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India | EMBO Symposium

on

"RNA viruses: Immunology, pathogenesis and translational opportunities"

National Capital Region, Faridabad

(28th - 30th March, 2018)

Organised by:

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ट्रांसलेशनल स्वास्थ्य विज्ञान
एवं प्रौद्योगिकी संस्थान

TRANSLATIONAL HEALTH SCIENCE
AND TECHNOLOGY INSTITUTE

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Scientist-G
National Institute of Animal Biotechnology (NIAB),
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India | EMBO Symposium

on

RNA viruses: Immunology, pathogenesis and translational opportunities

March 28 – 30, 2018, Faridabad, India

Several RNA viruses constitute the majority of emerging and re-emerging pathogens, however, there is very little focus on research into the biology and pathogenesis of RNA viruses in India. Studies on epidemiology and disease burden, risk factors, the immune response to RNA viruses, circulating virus strains and virus evolution, animal models of disease, antivirals and vaccines are strikingly absent. Emerging RNA viruses such as Zika virus and Crimean-Congo haemorrhagic fever virus are a matter of grave concern to India. Therefore, this symposium will play a key role in bringing together national and international RNA virus experts from diverse areas on one platform to guide the future of RNA virus research in India. This symposium will provide impetus on laying a strong foundation for both the fundamental and translational aspects of RNA virus biology with a focus on epidemiology, immune response, virus evolution and vaccine trials.

India | EMBO Symposium

RNA viruses: Immunology, pathogenesis and translational opportunities

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Programme

DAY 1 – MARCH 28, 2018

Session 1: RNA viruses: Epidemiology, disease burden and risk factors **Chair: V. Ravi, National Institute of Mental Health and Neurosciences, INDIA**	
9.00-9.15	Welcome Note **Guruprasad Medigeshi**
9.15-9.45	Keynote Address: "Capacity building for RNA virus research in India – ecosystem, challenges and way forward" **Maharaj Kishan Bhan**
9.45-10.30	Enteric virus strain diversity: epidemiology and impact on vaccine design and efficacy **Miren Iturriza-Gomara**
10.30-11.00	**Coffee break**
11.00-11.30	Arboviruses in India: where, when, how much, and what can be done? **Katherine Gibney**
11.30-11.45	Complement-mediated neutralization of Chandipura virus is through classical pathway dependent aggregation **Selected abstract: Kunnakkadan, U. et al.,**
11.45-12.00	HULC influences HCV replication and release in liver cells **Selected abstract: Geetika Sharma and Saumitra Das**
12.00-14.00	**Lunch and poster session**
Session 2: RNA virus infections: Focus on pathogenesis and evolution **Chair: Sudhanshu Vrati, Regional Center for Biotechnology, INDIA**	
14.00-14.45	MERS-coronavirus: from discovery to intervention **Bart Haagmans**
14.45-15.15	Role of complement during the pandemic influenza A(H1N1) 2009 virus infection **Arvind Sahu**
15.15-15.30	Uncovering viral surface hotspots in Dengue for targeted antibody discovery **Selected abstract: Anand, G.S. et.al.,**
15.30-16.00	**Coffee Break**
16.00-16.30	Ebola virus immunology then and now **Cesar Munoz-Fontela**
16:30-17.00	Pathogenesis of vascular leak in dengue **Gathsaurie Malavige**
17:00-17.30	**Meet the speakers of session 1 and 2**
17.30-19.00	**Poster session**
19.00-21.00	**Dinner**

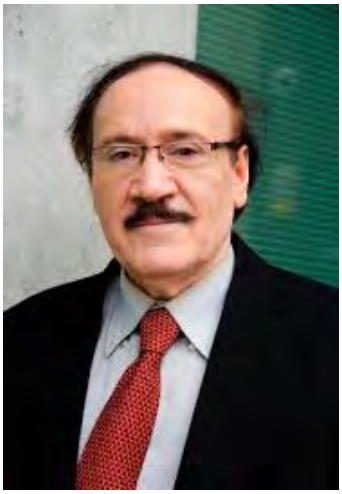
DAY 2 – MARCH 29, 2018

Session 3: Viral and immunological determinants in RNA virus infections **Chair: Shahid Jameel, Wellcome trust/DBT-India Alliance**	
9.00-09.45	Pandemic Influenza Viruses: Biology and Transmission **Kanta Subbarao**
9.45-10.15	Immune signatures of CD4 T cell subsets in healthy and dengue virus-infected individuals **Daniela Weiskopf**
10.15-10.45	Molecular basis for attenuation of Live attenuated Japanese encephalitis virus vaccine SA14-14-2 **Vijaya Satchidanandam**
10.45-11.15	**Coffee break**
11.15-11.45	Human CD8 T cell response in Dengue Virus Infections **Anmol Chandele**
11.45-12.15	Innate immune response to Flaviviruses **Nolwenn Jouvenet** **EMBO Young Investigator Lecture**
12.15-12.45	A systems biology approach to understand immune protection against hepatitis C virus **Fabio Luciani**
12.45-14.00	**Lunch and poster session**
Session 4: Vaccine development: new strategies for new markets **Chair: Gagandeep Kang, Translational Health Science and Technology Institute, INDIA**	
14.00-14.45	Crimean-Congo Haemorrhagic Fever (CCHF) Virus - A Growing Threat **Roger Hewson**
14.45-15.15	Designer VLP based tetravalent dengue vaccine candidate **Navin Khanna**
15.15-15.30	Role of immune cell subsets and identification of biomarkers in severe dengue **Selected abstract: Kar, M., et al.,**
15.30-16.00	**Coffee Break**
16:00 - 17:00	Panel Discussion: Road map for RNA virus research in India. Moderator: **Gagandeep Kang.** Panelists: **Bart Haagmans, Kanta Subbarao, Soumen Basak and Shahid Jameel**
17:00-17.30	**Meet the speakers of sessions 3 and 4**
17.30-19.00	**Poster session**
19.00-21.00	**Dinner**

DAY 3 – MARCH 30, 2018

Session 5: Clinical trials and models to test therapies and vaccines Chair: **Saumitra Das, Indian Institute of Science**	
8:30	Departure to NCR Biotech Cluster
9.00-09.45	Understanding Dengue Pathogenesis and Essential Areas for Research **Timothy Endy**
9.45-10.15	Human B cell response to dengue virus infection and lessons for vaccine development **Katja Fink**
10.15-10.45	**Coffee break**
10.45-11.15	Controlled Human Infection Models **Gagandeep Kang**
11.15-11.45	'Women in Science: An Indian Perspective' **Sandhya S. Visweswariah** **EMBO "Women in Science" lecture**
11.45-12.15	**Meet the speakers of session 5**
12.15 -12.45	**Tour of NCR Biotech cluster**
12.45-14.00	**Lunch**
14.00-15.00	**"Crystal gazing" talks by Session chairs - 10 min each** **V. Ravi, Sudhanshu Vrati, Shahid Jameel, Gagandeep Kang, Saumitra Das**
15.00-15.15	Closing Remarks **Nagendra Hegde**
15.15-15.45	**Coffee break and Departure**

Invited speakers



Maharaj Kishan Bhan

National Science Professor, Indian Institute of Technology (IIT), New Delhi
President, Jawaharlal Nehru Institute of Post-graduate Medical Education and Research (JIPMER), Puducherry
Former Secretary, Department of Biotechnology, Govt.of India

Dr. Bhan's research has focused on elucidating infection-nutrition cross talk and its impact on incidence and severity of mucosal infections and disease. He has achieved wide recognition for his research contributions that have impacted children's health and survival across the developing world. His notable contributions include the development of an indigenous rotavirus vaccine which is now introduced in to the national program of immunization, development of low osmolarity ORS, zinc as a treatment of diarrhea, understanding zinc deficiency and its association with enteric and respiratory infections. This has contributed to strategies to improve zinc intake through various strategies in medicine, public health and agriculture. The development of a rotavirus vaccine in India from a neonatal strain was a landmark effort in team science and affordable innovation in India and in global health.

As Secretary, Department of Biotechnology, he was one of the key architects of designing the biotechnology ecosystem in the country and transformation in translational research and academic-industry interaction. His efforts and ideas enhanced opportunities for developing leaders in biological science and medical science in India. He is a fellow of National Science Academics, and has been bestowed upon numerous national and internationl awards, including the Shanti Swarup Bhatnagar award, and the prestigious Padma Bhushan from the Govt. Of India.

Miren Iturriza-Gomara

Professor, Institute of Global Health and Infection, University of Liverpool, UK

Prof. Iturriza-Gomarra is a virologist with a particular interest in enteric virus infections, virus evolution and the use of molecular tools for diagnosing, monitoring and tracking infections. She studies molecular epidemiology of virus infections, and mechanisms involved in generating the diversity of virus types found in the human population. She is also interested in the study burden of disease from a syndromic point of view and on the application of molecular detection and pathogen characteriation for surveillance and the study of transmission of viruses.

Katherine Gibney

NHMRC EarlyCareerFellow, Doherty Institute, University of Melbourne and the Royal Melbourne Hospital, Australia

Dr Gibney is an Australian infectious diseases physician, public health physician and medical epidemiologist with an interest in arboviral diseases of public health importance. She completed her medical degree at the University of Melbourne (MBBS with honours 2001) and trained as an infectious diseases specialist in Victoria and the Northern Territory (FRACP 2010). During a 2-year applied epidemiology fellowship at the US Centres for Disease Control and Prevention, the Epidemic Intelligence Service, she was stationed at the Arboviral Diseases Branch in Fort Collins, Colorado (EIS 2009).
She then returned to Australia and completed a PhD in infectious disease epidemiology at Monash University (PhD 2016), as well as training as a public health physician (FAFPHM 2015). She continues to work in clinical infectious diseases and public health medicine.

Bart Haagmans

Group Leader, Department of Viroscience, Erasmus Medical Center, Rotterdam, the Netherlands

Dr. Haagmans is a virologist who did his training at the Utrecht University and holds a PhD from the same university. His main interest is the pathogenesis of emerging viral infections including SARS and the novel MERS coronavirus. He is a recognized leader in the field of coronaviruses and was involved in the characterization of the MERS-CoV genome and development of molecular and serological assays to detect MERS-CoV, but also different other viruses. He identified the receptor used by MERS-CoV to infect cells and was involved in studies that led to the identification of the intermediate host of MERS-CoV, the dromedary camel. Currently the focus of his research is the development of a MERS-CoV vaccine. In addition, he is actively involved in issues related to biosafety and biosecurity in laboratories but also during outbreaks such as MERS and Ebola.

