madhupriya, Division of Plant Pathology, IARI, New Delhi

Stamp release on occasion of Prof. Anupam Varma, 75th Birthday

Latest in Virology

Armed against Ebola

Less than two years ago, the world was rocked by the Ebola outbreak in West Africa. Wide spread occurrence of Ebola in largely due to lack of availability of drug or vaccine. Finally, we are armed with an effective vaccine against the deadly virus. The study published in Lancet heroes the VSV-EBOV vaccine, developed at Public Health Agency of Canada. The vaccine is based on a recombinant vesicular stomatitis virus genetically engineered to express Ebola virus glycoproteins to train the host immune system.

Close contacts of the infected individuals and Frontline workers were vaccinated either immediately or three weeks after the contact with the infected one. In 2,014 subjects vaccinated immediately, zero cases were reported. In those vaccinated later, there were only 16 cases. The vaccine had shown 100% effectiveness. The vaccine has been deemed "remarkable", "game-changer" and "extremely promising" because of its high efficacy.

Upcoming Conference of IVS

- The 8th International Geminivirus Symposium and 6th International ssDNA Comparative Virology Workshop, 7-10 November 2016 at New Delhi (Contact: Dr. Indranil Dasgupta; indranil58@yahoo.co.in)
- VIROCON 2016 at IIHR, Bengaluru (date will be announced later; Contact : Dr. M.K. Reddy; mkreddy@iihr.ernet.in; mkreddy60@gmail.com)

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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