VirusResearch News

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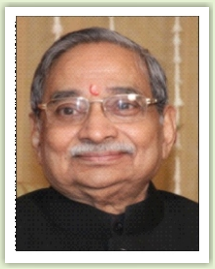
A Half Yearly Newsletter of Indian Virological Society

From IVS President Desk



Vaccine Advocacy

Vaccine Advocacy Forum was launched by 8 like minded people who believe that prevention is better than cure. India is still struggling with the disease burden of infections of bacterial, viral & zoonotic origin. The infrastructure of healthcare system in India is grossly legging behind in taking care of the massive disease burden in its huge population. The bed strength, healthcare professionals & available medicines are not adequate to take care of diseased population. In the light of above situation, the preventive strategies with the Vaccines available for the vaccine preventable diseases are most relevant in the form of both economy, saving of human morbidity & mortality. Preventive vaccine usage has paid risk dividends in infants & children by showing its positive effects on morbidity & mortality in this segment of population. It is high time to use these preventive vaccines in adults & older adults.

Vaccine Advocacy Forum strongly feels the need for advocacy programs for vaccination among policy planners, scientific bodies, medical professionals & public at large. To create total national health care for the country will be an up-hill task for any country including India. Therefore the easier and cheaper way will be to adopt preventive vaccine on mass scale to create an "Immune Population" and prevent the occurrence of preventable diseases both for human as well animals including birds. On the very face, this sounds big and difficult but in actual this is very much possible. It is to your knowledge small pox is a disease of past and totally eradicated from the world. Likewise Polio is also becoming the disease of past with no case reported from India. The available preventable vaccines adoption is a possibility. They are a safe, cost-effective, and efficient way to prevent sickness and death from infectious diseases. Vaccines have led to some of the greatest public health triumphs ever, including the eradication of naturally occurring smallpox from the globe and the near eradication of polio.

In this Issue

RESEARCH NEWS

Egg yolk antibodies emerge as tailored diagnostic and therapeutic antibodies

Lavleen Kumar Gupta

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The avian egg contains all of the necessary nutrients and growth factors required for the developing embryo, including antibodies that are transported from the blood of the hen into the egg yolk to provide immunity to the chick. Egg yolk antibodies, now called immunoglobulin Y (IgY), which is the functional equivalent of immunoglobulin G (IgG) in mammals. IgY is experiencing an enormous economic growth mainly due to the high specificity, strong avidity and low production costs as compared to mammalian antibodies. This economic advantage allows for its application not only in the design of diagnostic systems, but also in generating prevention and treatment products for various deceases, both in animals and humans.

The alarming increase of resistant microbes including viruses is today one of the biggest threats to both mankind and environment. The frequency of antibiotic resistance organisms has been on the rise at an alarming rate against a backdrop of decreasing numbers of new antibiotics being developed and added to the market. Simple and effective natural remedies of which IgY comprises the most potent and easily generated substitute to antibiotics. The extraordinary amount of antibody obtained from egg yolk opens the door also for using IgY antibody in human and veterinary medicine for therapeutic/prophylactic purposes.

IgY antibodies have been explored to use for research antibodies and have been found useful and reduce to non-specific background using mammalian antibody sources for mammalian or microbial antigen detection. IgY antibodies provide high specificity, strong avidity and low assay background have been utilized for many immunoassays. It is also ethically more attractive to produce chicken antibodies, as they are purified from the yolk, unlike the production of mammalian IgG antibodies, IgY production does not require bleeding of animals.

Recently, successful progresses have been achieved in Japan, Korea and Germany through industrialization of IgY technology. IgY has been shown to provide a safer, more efficient and less expensive method for managing disease-causing pathogens. IgY antibodies are obtained by immunizing the hen with the antigen of interest. A small amount of antigen in the milligram or microgram range usually elicits enough IgY response and the antibody titers persist over several weeks to several months.

IgY producing company is not visible in India and on the contrary, there are several research publications from academic institutions that give confidence for efficacy and scalability. IgYImmunologix has put her path forward for developing IgY technology with mandate to develop precision antibodies with designer antigens for research and diagnostics. With a clear vision of developing of meaningful antibodies, company values for customized and tailored antibodies for diagnostics and therapeutic potential.