Arvind Sahu

Scientist – G, National Centre for Cell Science, Pune, India

Dr. Sahu obtained his Ph.D. from Vallabhbhai Patel Chest Institute, University of Delhi, and post-doctoral training from the University of Pennsylvania, USA, and the University of Texas Health Sciences Centre, USA. He worked briefly at the University of Pennsylvania before joining NCCS in 2000. He has worked extensively on the role of complements during viral infections, focusing on understanding the role of intact

complement as well as individual pathways during viral infections. Recent studies his lab show that synergy between the classical and alternative pathways is critical for providing effective protection against the pandemic influenza A(H1N1)2009 virus infection. Further, our data indicate that complement synthesized by the splenic B cells and C3a receptor-mediated signaling plays a major role in providing the protection. In addition, his group is also studying viral complement evasion. Specifically, the laboratory identified a complement homolog encoded by Kaposi's sarcoma-associated herpes virus (KSHV), and showed that complement regulators encoded by KSHV and other viruses (vaccinia virus, variola virus, and Herpes virus saimiri) inhibit complement by targeting the C3-convertases. The group has also contributed to mapping of the functional domains, determinants and species specificity of these viral proteins. Currently, studies in the laboratory are directed towards understanding the molecular basis of complement inhibition by pox and herpes viral complement regulators and their role in viral pathogenesis.

Caesar Munoz-Fontela

Bernard Nocth Institute for Tropical Medicine, Hamburg

Dr. Munoz-Fontela graduated from the Complutense University of Madrid and obtained his doctoral degree in Microbiology and Parasitology from the same university in 2005. As a Fulbright postdoctoral fellow at Mount Sinai School of Medicine, New York, he discovered the chief role of p53 as a linker between innate and adaptive immunity to emerging RNA viruses such as influenza and Ebola virus. In 2011, Dr. Munoz-Fontela started his own independent group, first at the Heinrich Pette Institute in Hamburg, and since 2018 at the Bernhard Nocht Institute for Tropical Medicine in the same city. Research at the Munoz-Fontela laboratory is focused on the immunology of viral hemorrhagic fevers, in particular,filoviruses such as Ebola virus. The goal is to advance the field by providing insight into a poorly known aspect of viral hemorrhagic fevers, namely, the physiology of the host immune response *in vivo*, both in relevant animal models as well as in humans. The group's strategy is to model filovirus immunity in the BSL4 laboratory utilizing newly generated immunocompetent animal models including humanized mice. To validate this basic research in animal models, the group also studies human immunity to hemorrhagic fever viruses in the field. Dr. Munoz-Fontela has worked as consultant for the World Health Organization (WHO) Global Outbreak Alert and Response Network (Ebola, Lassa).

Gathsaurie Neelika Malavige

Professor – Dept. Of Microbiology, Director – Centre for Dengue Research, University of Sri Jayavardenapura, Sri Lanka ; Visiting Academic, MRC Human Immunology Unit, University of Oxford, UK

Dr. Malavige obtained medical degreefrom the University of Colombo, MRCP and FRCP from the Royal College of Physicians, UK, and D. Phil. From the University of Oxford. Her research interests are to understand host-virus interactions during pathogenesis of dengue. Specifically,

her work focuses on investigating the mediators that cause vascular leak in dengue. Prof. Malavige has identified a potential mediator and a clinical trial for a drug for the treatment is on progress.

Kanta Subbarao

Director of the WHO Collaborating Centre for Reference and Research on Influenza at the Peter Doherty Institute for Infection and Immunity and Professor, Department of Microbiology and Immunology, The University of Melbourne

Dr. Kanta Subbarao is a virologist and a physician with specialty training in pediatrics and pediatric infectious diseases. Previously, she was Chief of the Emerging Respiratory Viruses Section of the Laboratory of Infectious Diseases, NIAID, NIH in Bethesda (2002-2016) and Chief of the Molecular Genetics Sectionof the Influenza Branch at the CDC in Atlanta (1997-2002). Dr. Subbarao' s research is focused on newly emerging viral diseases of global importance including pandemic influenza, severe acute respiratory syndrome(SARS) and Middle East Respiratory Syndrome (MERS) coronaviruses. She is a Fellow of the American Academy of Microbiology and the Infectious Diseases Society of America. She serves on the Editorial Board of PLoS Pathogens and mBio.

Daniela Weiskopf

Instructor, La Jolla Institute of Allergy and Immunology, USA

Dr. Weiskopf graduated with a Masters in Microbiology from the University of Innsbruck, Austria, and a Ph.D. in Immunology from the Innsbruck Medical University, Austria. She obtained further training at the La Jolla Institute of Allergy and Immunology, USA. She has devoted her career for understanding the T cell response to viral pathogens and spent the last twelve years studying infectious viruses relevant to human health and disease. During her Ph.D., she performed research analyzing post-translational modifications of CMV-derived epitopes and modulation of the T cell immune response during aging. During her post-doctoral training under Dr. Alessandro Sette, her efforts were dedicated to characterizing human dengue virus-specific $CD8^+$ and $CD4^+$ T cell responses in two different cohorts: samples from areas with endemic dengue infection and following experimental vaccination developed by the National Institutes of Health. She has also established and published a mouse model of human DENV disease using HLA transgenic mouse strains. In light of the upcoming pandemics, she has begun to study the human T cell response to ZIKA virus, further broadening her interest in T cell responses in viral immunity in general and flaviviruses such as Dengue and Zika viruses in particular.

Vijaya Satchidanandam

Professor, Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, India

Prof. Sathchidanandam studies host-pathogen interactions and the molecular basis for pathogenesis of two human infectious agents *Mycobacterium tuberculosis* and Japanese encephalitis virus (JEV).

Her laboratory works on the biochemical and kinetic properties of the flaviviral replicase, and has demonstrated the unexpected presence of viral RNA synthesizing activity within the nucleus of cells infected with JE, Dengue and West Nile viruses. Currently, they are trying to understand the low abundannce JEV NS1' protein, which is a product of ribosomal frame shifting during translation of the viral polyprotein. In addition, Dr. Satchidanandam has recently analysed the human immune response to JEV antigens among individuals living in areas endemic to JEV, revealing the target antigens of human CD4+ and CD8+ T cell responses to be the capsid and envelope proteins, and non-structural proteins NS3 and NS1, respectively, as also the vital role played by interferon gamma and perforin in protection against encephalitis. They are currently studying the role of endoplasmic reticulum (ER)-localized viral protein folding pathways that modulate host cell ER stress and lead to autophagy, and have revealed that these pathways are essential for eliciting virus-specific CD8+ T cells, which are actively suppressed by wild type JEV.

Anmol Chandele

Assistant Professor, International Centre for Genetic Engineering and Biotechnology (ICGEB) & Emory Vaccine Center, New Delhi

Dr. Chandele received her PhD from the National Center for Cell Science, India and then her post-doctoral training in Dr. Susan Kaech's laboratory at Yale University. During this time, she studied the signals and factors that dictate the formation of immunological memory and why in certain cases the body fails to generate long-lived immunity. After this, she joined as a research scientist with Dr. Rafi Ahmed at the International Centre for Genetic Engineering and Biotechnology (ICGEB), India, and gained experience in human immunology research and extensively characterized dengue specific immune responses in patients from Thailand. Since 2013, she has dedicated extensive efforts in setting up state of the art human immunology research program in India. Her focus has been to better understand human immunity to infectious diseases of public health importance to the developing world. She is actively involved in developing and establishing cutting edge immunology platforms for quantitating innate and antigen-specific effector B and T cell responses in humans. Leveraging on the sustained collaborations with clinicians in India and researchers at Emory, the lab recently published the first comprehensive analysis on human CD8 T cell responses from dengue patients from India.

Nolwenn Jouvenet

Junior Group Leader, Virology Department, Pasteur Institute, Paris, France

Dr. Jouvenet studies the interaction between viruses and their hosts. During her Ph.D. at the Institute for Animal Health, UK, she studied the intracellular transport of African swine fever virus, leading to new insights into how the virus replicates in the cytoplasm, exploits and manipulates the cytoskeleton and its associated motors to move within, and escape from, infected cells. During post-doctoral work at the Rockefeller University, USA, she focused on the mechanisms of assembly and budding of retroviruses. She developed innovative fluorescent imaging approaches to study the dynamics of retrovirus assembly in live cells, and characterized the genesis of individual viruses in real time, from initiation of assembly to release from the host cell. She also studied antiviral innate immunity, and demonstrated that tetherin, one of the interferon-stimulated genes (ISGs), is capable of inhibiting the release of a broad spectrum of retroviruses and filoviruses.

At the Pasteur Institute, she is exploring flavivirus-host interactions that govern immunity and infection outcome using biochemical, virological and microscopic assays combined with high-throughput screening methods. More specifically, she is studying mechanisms of flavivirus entry into cells, virus-induced innate immunity and inflammation, and the interface between the cell and viral proteins whose functions are poorly defined. By doing so, she hopes to contribute to the development of effective vaccines and therapeutics to combat flavivirus-mediated diseases.

She was awarded the EMBO Young investigator prize in 2015.

Fabio Luciani

Senior Scientist, Kirby Institute for Infection and Immunity, and Associate Professor, School of Medical Sciences, University of New South Wales, Australia

Dr. Luciani obtained a PhD degree in Theoretical Biology (Humboldt University, Berlin). In September 2005 Dr Luciani moved to Australia for a postdoctoral position with Dr Mark Tanaka to work on stochastic models applied to molecular epidemiology of bacterial infection and on Bayesian statistical analysis of molecular epidemiology data. Since 2017 he is a National Health and Medical Research Council Research Fellow. His research interests include adaptive immune responses against pathogen infections, computational models for studying host-pathogen interactions, and bioinformatics analysis of high throughput next generation sequencing data. Recently, he has been involved into application of single cell technologies to study T and B cells. Dr. Luciani is author of >80 publications in the field of immunology, population genetics and molecular epidemiology, biophysics, bioinformatics, and statistics.

Roger Hewson

Scientific Leader, Viral Haemorrhagic Fevers & Arboviruses, and Head, WHO Collaborating Centre for Virus Research & Reference, Porton Down Institute, Public Health England; Theme Leader, Pathogen Discovery and Characterization Division, National Institute of Health Research, UK

Dr. Hewson received his B.Sc. in Biological Sciences-Biochemistry from the University of Exeter (1987) and DPhil. in Membrane Protein Biochemistry from the University of Oxford (1993 St Hughs'). Following postdoctoral work in molecular virology at the Karolinska Institute, Sweden, and the University of Wisconsin, USA, he joined the Special Pathogens Unit at the Centre for Applied Microbiology and Research to work on a range of zoonotic and arthropod borne viruses. He now leads his own research group – Virology & Pathogenesis, which is focused on public health aspects of virology, pathogenesis and emerging disease. His research includes an in vivo and in vitro capability with highly pathogenic viruses at containment levels 3 & 4 and a surveillance programme on emerging viruses with key collaborations in Africa, Central and South Asia, the Middle East, Africa the Caucuses and South America. He holds professorships at the London School of Tropical Medicine, Liverpool School of Tropical Medicine and the Institute of Tropical Medicine at the University of Nagasaki, Japan.

Navin Khanna

Senior Scientist and Group Leader, Molecular Medicine Division, International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi; Adjunct Professor, Emory University, USA; Adjunct Professor, Translational Health Sciences and Technology Institute (THSTI), New Delhi; Adjunct Professor, Charotar University of Science and Technology, Gujarat.

For the past 25 years, Dr. Khanna has been interested in genetically engineered bio-molecules of medical use. His research efforts in the area of in-vitro diagnostics have made a significant impact on the public health in India and many developing countries. His current interest includes tailored Dengue vaccine candidates.

Timothy Endy

Associate Professor and Chief of Infectious Diseases, Department of Medicine, Upstate Medical University, State University of New York, USA

Dr. Endy is an infectious disease specialist and is affiliated with multiple hospitals in the state of New York, including Crouse Hospital and Syracuse Veterans Affairs Medical Center. He received his medical degree from Uniformed Services University of the Health Sciences and has been in practice for more than 20 years. Dr. Endy has extensive experience in dengue hemorrhagic fever and emerging diseases. His

laboratory is currently developing an animal model for dengue hemorrhagic fever using humanized SCID mice.

Dr. Endy is a part of an interdisciplinary university and military team of researchers in the US and Thailand to conduct coordinated studies to determine the effect that vaccination with a candidate live-attenuated tetravalent vaccine will have on vaccine-related genetic changes on wild-type dengue virus and how these changes will determine risk for dengue hemorrhagic fever, virus transmission in the vaccinated and surrounding population, and its mosquito vector. The study offers a unique opportunity to understand long-term impact on the design of future dengue vaccines and conduct of dengue vaccine efficacy trials.

Katja Fink

*Principal Investigator, Singapore Immunology Network (SIgN), Agency for Science Technology and Research (A*STAR), Singapore*

Dr. Fink graduated in biochemistry from the University of Zurich and received her doctor in natural sciences (Dr. sc. nat) from the Swiss Federal Institute of Technology (ETH) where she studied antiviral immune responses in mouse models. In 2006 she joined the Novartis Institute for Tropical Diseases in Singapore and studied immune activation in dengue patients for the identification of dengue-specific markers of immunopathology that could potentially be targeted for the treatment of the disease. Dr. Fink joined SIgN in May 2009 as a Principal Investigator, continuing her work on dengue, with a focus on immune-therapy and immune memory.

Gagandeep Kang

Executive Director, Translational Health Science and Technology Institute (THSTI), India

Dr. Kang is an accomplished infectious disease biologist and vaccinologist who joined THSTI, an autonomous institute of the Department of Biotechnology, Govt. Of India, in August 2016. THSTI has a mission to conduct innovative translational research across disciplines to understand disease biology and accelerate development of concepts into products and strategies for public health. At THSTI, she is exploring the possibilities of establishing controlled human infection models for diseases relevant to India.

Prior to her appointment at THSTI, she was a Professor in the Division of Gastrointestinal Sciences at the Christian Medical College, Vellore. Over two decades, she built a research program that has conducted key studies to understand enteric infectious diseases in impoverished communities. Working in partnership with non-governmental organizations and the government, she has carried out phase I to III studies of rotaviral vaccines and provided laboratory support for vaccine development in India and for other developing countries. With the Indian Council for Medical Research and the World Health Organization,

she has supported the establishment of networks of sentinel hospitals and laboratories that carry out surveillance for rotavirus disease in children and ancillary studies.
She chairs the Immunization Technical Advisory Group for the WHO's South East Asian Region. She is an Independent Director of the Hilleman Laboratories, a partnership established to make affordable vaccines by Merck and the Wellcome Trust.

Sandhya S. Visweswariah

Professor and Chair, Molecular Reproduction and Developmental Biology, Indian Institute of Science, Bangalore, India

After completing her Masters degree in Chemistry from Indian Institute of Technology – Kanpur, Prof. Visweswariah obtained a PhD degree in Biochemistry from the Indian Institute of Science. After a stint in industry in Astra Zeneca India, she returned to the Indian Institute of Science where she is currently Professor and Chair of the Department of Molecular Reproduction, Development and Genetics, and Co-Chair of the Centre for Biosystems Science and Engineering. Her research interests have focused on the role of a receptor in the gut which is the target of bacterial toxins that cause diarrhoea amongst children and travelers to countries such as India. She has also identified and characterized novel proteins from mycobacteria that are involved in the metabolism of the second messenger, cAMP. Prof. Visweswariah is a JC Bose Fellow, a Fellow of the Indian National Science Academy and the Indian Academy of Science. She is currently a recipient of a Royal Society Collaborative Research Professorship, and is a Margdarshi Fellow supported by the DBT-Wellcome Trust India Alliance. She has graduated ~ 30 students who are now placed in academia and industry in India and abroad.

Abstracts – Invited speakers

01

RNA virus research in India - Capacity building and challenges

M.K. Bhan
National Science Professor, Indian Institute of Delhi, New Delhi, India

RNA viruses are probably the most versatile and complicated pathogens in terms of genetic material and evolution, modes of transmission to various hosts and persistence in the environment, and the emergence of new virus types and diseases. Drivers of emerging infectious diseases of viral origin include environmental changes, human behaviour, socio economic and demographic phenomena, food production, health care and microbial adaptation. To better understand and deal with these emerging and persistent threats, interdisciplinary crosstalk among virologists, epidemiologists, modellers, economists, ecologists, behavioural biologists and experts in environment is a critical need. The challenge is how to initiate and nurture such collaborative endeavors and networks, when the natural approach of academic scientists is often to work in peaceful isolation.

New strategies and tools are required for surveillance, prediction, early detection and prevention of potentially dangerous infectious diseases of viral origin. Safe and effective vaccines are required against many viral infections that cause significant disease burden or have the potential to cause epidemics or pandemics. Knowledge of correlates of protection will expedite vaccine development. Discovery and development related to difficult to make vaccines, diagnostics and biomarkers also requires collaboration among biologists, microbiologists, chemical and material scientists, engineers and others. The knowledge of disease burden and molecular epidemiology, and the measurement of changing trends is currently inadequate. This needs investment and sustainable institutional arrangements and quality, well-trained human resource. Our research must be directed to create success in knowledge generation and tool development but also a better understanding of failed efforts.

02

Enteric virus strain diversity: epidemiology and impact on vaccine design and efficacy

Miren Iturriza-Gomara
University of Liverpool, Liverpool, United Kingdom

Diarrhoeal disease remains the second most common cause of childhood mortality, with viruses being major aetiological agents globally. Rotavirus is the commonest cause of severe diarrhoeal disease in children and a major contributor to child morbidity and mortality. The global introduction of rotavirus vaccines has had significant impact in reducing this burden, although efficacy varies significantly between high and low income settings. Noroviruses are also globally distributed, but patterns of disease appear to be different between high and low income countries. These two viruses are globally distributed, and strain surveillance has highlighted the diversity of co-circulating strains within and between populations and through time. Strain diversity has been an important consideration for the design of the rotavirus vaccines currently in use and also for the design of norovirus vaccine candidates. Current rotavirus vaccines confer high degree of cross-protection, but evidence from both high and low income countries shows some differences in efficacy against heterotypic strains that can also be explained by long terms observations and analysis of strain diversity in non-vaccinated populations. This talk will summarise some of the data on RV strain diversity pre-and post-vaccine introduction in high and low income settings and some of the challenges currently facing the design of a norovirus vaccine and vaccination strategy in different populations.

03

Arboviruses in India: where, when, how much, and what can be done?

Katherine Gibney
The Peter Doherty Institute of Infection and Immunity, University of Melbourne and the Royal Melbourne Hospital, Melbourne, Australia

Arboviruses cause a huge burden of disease in India, but the epidemiology is never static. Both dengue and Japanese encephalitis viruses are endemic to India, with focal epidemics and an overall increasing trend. Chikungunya was first recognised to cause disease in India in the 1960's but then disappeared as a public health threat for more than four decades. It re-emerged a decade ago, with 1.4 million chikungunya cases reported in India in 2006. Zika virus, the cause of microcephaly and other serious congenital malformations, has more recently been identified in India, with potentially devastating outcomes for the babies of women infected during pregnancy. \

The economic cost of dengue virus alone has been estimated to exceed $1billion per year in India. Although much of the clinical and public health focus has been on the acute arboviral illnesses, long-term sequelae are often under-recognised and contribute substantially to the overall burden of arboviral diseases, reported in disability adjusted life years (DALYs).

Efforts to curb the public health burden of arboviruses are ongoing. Vaccines range from the established (Japanese encephalitis) to emerging (dengue) and under development (chikungunya and Zika). Alternate prevention interventions likewise vary from established (mosquito control) to emerging strategies (Wolbachia-infected mosquitoes).