RT-LAMP: A rapid, specific and sensitive diagnostic assay for chilli veinal mottle virus

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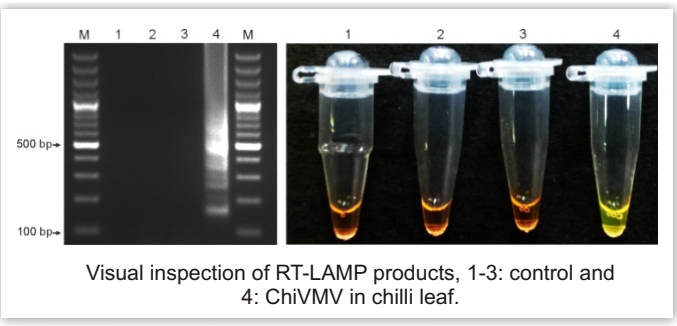
Chilli veinal mottle virus (ChiVMV, genus *Potyvirus,* family *Potyviridae*) is an economically important virus infecting chilli crop throughout eastern Asia. ChiVMV is a major limiting factor of chilli production in North-East India. The use of conventional phytosanitary practices is often inefficient against ChiVMV because it spreads rapidly in the field through aphids. Disease control is still largely based on the rouging of infected plants and controlling aphid vectors. Therefore, an early and efficient detection of the virus is necessary to prevent further spread of the virus in the fields. The available methods for detecting ChiVMV are time consuming and require expensive equipment, sophisticated laboratory set up and highly skilled personnel. However, reverse transcription loop-mediated isothermal application (RT-LAMP) is a novel gene detection method that amplifies nucleic acids with high specificity, efficiency and rapidity under isothermal conditions. It is cost effective, user-friendly and can be carried out in a simple laboratory set up using a water bath or heat block. We developed a RT-LAMP for specific and sensitive detection of ChiVMV.

The primer set composed of two outer primers (F3 and B3) and two inner primers [FIP (F1c+F2) and BIP (B1c+B2)] were designed from large nuclear inclusion protein (Nib) region of ChiVMV isolate KC-ML1 from Meghalaya (accession number KM222501) using the online PrimerExplorer V4 software (http://primerexplorer.jp/e/). The optimized RT-LAMP reaction mixture (Table 1) was incubated in a thermo cycler (Eppendorf, Germany) at 63°C for 60 min, and then heated at 80°C for 10 min for termination. The amplification products (3.0 µl) were detected using 2% agarose gel, as well as, by visual detection based on colour differentiation between positive and negative reactions using 1.0 µl SYBR Green I (1000×) (Invitrogen, USA) per 10 µl reaction mixture. The successful RT-LAMP reaction produced ladder-like bands, as detected by agarose gel electrophoresis, only in case of ChiVMV infected samples. In addition, the colour of RT-LAMP products changed from orange to green in positive samples by adding SYBR Green I, while the colour remained orange for the healthy plant and water controls.

The specificity of RT-LAMP to ChiVMV was evaluated including other chilli infecting potyviruses *viz. Pepper veinal mottle virus* (PVMV)*, Tobacco eatch virus* (TEV), *Potato virus Y* (PVY), *Pepper mottle virus* (PepMoV), *Pepper yellow mosaic virus* (PepYMV), and *Pepper severe mosaic virus* (PepSMV). Both agarose gel electrophoresis and SYBR Green I staining showed an obvious amplification only in ChiVMV positive sample. Thus, the negative cross-reactivity of RT-LAMP primers to other chilli-infecting potyviruses proved high specificity of the developed primers. For comparison of relative sensitivity of RT-LAMP to that of RT-PCR for ChiVMV detection, the template RNA from chilli leaves infected with ChiVMV was serially diluted 10-fold (*i.e.* from 100 ng to 0.00001 ng) and was used independently in both assays. The RT-LAMP assay was found to be 100 times more sensitive.

Overall, the developed assay can be a potential diagnostic tool for rapid, specific and sensitive screening of chilli plants, even at a very initial stage of virus infection. Moreover, the assay will be useful in places like north-east India, where laboratory facilities and other resources required for conventional molecular diagnostic techniques are lacking.



Visual inspection of RT-LAMP products, 1-3: control and 4: ChiVMV in chilli leaf.