04

MERS-coronavirus: from discovery to intervention

Bart Haagmans
Erasmus Medical Centre, Rotterdam, Netherlands

Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in the human population in the Arabian Peninsula and continues to cause outbreaks five years after its identification. More than 2000 cases in 27 countries have been reported with a 35% case fatality rate. Serological and molecular evidence indicates that dromedary camels act as reservoirs for MERS-CoV, posing a continuous risk of virus spill-over to in-contact people, such as those working in slaughter houses and animal farms. Therefore, it is crucial to understand the biology of MERS-CoV and develop intervention measures. We identified the MERS-CoV receptor, dipeptidylpeptidase-4 (DPP4), using Fc-tagged fragments of the spike (S) protein in an immunoprecipitation assay followed by mass spectrometry. DPP4 is expressed in the lower respiratory tract of humans, consistent with human fatalities associated with lower respiratory tract infection. The S protein, a type I trimeric viral envelope glycoprotein, interacts with DPP4 through the S1 subunit, the receptor binding domain of which is the major target of virus neutralizing antibodies. We also demonstrated that a modified vaccinia virus Ankara virus vaccine expressing the S protein confers mucosal immunity in dromedary camels. Significant reduction of excreted infectious virus and viral RNA transcripts was observed in vaccinated animals upon MERS-CoV challenge as compared to controls. The MVA-S vectored vaccine may also be tested for protection of humans at risk, such as healthcare workers and people in contact with camels. Such a One Health approach combining efforts to target both humans and dromedaries may be needed to tackle this zoonotic outbreak.

05

Role of complement during the pandemic influenza A(H1N1) 2009 virus infection

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The pandemic influenza A(H1N1) 2009 virus caused significant morbidity and mortality worldwide and is now circulating seasonally, necessitating the need to understand the host factors that influence its control. The complement system provides protection against seasonal influenza virus infection. We dissected the role of intact complement as well as of its individual activation pathways by infecting mice deficient in various complement components. Infection of C3-/- mice resulted in increased viral load and 100% mortality, which could be reversed by transfer of naïve wild-type (WT) splenocytes, purified splenic B cells, or immune sera from WT, but not C3-/- mice. Blocking of C3a and/or C5a receptor signaling in WT mice and use of C3aR-/- and C5aR-/- mice showed significant mortality after blocking/ablation of C3aR, with little or no effect after blocking/ablation of C5aR. Intriguingly, deficiency of C4 and FB resulted in partial mortality (24%-32%) suggesting a necessary cross-talk between the classical/lectin and alternative pathways for providing effective protection. In vitro experiments indicated that activation of the classical and alternative pathways in concert, owing to coating of viral surface by antibodies, is needed for efficient virus neutralization. Examination of virus-specific complement-binding antibodies in virus-positive subjects showed that their levels vary among individuals. Together, these results indicate that cooperation between the classical and alternative pathways not only results in efficient neutralization of the pandemic influenza virus, but also leads to the optimum generation of C3a, which when sensed by the immune cells along with the antigen, culminates in generation of effective protective immune responses.

06

Ebola virus immunology then and now

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Since its initial emergence in 1976 in northern Democratic Republic of Congo (DRC), Ebola virus (EBOV) has been a global health concern due to its virulence in humans, the mystery surrounding the identity of its host reservoir and the unpredictable nature of Ebola virus disease (EVD) outbreaks. Early after the first clinical descriptions of a disease resembling a 'septic-shock-like syndrome', with coagulation abnormalities and multi-system organ failure, researchers began to evaluate the role of the host immune response in EVD pathophysiology. Here, we will discuss how data gathered during the last 40 years in the laboratory as well as in the field have provided insight into EBOV immunity. From molecular mechanisms involved in EBOV recognition in infected cells, to antigen processing and adaptive immune responses, we will evaluate current knowledge on the main immune barriers of infection as well as outstanding research questions.

07

Pathogenesis of vascular leak in acute dengue infection

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Endothelial dysfunction leading to vascular leak, typically evident 3-6 days post-onset of illness, is the hallmark of severe dengue. This critical phase usually occurs during resolution of viraemia, suggesting that vascular leak is likely a result of inflammatory mediators or pathogenic antibodies, rather than by the virus or viral proteins. Cytokines such as TNFα and IL-1β, which are elevated in the critical phase, along with mediators released from cross-reactive T-cells are thought to contribute to vascular leak. However, recent data suggests that virus-specific T-cells are more likely to be protective rather than contributing to pathology. Many inflammatory lipid mediators, such as platelet activating factor (PAF), leukotrienes and secretory phospholipase A2, are elevated in acute dengue infection. Mediators such as vascular endothelial growth factor (VEGF) and angiopoietin-2 are elevated in patients with dengue haemorrhagic fever (DHF), exerting their action in part by inducing the activity of phospholipases, which have diverse inflammatory effects including generation of PAF. Dengue virus NS1 disrupts the endothelial glycocalyx, and anti-NS1 antibodies protect against vascular leakage in mouse models. However, our data shows that anti-NS1 antibodies rapidly rise in patients with secondary dengue with DHF and not during the critical phase of DF. Therefore, the role of anti-NS1 antibodies in the pathogenesis or protection against dengue should be further evaluated. While dengue vaccines that elicit anti-NS1 antibodies are likely to be helpful in reducing disease pathogenesis, drugs that block down-stream immunological mediator pathways such as PAF may also be beneficial in the treatment of severe disease.

08

Pandemic Influenza Viruses: Biology and Transmission

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The epidemiological success of influenza A viruses relies on the ability to transmit efficiently from person-to-person via respiratory droplets. Airborne transmission of influenza viruses requires efficient replication and release of infectious particles into the air. Influenza A virus infection is mediated via binding of the viral haemagglutinin (HA) to terminally attached a2,3 or a2,6 sialic acids on cell surface glycoproteins. Human and influenza A viruses preferentially bind a2,6- versus a2,3-linked sialic acids, respectively, on complex glycans on airway epithelial cells. Historically, influenza A viruses with preferential association with a2,3-linked sialic acids have not been transmitted efficiently by the airborne route in ferrets. We used a ferret model to study the viral properties governing airborne transmission of the 2009 pandemic H1N1 (H1N1pdm) influenza virus. We demonstrated that the Eurasian-origin NA and M gene segments contributed to efficient airborne transmission of the H1N1pdm virus. We then engineered the H1N1pdm virus to preferentially bind a2,3-linked sialic acids. Airborne transmission was associated with rapid selection of virus with a change at a single HA site that conferred binding to long-chain a2,6-linked sialic acids, without loss of a2,3-linked sialic acid binding. The transmissible virus emerged in experimentally infected ferrets within 24 hours after infection and was remarkably enriched in the soft palate, where long-chain a2,6-linked sialic acids predominate on the nasopharyngeal surface. Notably, presence of long-chain a2,6-linked sialic acids is conserved in ferret, pig and human soft palate. Our data demonstrate complex viral and host interactions that influence the transmissibility phenotype of influenza viruses.

09

Immune signatures of CD4 T cell subsets in healthy and dengue virus-infected individuals

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Dengue virus (DENV) is the etiologic agent of dengue fever (DF), the most significant mosquito-borne viral disease in humans. Transmission occurs in more than 100 countries leading to almost 400 million infections a year. Dengue virus is a rapidly spreading pathogen with unusual pathogenesis and protective immunity is poorly understood. In particular, the role of CD4 T cells in dengue infections is less clear. We defined HLA-restricted CD4+ T cell responses resulting from natural infection with dengue virus in a hyper-epidemic setting and described a subset of memory T cells re-expresses CD45RA (TEMRA) after antigenic stimulation. CD4 TEMRA cells are found with highly variable frequencies in different individuals and have been implied in providing protective immunity against infections with pathogens such as dengue virus (DENV). Applying immune profiling methods, we found that not only the frequency but also the phenotype of CD4 TEMRA cells was heterogeneous between individuals. A subset of TEMRA cells displayed a transcriptional and proteomic program with cytotoxic features that is distinct from effector memory T cells. Moreover, this subset showed higher levels of clonal expansion and contained the majority of DENV- specific TEMRA cells. Overall, this study reveals the heterogeneity of CD4 TEMRA cells and provides new insights into T cell responses against DENV and potentially other emerging pathogens.

10

Molecular basis for attenuation of Live attenuated Japanese encephalitis virus vaccine SA14-14-2

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Diseases caused by flaviviruses including dengue fever, Japanese encephalitis, West Nile encephalitis and yellow fever have become increasingly frequent over the last couple of decades, aided by global warming and expanding geographies of the mosquito vector.
The extremely safe and efficacious live attenuated vaccine for Japanese encephalitis virus (JEV) SA-14-14-2, licenced in China in the early 1990s and subsequently by other S.E. Asian countries, was introduced in 2006, by the Indian government to combat increasingly severe annual epidemics of encephalitis in Eastern U.P. and S. India.

We observed impressive enhancement in human CD8+ T cell responses in vaccines relative to volunteers naturally exposed to circulating strains of JEV. Using cell lines that support JEV infection, we queried the molecular basis underlying the generation of enhanced CD8+ T cells by the live vaccine SA-14-14-2. Our studies revealed that the vaccine virus induced severe ER stress, viral protein was rapidly degraded in vaccine virus-infected cells and was differentially recognized by a panel of monoclonal antibodies.
Sustained activation of the ER stress sensor PERK in vaccine virus-infected cells led to prolonged phosphorylation of eIF2α and activation of several autophagy markers. Interestingly, we also observed active dephosphorylation of eIF2α and inhibition of end stage autophagy in WT JEV infected cells.

Our results can guide the rational design of efficacious vaccines against not only flaviviruses such as Zika virus, dengue virus and West Nile virus but also other pathogenic viruses belonging to other families, based on activation of autophagy triggered by activation of ER stress from engineered misfolded viral glycoproteins.

11

Human CD8 T cell response in Dengue Virus Infections

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Dengue virus infections have exponentially increased making them a major public health challenge throughout the world. Clinical spectrum of the disease occurs with varying symptoms ranging from fever, hemorrhage and/or shock that are often associated with mortality, especially among children. Human immune responses have been implicated in both pathology and protection of dengue, but how the balance is maintained between these opposing functions is not well understood. Recent estimates suggest that India has now become the country with highest dengue burden worldwide. However, human immunity remains poorly characterized in patients from India.
We performed the first holistic and comprehensive analysis of effector CD8 T cell response in dengue confirmed children from India. We found that CD8 T cells expanded massively, and expressed markers indicative of antigen-driven proliferation, activation, tissue homing and cytolytic effector functions. Analysis of the functionality of these activated CD8 T cells in response to dengue-derived peptides revealed that only a very small fraction of these effector CD8 T cells produced Interferon gamma. Transcriptomics revealed down-regulation of key molecules involved in T cell receptor signaling pathway. Despite this loss of functionality during the febrile phase of dengue, patients with severe disease had higher frequencies of effector CD8 T cells suggesting that an ongoing effector CD8 T cell response is associated with dengue-induced pathological consequences. Taken together, these data open novel directions for better understanding of the role of CD8 T cells in protection versus pathology during dengue disease.

12

5’ region of the genome of Dengue virus are RIG-I ligands

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Upon activation through the recognition of viral RNA, cytoplasmic RIG-I-like-receptors (RIG-I), such as RIG-I and Mda5, triggers an interferon-mediated antiviral response. To identify the exact Dengue virus (DENV) RNA sequences recognized by RLRs in human cells, we used riboproteomic approach combined with next-generation sequencing (NGS). Kinetic of replication of two DENV clinical isolates in cells stably expressing STrEP-tagged (ST)-RIG-I or ST-Mda5 proteins were characterized. Silencing approach revealed that, in our system, RIG-I was the primary sensor for DENV. Consistently, viral RNAs that co-purified with ST-RIG-I were immunostimulatory, whereas the ones bound to ST-Mda5 were not. Analysis of NGS data of RNAs bound to RLRs suggests that RIG-I recognizes specifically the 5’ region of the DENV genome. No specific enrichment of viral RNAs was found attached to Mda5. NGS data were validated by quantitative PCR and by experiments performed with DENV RLR ligands produced in vitro. These experiments also demonstrated that 5’-triphosphates are necessary for recognition of DENV genomes by RIG-I.

Our data identifies, for the first time, DENV RNA signatures recognized by RIG-I in the context of infection. We propose that the 5’ region of DENV nascent transcripts that are produced during viral replication and before capping are RIG-I ligands.

13

A systems immunology approach reveals novel molecular signatures associated to cytotoxic T cell responses with implication for immune protection against hepatitis C virus

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CD8+ T (CTL) cells play a pivotal role in protection from viral infections. Better understanding of heterogeneous T-cell subset phenotypes evolving during an immune response is needed for development of T-cell-based vaccines that can provide long-term protection. Viruses causing chronic infections such as HIV and HCV, trigger a T-cell response characterised by functional exhaustion, and accumulation of immune escape variants. We have developed a single cell approach to identify and link functional phenotype, gene expression profile, TCR diversity and T-cell avidity with the onset of exhaustion and its relationship with immune escape.

We analysed a cohort of prospectively followed subjects from acute primary HCV infection to disease outcome (clearance and chronicity). Antigen-specific (Ag-) CTL were identified via epitope discovery and functional validation via IFNγ-ELISPOT. Index sorting was utilised to link surface phenotyping with gene expression profile. Full-length TCRαβ repertoire was identified from scRNAseq data using novel tools. Exhaustion was detected early on in the acute phase of infection and in CTL targeting conserved but also immune escape epitopes. Single cell RNAseq showed a distinct gene expression profile of exhausted T-cells compared to the less differentiated subsets. In contrast, Ag-CTL in subjects that cleared HCV, polyfunctional (IFNγ, granzyme, and perforin) responses were found, with a highly diverse TCR repertoire. We discovered a novel T-cell clone, characterised by high avidity for viral epitope, elevated IFNγ production and polyfunctional phenotype.

This systems immunology approach provides new routes to investigate complex T-cell dynamics and to identify new mechanisms to exploit in vaccine and immunotherapy.

14

Crimean-Congo Haemorrhagic Fever (CCHF) Virus - A Growing Threat

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Crimean Congo haemorrhagic fever (CCHF) virus constitutes a group of viruses in the Genus Orthonairovirus, classified within the family Nairoviridae within the large order Bunyavirales. Published descriptions of major epidemics, outbreaks and the ecology of CCHF underline the very wide distribution of the virus, which stretches over much of Asia, extending from the Xin Jiang region of China including, India and Pakistan to the Middle East and southern Russia, and to focal endemic areas over much of Africa and parts of south-eastern Europe. Tick vectors of the virus are important players in the spread and distribution of disease and seasonal and ecological factors contribute to the growing burden of CCHF. Important anthropogenic factors also play a role and may further increase the threat posed by CCHF in the coming years. Starting from a historical perspective the presentation will include recent emergences including in India, virus genetics in the context of diagnostics and close with an update on vaccine intervention.

15

Designer VLP based tetravalent dengue vaccine candidate

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International Centre for Genetic Engineering and Biotechnology, New Delhi, India

A safe and effective dengue vaccine has remained elusive despite several decades of research efforts. Whereas a live attenuated vaccine is commercially available, there are several whole virus based dengue vaccines at advanced stages of clinical trials. There is still a need for new vaccine candidates which in addition to eliciting tetravalent neutralizing antibodies must also be free of enhancement risk. We report a novel non-replicating tetravalent Dengue Subunit Vaccine (DSV4), capable of eliciting tetravalent seroconversion in experimental animals without evidence of infection enhancement. DSV4 was designed by in-frame fusion of the non-immunodominant but functional DENV envelope domain III (EDIII) of all four DENV serotypes and hepatitis B surface (S) antigen, co-expressed with unfused S antigen in Pichia pastoris. DSV4, purified using conventional chromatographic techniques, formed virus-like particles (VLPs), as assessed by dynamic light scattering and electron microscopic analyses. DSV4 VLPs displayed EDIIIs of all four DENV serotypes based on probing with a battery of serotype-specific conformational anti-EDIII antibodies. DSV4 VLPs were highly immunogenic, inducing potent and durable neutralizing antibodies against all four DENV serotypes encompassing multiple genotypes, in mice and macaques. DSV4-induced murine antibodies suppressed viremia in AG129 mice and conferred protection against Antibody-Dependent Enhancement, even at sub-neutralizing concentrations. The lack of ADE was supported by suppression of the production of pro-inflammatory cytokines such as TMF-α and IL-6. Our approach can circumvent the risk of inducing disease-enhancing antibodies while eliciting effective tetravalent seroconversion. DSV4 has a significant potential to emerge as a safe, efficacious and inexpensive subunit dengue vaccine.

16

Understanding Dengue Pathogenesis and Essential Areas for Research

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Dengue virus (DENV) is the most common arboviral infection in the tropical and subtropical regions of the world, resulting in subclinical to classic dengue fever (DF) to a more severe form of dengue hemorrhagic fever (DHF). There currently is one registered vaccine against dengue infection, Dengvaxia, which is registered in 12 countries. There is no antiviral therapy or immunomodulator that can be used to diminish disease severity. The World Health Organization considers the development of an effective DENV vaccine a high priority. Presently, the development of dengue vaccines is limited by (i) the absence of a preclinical disease model, (ii) no available validated human challenge model and (iii) no established immune correlate of risk or protection. Understanding dengue pathogenesis is essential in identifying biomarkers to identify those at risk for severe forms of dengue illness; identifying potential therapeutic interventions for treating severe dengue; and in developing improved dengue vaccines that will provide durable tetravalent protection. In this talk, 25 years of dengue research conducted in Thailand will be reviewed as well as current research on the pathogenesis of dengue. Key areas for future research will be identified and discussed.

17

Human B cell response to dengue virus infection and lessons for vaccine development

Katja Fink
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Antibodies are an essential component of successful anti-viral vaccines. Serum antibodies are constantly produced by long-lived plasma cells and ideally neutralize all virus particles entering the body during an infection. Since this response is hardly ever perfect, that is sterilizing immunity cannot be achieved, the activation of memory B cells after an infection is equally important. Interestingly, only a fraction of memory B cells with a limited epitope coverage is activated and differentiates into antibody-producing plasmablasts. I will discuss the complexity and functional relevance of naive B cell activation and memory B cell re-activation during a human viral infection in patients with dengue infection and how we can use this knowledge to assess dengue vaccines.

18

Controlled Human Infection Models

Gagandeep Kang
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Controlled human infection model (CHIM) studies are of pivotal importance in vaccine development, being used for a range of studies, including proof of concept, pathogenesis, down-selection and immunogenicity. However, they have seldom been carried out in low- and middle-income countries (LMIC) to date, where the greatest burden of vaccine preventable illness is found. Discussions in India have considered the benefits and barriers to CHIM studies. Benefits include improved vaccine effectiveness and in country capacity development in the domains of clinical and laboratory testing as well as governance. Barriers include social considerations, acceptability, safety and regulatory issues, but framework are being developed by which ethical, laboratory, scientific and governance issues may be addressed by investigators planning CHIM in LMIC.

19

Women in Science: An Indian Perspective

Sandhya Visweswariah
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In an ideal world, science is conducted with no regard to country of origin, or sex of the researcher. In other words, there is a level playing field. However it is now acknowledged that women are underrepresented in STEM across the world. Why is this the case, when statistics show that there is little to distinguish between boys and girls in terms of their proficiency in STEM while at school? In fact, in countries like India, girls consistently out-perform boys at high school level. What happens later? Why are there so few women in senior positions in India, and indeed around the world? I will attempt to answer these questions based on my own career, as well as observing the career trajectories of my younger colleagues, both men and women. There are no clear solutions, but ideas emerge for the way forward, so as to ensure a more diverse, and perhaps more compassionate, work place in future.

Abstracts – Participants' presentations

P01

Complement-mediated neutralization of Chandipura virus is through classical pathway dependent aggregation

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Rationale
The complement system is a potent barrier faced by animal viruses. Virus-complement interactions can lead to virus neutralization or the inverse upon complement modulation. Chandipura virus (CHPV) causes fatal pediatric encephalitis across India. After local infection, CHPV disseminates to the central nervous system causing marked damage. This study aims to unravel the effect of human complement on CHPV.

Methodology
CHPV (NIV, Pune) was cultured and titrated in vero cells. Complement susceptibility was tested with normal human serum (NHS) or heat-inactivated NHS by either varying concentrations or time followed by plaque assay. Complement specificity and dissection of pathways responsible for CHPV neutralization were tested with respective complement depleted NHS or reconstituted with either C3, C4, C8, C1q or factor B (Complement Technology, Tyler, Tx). Complement reconstitution was analyzed using hemolytic assays with rabbit or sheep RBC's against NHS. Mechanism of neutralization was assessed with Transmission Electron Microscopy (TEM, Jeol) and sucrose gradient ultracentrifugation (Beckman)

Results and Discussion:
Neutralization of CHPV by NHS (not HI) was concentration and time-dependent. Neutralization was complement dependent as CHPV was resistant to C3 depleted serum. Neutralization was classical pathway dependent as EGTA treated serum, C4, C1q depleted sera had no effect while Factor B depleted serum had. The terminal pathway was ineffective against CHPV as it was sensitive to C8 depleted NHS. A marked shift in sucrose gradient suggested an aggregation-mediated mechanism of neutralization further validated by TEM. Thus, CHPV susceptible to classical pathway results in complement component association and neutralization by aggregation.