Role of gammaherpes viruses for deregulating cell signaling and immune surveillance in cancers

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Tumor viruses have evolved unique strategies for modulating the expression of several cellular genes and signaling networks to enhance persistence, latency and survival of infected cells. Among them, gammaherpesviruses, specifically Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) are associated with a wide range of human malignancies. EBV latent antigen 3C (EBNA3C) is particularly essential for EBV-induced immortalization of B-cells. Interferon regulatory factors (IRFs) were considered as key transcription factors of the IRF family that regulate diverse functions in B cell development. Our work also revealed that EBNA3C stabilize IRF4, which leads to downregulation of IRF8 by enhancing its proteasome-mediated degradation. Interestingly, si-RNA mediated knock-down of endogenous IRF4 results significant reduction in proliferation of EBV-transformed lymphoblastoid cell lines (LCLs), as well as augmentation of DNA damage-induced apoptosis. These studies thus add a novel molecular link by which EBV deregulates cellular activities, through differential regulation of IRF4 and IRF8 activities increasing the potential for therapeutic intervention against EBV-associated cancers. Notably, oncogenic serine/threonine kinase PIM-1 is linked to vital cellular functions. Our comprehensive research explored the molecular mechanism by which EBNA3C enhances Pim-1 expression in EBV-infected primary B-cells. We observed that EBNA3C physically associates with Pim-1 through its amino-terminal domain, and forms a molecular complex in B-cells. Moreover, EBNA3C stabilize Pim-1 through abrogation of the proteasome/Ubiquitin pathway to enhance Pim-1 mediated phosphorylation of p21 at Thr145 residue. Henceforth, our study not only describes the critical role of oncogenic protein Pim-1 in EBV-mediated progression of lymphoma but also opens up novel insights into cellular oncogenic kinase-targeted therapeutic intervention in EBV-associated human malignancies. Like EBV, KSHV also directly influences T-lymphocyte activation via targeting the level of MHC-II expression. Particularly, KSHV encoded LANA (latency associated nuclear antigen) is known to evade MHC-I peptide processing, however, the effect on MHC-II remained unclear. We investigated that KSHV-LANA down-regulates MHC-II expression and presentation by inhibiting the transcription of MHC II transactivator (CIITA) promoter pIII and pIV in a dose-dependent manner. Moreover, our study demonstrated the role of LANA for evading MHC-II presentation and suppressing CIITA transcription to provide the unique strategy of KSHV escape from immune surveillance by cytotoxic T-cells. The research work on gammaherpesviruses associated malignancies was initiated in the Department of Microbiology and Tumor Virology Program, Abramson Comprehensive Cancer Center, Perelman School of Medicine at the University of Pennsylvania and at present

the work is being carried out at the Amity Institute of Virology and Immunology, Amity University, Uttar Pradesh, India. Overall, our research will help to provide several lines of evidence as to the mechanisms of differential regulation of host factors during gammaherpesvirus-mediated oncogenesis and also to come up with the potential combating approaches

CRISPR/Cas9 technology for plant virus resistance

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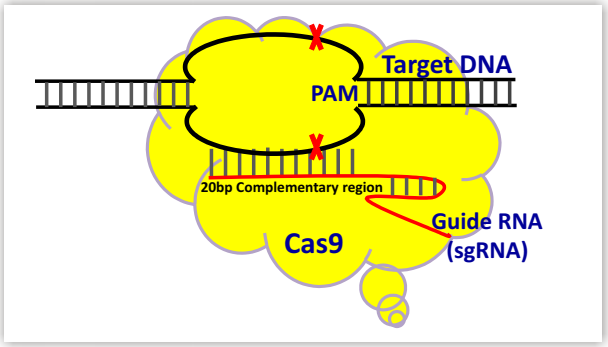
Globally, plant viruses cause extensive damage to the crop plants resulting in huge reduction in the food production. There are several approaches to develop virus resistant crop plants, one of which is introgressing virus resistance genes from wild relatives by classical crop breeding. Advances in gene mapping technologies has resulted in identification of several virus resistant genes, however crossing incompatibilities has hampered their introgression through plant breeding. The recent advent of genome editing technologies has ushered a new era of transgenic technologies for crop improvement. By using genome editing one can directly edit or introduce the alleles to confer virus resistance directly in the crop plants. This saves the time taken for several generations of back crossing and also preserves the plant ideotype.

Since its discovery in 2013, the RNA-guided gene/genome editing using the CRISPR-Cas9 (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats) derived from the bacterium *Streptococcus pyogenes* has revolutionised the genome editing technology in plants. The basic function of CRISPR-Cas9 system in the bacteria is to provide immunity against the invading phages and conjugative plasmids. The CRISPR-Cas9 technology essentially works by non-homologous end joining repair of the double-strand breaks in DNA, which results in insertions or deletions of few base pairs in the target sequence. The CRISPR-Cas9 based editing has been demonstrated in various plant families such as *Solanaceae*, *Cruciferae*, *Poaceae* and *Fabaceae*. CRISPR/Cas9 technology is also being explored to develop personalized therapies for individual patients with HIV-1 variants (Hu *et al.*, 2014).