Presentation type: Oral presentation

P02

HULC influences HCV replication and release in liver cells

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Hepatitis C Virus (HCV) is a liver-specific pathogen which manipulate the host machinery to establish a successful chronic infection. During progression to hepatocellular carcinoma, the HCV replication in the transformed cells decreases. However, the reason for this decrease is still unknown. HULC is a liver specific long non-coding RNA which gets up-regulated in the hepatocellular carcinoma tissues. We performed HULC knockdown and over-expression studies and found that HULC is infact a negative regulator of HCV replication. We found that the knockdown of HULC could recover miR-122 levels in HepG2 cells and support HCV replication. Further, we established that the increase in HULC levels during HCV infection helps the loading of HCV-core protein onto lipid droplets (LDs) thereby aiding the release of viral particle. We found that HULC contributes to the increase in number and the average size of LDs during HCV infection. We have also extended the study to establish the mechanism by which HULC gets upregulated during infection. We show that HCV-core protein can interact with the transcription factor RXRA and increase the RXRA-mediated transcription of HULC. The up-regulated HULC also contributes to the increased lipid biogenesis during HCV infection. Our study offers fresh insights into the disease progression and pathogenesis caused after HCV infection.

Presentation type: Oral presentation

P03

Uncovering viral surface hotspots in Dengue for targeted antibody

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Amide hydrogen/deuterium exchange mass spectrometry (HDXMS) is a powerful probe for monitoring dynamics of proteins and macromolecular complexes in solution. Here, we describe dynamics of dengue viral particles in solution and map effects of human host-specific perturbations including interactions of specific antibodies. Dengue serotype 2 (DENV2) has been shown to undergo a large expansion in viral capsid size at the human host temperature of 37 °C by cryo-EM. In contrast, temperature-induced capsid expansion was not observed in the other three serotypes at 37 °C. In order to localize serotype-specific differences associated with temperature shifts and antibody interactions, we have used HDXMS to monitor dynamics of whole DENV1 and 2 particles in solution and to capture temperature and antibody-specific changes at peptide resolution at 37 °C and further at 40 °C (mimicking high fever). Our results indicate that DENV2 show high intrinsic and non-uniform dynamics across the C, E and M-proteins compared to DENV1 at 28 °C. At 37 °C, DENV2 shows temperature-specific changes with the biggest change at the E- intradimeric interface while DENV1 did not show any temperature specific-changes consistent with cryo-EM. These changes are due to specific assemblies of E-protein on the viral particle rather than isolated unassembled E-proteins in solution and underscore the importance of protein quaternary contacts, packing of lipid bilayer and RNA genome. The increased dynamics and temperature-dependent expansion highlight the potential for whole virus HDXMS in exposing hidden linear epitopes for targeted antibody discovery.

Presentation type: Oral presentation

P04

Role of immune cell subsets and identification of biomarkers in severe dengue

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Severe dengue can manifest with hemodynamic disturbances including thrombocytopenia and platelet dysfunction; mechanisms of which are currently obscure. Our study attempts to investigate the dynamics of dengue virus (DENV) infection in subset of blood cells from dengue patients and also in vitro in platelets and endothelial cells (ECs). In vitro infection studies indicated that primary ECs were less permissive for DENV infection as measured by very low viral titers in culture supernatants of infected cells. Ex vivo infection of platelets isolated from healthy donors failed to show productive DENV infection. Platelets isolated from patients were also negative for DENV antigen. In DENV patient samples, only B cells and monocytes showed the presence of DENV-E antigen and negative strand RNA intermediates by flow cytometry and RT-PCR respectively. We observed a significantly higher association of platelets with CD14+CD16+ immune cells in DENV patients as compared to controls suggesting a mechanism for platelet clearance. Analysis of plasma samples from dengue patients with varied clinical symptoms by Isobaric Tags for Relative and Absolute Quantitation (iTRAQ) showed significant modulation of platelet basic protein, retinol-binding protein 4, histidine rich glycoprotein, serum amyloid A1 and A2, apolipoprotein E, galectin 3 binding protein, ficolin 3, leucine-rich alpha 2 glycoprotein, lipopolysaccharide binding protein, C-reactive protein indicating the potential role for these proteins in severe dengue and could also be used as biomarkers of disease severity.

Presentation type: Oral presentation

P05

High expression of B Lymphocyte Stimulator (BLyS) correlates with poor viral neutralization activity and disease progression in HIV-1 infected children

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Introduction: Multiple B cell defects have been reported during HIV-1 infection. B lymphocyte stimulator (BLyS), a key regulator of B cell homeostasis is expressed by dendritic cells (DCs). Hence understanding the role of DCs in modulating the B cells phenotype and viral neutralization in HIV-1 infected children is important to develop strategies for restoration of B cell function.

Materials: A total of 38 HIV-1 infected children and 25 controls were recruited. Based on CD4 counts and years of infection, they were categorized as long term non-progressors (LTNPs) (n=20), and progressors (n=18). DC, B cell subsets, BLyS expression on DCs were determined by flow cytometry. Plasma levels of B cell growth factors were measured by ELISA and viral neutralization activity was determined.

Results: Lower (%) of myeloid DCs (mDCs), plasmacytoid DCs (pDCs) and high expression of BLyS on mDCs were observed in progressors than controls. Expression of BLyS on mDCs and in plasma were significantly higher in progressors vs. controls. We observed lower % of resting memory and naïve B cells and higher % of mature activated and tissue like memory B cells in progressors vs. controls. Higher plasma levels of IL-4, IL-6, IL-10, IgG and IgA were observed in progressors. High levels of BLyS in progressors correlated with poor viral neutralization. Progressors, upon initiation of antiretroviral therapy showed reduction in BLyS levels that correlated with improvement in viral neutralization.

Conclusion: Our study demonstrates that reduction in BLyS levels correlates with restoration of B cell function (viral neutralization) in HIV-1 infection.

Presentation type: Poster presentation

P06

Nuclear receptor co-repressor NCoR1 controls tolerogenic and antiviral program in dendritic cells upon TLR9 stimulation

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CD8α+ Dendritic cells (DCs) are the major conventional DCs that cross-present antigens to T cells upon encounter with intracellular pathogens. Besides they also educate naïve T cells to differentiate into effecter subtypes like Th1, Th2 or Tregs. Our major objective is to understand how DCs initiate a variety of immune responses at transcriptional level upon pathogenic stimulation. We found nuclear receptor co-repressor NCoR1 as a master repressor of tolerogenic responses in DCs. To explore the underlying mechanism we performed integrative genomic analysis of NCoR1 depleted cells. Interestingly we identified an enriched anti-viral network in activated NCoR1 KD DCs. These DCs secrete high IFNβ1 and inhibit viral infection by generating a cascade of anti-viral responses against negative strand RNA viruses like Sendai, VSV and NDV. We validated the findings ex vivo and in vivo using control and NCoR1DC-/- animals. Integrative genomics identified that PU.1 recruits NCoR1 in these DCs at global scale. NCoR1 has been reported to form complexes with diverse HDACs to repress their target genes. Therefore we speculated that differential HDACs in complex with NCoR1 might be controlling these dichotomous antiviral vs tolerogenic responses. Our preliminary HDAC inhibitor screen in CD8α+ DCs showed some promising results. Here we will discuss further how we identified the mechanisms underlying the transcriptional control of these diverse responses by NCoR1 in DCs.

Presentation type: Poster presentation

P07

Recruitment of CD46 and CD55 by Vesicular Stomatitis Virus - a specific modulatory function adopted by the virus to evade complement mediated neutralization

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The complement system, an integral arm of the innate immunesurveillence system, rapidly recognizes and eliminates invading pathogens. Ability of viruses to activate or counteract complement system contributes to/dictates the degree of viral pathogenesis. Vesicular stomatitis virus (VSV), a prototypic rhabdovirus activates the classical pathway of complement in an antibody dependent or independent manner. To counter complement, VSV recruits at least two membrane associated regulators of complement activation (RCA), CD55 and CD46, providing VSV resistance against complement. The delay in neutralization was much greater in VSV-CD55 than VSV-CD46. The objective of this study was to understand the specificity of RCA recruitment by VSV.

Time course experiments in HeLa infected with VSV (0.1 and 10 MOI) followed by western blotting showed constant levels of CD55 from 0-24h, but a decline or absence of CD46 from 12 h post infection. Semi quantitative RT-PCR showed that CD46 transcripts declined from 12 hpi, however this was delayed with CD55. Immunofluorescence and quantitative PCR further validated the differential levels of CD55/CD46 protein and transcript respectively. To test if the differential expression of CD55 and CD46 is due to modulation of antiviral responses by VSV, microarray analysis was done. Initial analysis showed marked down regulation of key cytokines like IL-6 and other transcription factors associated with CD46. Although a decline in transcript levels was observed, sustained levels of CD55 protein in infected cells correlated with CD55 abundance in virion and superior resistance. This recruitment of RCAs is a specific function of VSV to confer resistance against complement.

Presentation type: Poster presentation

P08

RelA/NF-κB promotes Chandipura virus growth in host cells

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Chandipura virus (CHPV) is an emerging human pathogen associated with several of the recent encephalitis outbreaks in India. CHPV belongs to Rhabdoviridae family and vesiculovirus genera and possesses a single-strand negative sense RNA genome. In general, endosomal TLR3 as well as cytosolic RIG-I recognize invading RNA viruses and transmit signals for activating RelA/NF-κB and IRF3 transcription factors. These factors then collaborate to induce the expression of a diverse set of anti-viral genes, including that encodes Interferon-β. However, engagement of the cell-signaling pathways specifically during CHPV pathogenesis remains poorly characterized. Here, we utilized genetically tractable and biochemically amenable mouse embryonic fibroblast (MEFs) based system for investigating host responses to CHPV. Our analyses substantiated that CHPV mediates activation of the RelA/NF-κB dimer and also induces the IRF3 activity. Indeed, genetic deficiency of RelA led to the diminished production of Interferon-β in response to CHPV infection. Unexpectedly, we observed that RelA deficiency also reduced the yield of progeny CHPV particles despite abrogated IFN-β expressions. Our mechanistic study clarified that this pro-viral RelA/NF-κB function was linked to the ability of the RelA factor in suppressing virus-induced cell death. In sum, our results suggest that the multifunctional transcription factors RelA/NF-κB, known for its involvement in the anti-viral host immune responses, plays a pro-viral role during CHPV infection.