Many of the plant virus resistance genes are recessive in nature, including the eukaryotic translation initiation factors *eIF4E*. The complex of such initiation factors and other host factors, bind to the viral 5' cap structure and the 3' poly-A tail of mRNA for translation. The plant viruses, potyviruses in particular, associate with these proteins, through the viral-encoded protein (VPg). The copy numbers of the *eIF4E* gene and its isoforms vary among plant species. Broad spectrum virus resistance has been displayed by silencing the *eIF4E* gene in tomato and melon.

A virology group lead by Dr. Amit Gal-On from Volcani Center in Israel, recently employed CRISPR/Cas9 strategy to mutate the cucumber *eIF4E* gene at two different sites by designing two sub genomic RNAs or single guide RNAs (sgRNA1 & sgRNA2) for site specific sequence recognition (Chandrasekaran *et al.*, 2016, *Molecular Plant Pathology*). The non-transgenic T_3 generation homozygous plants with deletions in the *eIF4E* gene were challenged with diverse viruses of the family *Potyviridae* such as *Cucumber vein yellowing virus*, *Zucchini yellow mosaic virus* and *Papaya ring spot mosaic virus-W*. The virus challenge experiments showed that the progenies of these T_3 generation mutant plants showed broad virus resistance compared to the susceptible control plants.

In addition to the control of RNA plant viruses, the DNA plant viruses, family (*Geminiviridae)*, which includes the most notorious *Tomato yellow leaf curl virus* and the *Cabbage leaf curl virus* have also



been controlled by employing CRISPR/Cas9 technology (Ali *et al.*, 2015; Yin *et al.*, 2015). However in this case the sgRNAs had sequence homology with the geminiviral sequence, in contrast to the host genes in the case of potyviruses. Thus by using an array of sgRNAs, the CRISPR/Cas9 technology can be used for control of multiple and diverse viruses infecting a crop plant.

VIROLOGY NEWS

The influenza pandemic and vaccines

Rahul Shekhar

Influenza is a contagious viral disease caused by the well known influenza virus - a RNA group of viruses belonging to family Myxovirus. Influenza virus has over 300 different serotypes of respiratory viruses which affect the upper respiratory tract. It has 3 subtypes - A, B and C, of which A and B is the most subtypes of seasonal influenza. An influenza pandemic can occur depending on the mutation of the surface antigen, H&N. In 2009, the WHO declared the Mexican swine flu virus (pH1N1) as the reason for the influenza pandemic. This virus despite having low mortality caused great chaos due to its highly invasive character. It spread to over 200 countries within the time span of 3 months, marking the beginning of the pandemic. Due to its invasive character it successfully replaced the original H1N1 in circulation.This deadly virus has higher mortality in India due to lack of awareness and preventive measures. According the WHO report released on October 05 2015, many countries in the world had reported low levels of influenza, however, India reported increased activity mainly due to the A(H1N1)pdm09 virus present in the country.

There are preventive vaccines that have proven to be quite efficacious in combating influenza in the past 60 years. The vaccine required depends on the person and need. There is a split/subunit vaccine, an attenuated and live killed vaccine and even a tissue culture vaccine for those allergic to egg. The influenza vaccine (3 - strain) used to contain two immunologic Influenza type A strains and one Influenza type B strain. Lately, it was observed that the just one type B strain wasn't providing satisfactory results due to which another was added to give better coverage from the type B strain. Now there is a 4 - strain vaccine that protects the people better and studies have shown that the 4 - strain vaccine causes side effects analogous to those caused by the 3 - strain vaccine.

EBV hijacks gene enhancers to promote carcinogenesis

Lohit Khera, University of Delhi South Campus

Epstein-Barr Virus (EBV) is known to infect more than 90% of human population and cause a number of lymphoid and epithelial cancers including Burkitt's and Hodgkin's lymphoma. The virus infects human B cells, and can transform them into cancerous cells hence causing lymphomas. Recently, a team of Scientists from University of Sussex has made an important development in finding the mechanism of carcinogenesis by EBV. They reported that EBV could take control of certain DNA regions called enhancers. Enhancers are small DNA sequences that loop out of long intervening DNA stretch and control activity of genes located far away. Two of the enhancers associated with EBV have been identified to control the MYC gene and BCL2L11

gene. The former is a known key driver in development of lymphoma and the latter is an apoptosis suppressor. It was seen that while the virus increases the contact between with MYC enhancer and the controlled gene to up-regulate gene activity; the contact between BCL2L11 enhancer and its controlled gene is blocked by EBV to down-regulate gene activity. Not only this, the team also discovered that a specific drug could reverse the blocking of BCL2L11 enhancer, leading to apoptosis in infected cells, hence preventing transformation to cancerous cells. More work is required to find out more such interactions with enhancers of other onco-genes and mechanism of these interactions.