Presentation type: Poster presentation

P09

iTRAQ-based proteome analysis of autophagy-mediated host response to Japanese encephalitis virus infection

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RATIONALE
Japanese encephalitis virus (JEV), belonging to family Flaviviridae, is the leading global cause of virus-induced encephalitis. The focus of our research is to understand how the cellular autophagy pathway and/or its components interact with and influence the JEV life cycle and the host response. Studies from our laboratory have shown that autophagy restricts JEV replication and delays virus induced cell death. The cellular stress responses- oxidative stress and ER stress are the major inducers of autophagy during JEV infection. An understanding of how autophagy influences virus pathogenesis has the potential to impact the development of anti-virals and/or vaccines against JEV.

METHODOLOGY
We have performed an iTRAQ- based proteome analysis of JEV infected-wild-type (WT) and autophagy deficient- atg5 knockout Mouse embryonic Fibroblasts (MEFs).

RESULTS
Our studies show that autophagy induction during JEV infection requires virus replication. Significant transcriptional differences were observed in the activation of innate immune markers between WT and atg5-/- MEFs. iTRAQ-mass spectrometry analysis demonstrated that ATG5 protein deficiency affects biological pathways like autophagy, lysosomal degradation, proteasome machinery, immune responses, apoptosis, metabolism and cell adhesion. We observed significant upregulation of inflammation, JAK-STAT, TLR, chemokine signaling and apoptosis pathways during JEV infection. However all these were differentially regulated between WT and atg5 -/- MEFs. Several Immune related proteins like C5, IRF7, Csf1 and RIPK2 are exclusively over-expressed in WT MEFs but not in atg5-/- MEFs during JEV infection.

CONCLUSION
We demonstrate a crucial role for autophagy/ATG5 in influencing signaling pathways and host immune response during JEV infection.

Presentation type: Poster presentation

P10

Circulating microRNA profiles of Dengue virus infection

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MicroRNAs (miRNAs) are small (19-21nt. length), noncoding, highly conserved, single-stranded RNAs which can precisely regulate gene expression by targeting messenger RNAs (mRNAs). miRNAs are found to be extremely stable in extracellular fluids of mammals, such as blood plasma, serum, etc. The expression profiles of these circulating miRNAs have immense potential for use as novel minimally invasive markers in monitoring human diseases. Here, we present for the first time Dengue virus-induced changes in circulating miRNA populations in plasma of dengue patients. Using high-throughput small RNA sequencing, the levels of circulating miRNAs analyzed between patients with uncomplicated dengue infection (DI, n=9), Dengue with a warning sign (DWS, n=14), and severe dengue (DS, n=16). We also included plasma for small RNA sequencing from nine dengue patients followed–up at multiple time points.
Using mirdeep2 and edgeR packages, total 89 microRNAs detected in all three groups in at least two-thirds of samples. These miRNAs could target 77 genes in PBMC significantly differentially expressed in severe dengue cases. Through Pathway enrichment analysis, we discovered that HALLMARK HEME METABOLISM" was one of the highly enriched pathways that could be affected by the dysregulated microRNAs. Altered Heme metabolism could induce neutrophil activation. Comparing miRNA profile with follow-up samples, we identified few miRNAs as disease progression marker which requires further validation in a large number of patients' samples.
Our study provides insight into the potential functions and regulatory interactions of miRNAs, which with additional experiments, will facilitate translational research.

Presentation type: Poster presentation

P11

Prohibitin-2 mediates DENV-3 entry on human neuroblastoma (SH-SY5Y) cell line

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Introduction: Dengue virus (DENV) not classified primarily as neurotropic virus, however in severe cases of dengue fever several reports of neurological involvement have been documented over the years. DENV-2 and DENV-3 are most commonly reported to cause neurological manifestations. The comparative ability of DENV serotypes to infect human neuroblastoma cell line is not known. We compared the infectivity of the DENV serotypes and the interacting proteins involved in binding and entry on SH-SY5Y cell line.

Objective: To evaluate possible mechanism in the neurological involvement of DENV serotypes using human neuroblastoma cell line as a model.

Methodology: DENV growth kinetics on SH-SY5Y cells was determined by plaque assay and C6/36 (Aedes albopictus) cells were taken as positive control. Virus overlay protein binding assay (VOPBA) was performed followed by mass spectrometry to identify the DENV interacting proteins in membrane fractions of SH-SY5Y cells. Peptides were analysed by LC-MS/MS orbitrap fusion platforms & Data were searched against human protein database using SEQUEST algorithm. Interacting proteins were confirmed by Infection inhibition and Coimmunoprecipitation assay.

Results: By using VOPBA followed by LC-MS/MS analysis, we identified prohibitin-2 as a putative receptor and vimentin as an interacting protein for DENV-3. However prohibitin-1 was found as an interacting protein for DENV-2. Further, anti-prohibitin-2 antibodies were able to block DENV-3 entry into the SH-SY5Y cells. Indirect immunofluorescence and flow-cytometry analysis demonstrated localization of prohibitin-2 on SH-SY5Y cell surface as well as in cytoplasm. Coimmunoprecipitation followed by Western blot analysis reconfirmed the interaction between prohibitin-2 and DENV-3-E protein.

Conclusion: Prohibitin-2 is the first characterized human neuroblastoma cell expressed DENV-3 virus receptor protein.

Presentation type: Poster presentation

P12

Nonsynonymous variant rs117648444 (P70S) acts as confounder in association of IFN-λ locus variants with response to IFN-RBV therapy in HCV 3 Infected patients

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Hepatitis C, caused by Hepatitis C Virus (HCV) is one of the major public health problems in India as well as in the world. Several genome-wide association studies carried out in different world populations found that polymorphisms present in interferon lambda locus (IFNL) are associated with clearance of both treatment-induced and natural clearance of HCV. Further case-control studies have shown that the dinucleotide variant (rs368234815) that gives rise to a functional IFN-λ4 may be the actual causal variant. Paradoxically, potential to express IFN-λ4 was associated with the lower clearance of HCV. A low-frequency nonsynonymous variant rs117648444 is present on the 70th position of IFN-λ4 protein (P70S) that affects the activity of the protein. In the current study, we produce genetic evidence to reinforce IFN-λ4's function as the prime causal mechanism. We used a cohort of genotype 3 HCV-infected patients, from different parts of India who underwent IFN-RBV therapy. Unexpectedly rs368234815 was not associated with sustained virological response (SVR). Subsequent analysis revealed that a negative confounding effect of rs117648444 was responsible for above result. Further, we found that IFNL locus variants are subject to either a positive or a negative confounding effect by rs117648444 which depends on the linkage of the IFNL variants with the minor allele "A" of rs117648444. In conclusion, we show that in future case-control studies involving IFNL locus variants, the confounding effect of rs117648444 should be taken in to account to appreciate the true effect of IFN-λ4 on the phenotype.

Presentation type: Poster presentation

P13

Mathematical modelling of early passive immunization with HIV-1 broadly neutralizing antibodies

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In a striking recent study, short-term early exposure to broadly neutralizing antibodies (bNAbs) was found to provide lasting control of HIV-1 infection, raising new hopes of achieving functional cure of HIV-1. A mechanistic understanding of how this lasting viral control is achieved remains unknown, precluding rational design of passive immunization protocols. We argued that bNAbs exert pleiotropic effects on HIV-1 that lead to this control. bNAbs can enhance viral clearance, upregulate antigen presentation, and prevent CTL exhaustion by lowering viremia. To test our hypothesis, we constructed a mathematical model of viral dynamics following early bNAb administration that incorporated the above mechanisms. The model quantitatively captured measured mean changes in viremia in untreated and treated macaques, giving us confidence in the model. The model predicted two distinct long-term fates of infection: high viral burden and viremic control. The model showed that early administration of bNAbs could orchestrate a transition from the high viremic to the controller state via the effects above, marking successful response to bNAb therapy. Model predictions were in quantitative agreement with data from macaques and demonstrated this transition. A framework thus emerges for the rational optimization and engineering of bNAb therapy.

Funding

The authors acknowledge support from the Wellcome Trust/DBT India Alliance Senior Fellowship (IA/S/14/1/501307).

Presentation type: Poster presentation

P14

International Vaccine Institute approach in developing RNA virus vaccines platform and vaccine evaluation system

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Emerging and re-emerging RNA viruses becomes major threat in Asian continent and International Vaccine Institute (IVI), with vision of developing countries free of suffering from infectious disease, initiated research for vaccine development and establishing vaccine evaluation platform. The major area where IVI initiated research includes development of VSV (vesicular stomatitis virus) and chimpanzee adenovirus vector-based Zikavirus vaccine development and evaluation. The approach includes construction of VSV and chimpanzee adeno vector-based Zikavirus vaccine candidates and in-vitro candidate vaccine evaluation (virus titer, antigen expression level, etc.). Further evaluation of immunogenicity in small animals including assessment of neutralizing antibodies, Cell-mediated immune response, memory T-cell response etc. Final the vaccine candidate selected in animal studies will be evaluated in clinical efficacy trial in human.
IVI is also involved in developing multiplexed assay for detecting antibodies to Flavi and arboviruses as part of surveillance in Asia and Latin America. Further clinical vaccine evaluation platform being established at IVI including flow cytometry based neutralization assay for dengue and zikavirus, pseudovirus evaluation system for MERS coronavirus.
Other area were IVI is working in development of bi-valent Hepatitis A Vaccine (HAV) vaccine including genotype I and III, considering reports of high HAV prevalence in Korea and South East Asia. In initial phase, reverse genetics and virus rescue strategy will be adapted to generated Bi-valent HAV vaccine candidate. Second phase involves scaling up and developing upstream and downstream strategy for HAV vaccine development.

Presentation type: Poster presentation

P15

Polymorphism of HLA and immunomodulation of inflammatory cytokines in influenza virus infected population of Assam, Northeast India.