Recently held conference

The 8th International Gemini virus Symposium and the 6th International ssDNA Comparative Virology Workshop was held at New Delhi from 7th -10th November 2016. Gemini viruses are small single-stranded DNA viruses, which have emerged as significant problems in a large number of crops worldwide. A total of 190 delegates, representing 23 countries, attended the symposium. The symposium was divided into four broad themes: Diversity, Evolution and Pathogenesis; Vector-virus interactions; Host-virus interactions and Strategies for disease management. There were 63 oral presentations and 113 poster presentations spread out over the four days of the symposium. During the symposium, Prof. Anupam Varma and Prof. V. Muniyappa, both of whom have contributed enormously to geminivirus research in India, were felicitated for their contributions. A special feature of the symposium was the Geminivirus Study Group Workshop, in which experts presented the latest status on the classification of geminiviruses, the vector transmission of the newly identified capulaviruses and the newdeltasatellites found associated with geminiviruses.

The symposium saw participation from 70 delegates from abroad and 120 from India. The delegates from India represented universities, national research institutes and seed companies. It presented unique opportunities for a large number of delegates to share their research, build academic and professional contacts, learn about the latest developments in the field and interact with peers, who have decades of research and mentoring experience in geminiviruses and single-stranded DNA viruses.

Editorial Board

Forthcoming Publications: Book

A Century of Plant Virology in India

Bikash Mandal, GP Rao, VK Baranwal and RK Jain

IVS is bringing out a reference book on the works on plant virology carried out during last 100 years in India. The book contains six major sections: capacity building, genus wise virus characterization, virus-vectors, virus diagnosis, virus management and eminent Indian virologist.

Encyclopedia of Plant Viruses and Viroids

K Subramanya Sastry, Bikash Mandal, John Hammond, SW Scott and RW Briddon

The encyclopedia provides description of viruses and viroids of plants known at a global level till 2016. More than 1300 plant viruses and viroids are included in this encyclopedia. The unique aspect of this encyclopedia is that the plant species are arranged in alphabetical order and all the globally known viruses and viroids that infect each plant species are described.

IVS 25th Anniversary - Silver Jubilee Year Lectures

1. **"Management of Potato Virus Y"** by Dr. Mathuresh Singh, Director, Agricultural Certification Services Inc., Fredericton, Canada, held at the Division of Plant Pathology, IARI, New Delhi on 10th March 2016.
2. **"Banana bunchy top virus: control through resistance"** by Prof. James Dale, Professor, Queensland University of Technology, Australia, held at the Division of Plant Pathology, IARI, New Delhi on 10th November 2016.
3. **"Identification of the nanovirus transmission helper protein and DNAs associated with coconut foliar decay disease"** by Prof. Bruno Gronenborn, Professor,Institute for Integrative Biology of the Cell, France, held at Division of Plant Pathology, IARI, New Delhi on 11th November 2016.

IVS Awards 2016

IVS Fellows 2016
PLANT VIROLOGY
Dr. K Subramanya Sastry; Hyderabad
Dr. A. Ishwara Bhat; ICAR-IISR, Kerala
MEDICAL VIROLOGY
Dr. Tathagata Choudhuri;Visva Bharati, Santiniketan
ANIMAL VIROLOGY
Dr. Baldev Raj Gulati; ICAR-NRCE, Hisar

IVS Life-time Achievement Awards 2016
Professor H.N. Verma, Vice Chancellor, Jaipur National University, Jaipur
Dr.Vishwa Mohan Katoch, Ex-DG, ICMR
Professor Gaya Prasad, Vice chancellor, SVPUA&T, Meerut

IVS-NIV Travel Bursary Awards 2016
Swati Gupta; Ph.D. Student, DIPAS, DRDO, Delhi
KC Rane; Ph.D. Student, MGM University, Mumbai
Saurabh Dubey; Ph.D. Student, , IARI, New Delhi
Renuka Sharma; Ph.D. Student, AMITY University, Noida

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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