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Background: Influenza virus infection is a highly contagious respiratory illness worldwide. HLA plays a vital role in the disease outcome. This study aimed to evaluate HLA polymorphism in signalling immune response in influenza virus infected population of Assam.
Method: Throat/Nasal swab and blood were collected from patients with influenza like illness during August 2016 to August 2017. Samples were screened by Real Time PCR for influenza viruses. HLA typing was done using PCR-SSP method for allele detection of cases and controls. Plasma cytokine levels (IL-6, IL-8, IL-10, IFN-γ and TNF-α) were analyzed using Flow cytometry.
Result: Out of 510 ILI samples (Male 246 and Female 264), 9.8% (50/510) were found positive for influenza viruses (Inf A 56%, 28/50 and Inf B 44%, 22/50). A total of 7 alleles of HLA-A, 16 alleles of HLA-B and 11 alleles of HLA-DRB1 locus were identified. The most common alleles in cases were HLA-A*11 (95.24 %), HLA-B*35 (20.83%) and HLA-DRB1 (54.55%) as compared to controls, 38.09%, 0.00% and 66.67% respectively. Cytokine levels of IL-6 and IL-10 were significantly increased in cases (p=0.017 and p=0.017) compared to controls. IL-10 and TNF- α were also higher in cases. While IFN- γ level was found to be higher in the control group.
Conclusion: In this preliminary study, HLA-A*11, B*35 and DRB1*15 in the study population is suspected to be a risk factor in conferring susceptibility to influenza virus infection along with elevated cytokine expression. A larger extended study may provide insights for HLA restricted peptide based vaccines.

Presentation type: Poster presentation

P16

VIRAL NEUTRALIZATION POTENTIAL OF SOME CONSERVED B-CELL EPITOPES OF DENGUE STRUCUTRAL PROTEINS

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The current research aims to explore the virus neutralizing potential of some linear B-cell epitopes from dengue virus (DENV) structural proteins, which are conserved across the four DENV serotypes. Five from the Envelope (E), 1 from Pre-membrane (prM), and one from the Capsid (C) protein, P1-P7 respectively, were studied. Peptides to represent these B-cell epitopes were commercially synthesized, and were used to generate antibodies in 3 mice (Balb/c) batches. The whole E protein was used as a positive control and the adjuvant alone was used as the negative control. The mice were bled after two immunization boosts with the peptides. The presence of antibodies for the epitopes, in the collected mice antisera, was confirmed using ELISA assays conducted against the corresponding peptides. The mice antisera were then subjected to microneutralization assays against all the four DENV serotypes. The antisera with an EC50 neutralization $\geq$ 40 times the serum dilution, was considered as neutralizing. All the three batches of mice antisera of the positive control showed EC50 neutralization titres above 40 against all the four DENV serotypes, whereas none of the antisera of the negative control was neutralizing. Antisera of all the E protein epitopes neutralized all the four DENV serotypes, at moderate levels. Anti-PrM sera were neutralizing mainly against DENV1 and DENV3. None of the anti-C sera was neutralizing for any of the DENV serotypes. The study identifies five conserved, moderately-neutralizing, linear B-cell epitopes from the DENV E protein, which also have locations with pathological importance.

Presentation type: Poster presentation

P17

Role of Non- Structural Protein 1 (NS1) Specific Antibodies In Dengue Disease Pathogenesis

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Objective: The role of NS1 specific antibodies in the pathogenesis of dengue is poorly understood. We sought to investigate responses to NS1 in patients with Dengue Fever (DF) and Dengue Hemorrhagic fever (DHF).

Method: Antibody responses to NS1 were assessed in serum samples obtained daily, throughout the illness in patients with acute secondary dengue infection due to dengue virus (DENV) serotype 1 (DF =15, DHF=12) and DENV2 (DF =19, DHF=24) by an in-house ELISA. The antibody responses were also assessed to DENV1 and DENV2 overlapping peptides of NS1 in patients with DF (n=10) and DHF (n=10) during the critical phase of illness.

Results: The overall antibody responses to NS1 antibodies were significantly higher in patients with DHF compared to those with DF for both DENV serotypes, as the patients entered the critical phase of illness. The difference in the NS1 antibody levels was most significant on day 7 for patients with DENV1 infection ($p=0.0008$) and on day 6 for DENV2 infection ($p=0.0007$). In both DENV1 and DENV2 secondary dengue infection, patients with DF had significantly higher antibody titres to different regions of NS1 compared to those with DHF.

Discussion: NS1 specific antibodies increase more significantly in patients with severe forms of dengue, coinciding with the onset of the critical phase, which suggests that they could be involved in disease pathogenesis. In contrast, patients with milder forms of dengue appear to have a different antibody repertoire recognizing different regions of NS1, compared to those with DHF.

Presentation type: Poster presentation

P18

Analysis of Hemagglutinin and Neuraminidase gene of 2014-2015 Influenza A (H1N1) pdm09 virus from Kerala, Southern India

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Influenza A pandemic continues to be of significant concern in many parts of the world. Even though a vaccine is available the efficiency of the vaccine against currently circulating strains has been questioned. In India, starting of 2014, health officials have observed a significant increase in the number of H1N1 cases compared with seasonal H1N1 activity in this region. Approximately 34,351 cases and 2,097 deaths were observed. There are relatively limited genetic data available for this virus from Asian countries. In this study, we describe the genetic analysis of Hemagglutinin (HA) and Neuraminidase (NA) gene of influenza A (H1N1) pdm09 viruses isolated from Kerala between January through December 2015. The phylogenetic analysis showed the hemagglutinin sequence belonged to sub clade 6B.1. A study from Massachusetts Institute of Technology (MIT) in 2015 reported novel mutations K166Q, T200A and D225N in haemagglutinin gene of 2014 Indian strain. However, in all the pandemic strains of 2014–2015 reported from Kerala, D225N mutation was not observed, but confirmed the presence of K166Q and T200A mutations. Antigenic analysis showed that antibodies to A/Ca/04/09 cross neutralized the drifted virus with K166Q mutation in HA gene. Neuraminidase gene of the analyzed strains did not show any classical drug resistance mutations H275Y and N295S suggesting continuation of Tamiflu® as drug of choice. The amino acid sequences of HA and NA gene segments from 2015 A(H1N1)pdm09 isolates identified several new mutations compared to the 2009 A(H1N1)pdm09 strains, which may have contributed towards enhanced virulence, compared to 2009 A(H1N1)pdm09 strains.

Presentation type: Poster presentation

P19

Pichia pastoris-expressed Zika Envelope Domain III-Based Particulate Vaccine Candidate

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Recent worldwide outbreaks of zika virus (ZIKV) and its association with severe neurological complications underscore the need for a safe and effective ZIKV vaccine. The existence of the phenomenon of antibody-dependent enhancement (ADE) due to the close antigenic relationship of ZIKV with dengue viruses (DENVs) makes it necessary that a ZIKV vaccine should not mediate ADE of DENV infection and vice versa.
We report a novel Zika Subunit Vaccine (ZSV) which displays multiple copies of ZIKV envelope domain III (EDIII) on the surface of Hepatitis B surface (S) antigen virus-like particles (VLP). ZSV was obtained by co-expression of two recombinant proteins, of which one was an in-frame fusion (ZS) of four copies of ZIKV EDIII and one copy of hepatitis B surface (S) antigen and the other was unfused S antigen, in the methylotrophic yeast Pichia pastoris. ZS and S proteins were co-purified, to near homogeneity, using conventional chromatography from induced P. pastoris cells. Purified ZSV was found to contain VLPs based on dynamic light scattering and transmission electron microscopic analyses. These were highly immunogenic in BALB/c mice inducing antibodies that were specific to ZIKV EDIII but not to any of the DENV EDIIIs, based on indirect ELISA. Efforts are underway to determine ZIKV-neutralizing antibody titers in the murine immune sera. ZSV-induced antibodies did not enhance a sub-lethal DENV-2 infection in AG129 mice. Our preliminary data suggest that the VLP format of ZSV and the lack of DENV enhancement in vivo warrant further exploration of this novel vaccine candidate.

Presentation type: Poster presentation

P20

Association of single nucleotide polymorphisms in TNFA and IL10 genes with disease severity in severe influenza A/H1N1 pdm09 infections

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Rationale: Susceptibility to severe influenza A/H1N1 pdm09 influenza is multi-factorial involving pathogen, host and environmental factors. While the viral factors influencing disease severity has been extensively studied, there is dearth of data on the influence of host genetic variations on susceptibility to severe influenza A/H1N1 pdm09 infections in India.
Objective: To find out whether single nucleotide polymorphisms (SNPs) in the genes coding for pro-inflammatory and anti-inflammatory cytokines are associated with severe influenza A/H1N1 pdm09 disease
Methods: Blood samples from 246 patients infected with influenza A/H1N1 pdm09 were collected. The patients were classified into mild and severe based on the presence or absence of severe acute respiratory illness. TNFA (-308) and IL10 (-1082) SNPs were genotyped by PCR-SSP while IL10 (-592) and IFNG (+874) were genotyped by PCR-RFLP methods.
Results: A significantly higher frequency of G/A genotype of TNFA (-308) polymorphism was observed in severe cases [Odds ratio (OR) with 95% confidence intervals (CI) 3.05 (1.27-7.31)] and fatal cases [OR with 95% CI 3.28 (1.16-9.29)] as compared to mild cases and survived cases respectively. In an over dominant model, IL10 (-592 was significantly associated with fatality in H1N1pdm09 infections [OR with 95% CI 2.63 (1.08-6.37)]. When IL10 haplotypes were analyzed, a significantly higher frequency of IL10 A-C and G-A haplotypes were found in fatal cases compared to survived cases.
Conclusion: The preliminary results suggest that SNPs in the IL10 and TNFA genes might be associated with disease severity in influenza A/H1N1 pdm09 patients.
Funding: The study was funded by Department of Biotechnology, Government of India (BT/PR8957/MED/12/630/2013).

Presentation type: Poster presentation

P21

Differences in response to the dengue virus by primary human monocytes in those with varying severity of past dengue infection

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Objective: We sought to investigate the differences in initial immune responses to the DENV in those who had previously had either severe disease (PSD) or asymptomatic disease (PAD) following a secondary dengue infection.
Method: Monocytes from healthy individuals who had either PAD (n=6) or PSD (n=6) were infected at MOI one, with all four DENV serotypes following incubation with autologous serum. 48-hours post infection, levels of IL-10, IL-1β, IL-6, IL-8, TNF-α, VEGF, IL-18, PAF and viral loads were measured in culture supernatant and expression of IFNA-1, IFNA-2, IFNB-1, RIG-1, TRIM-25, ISG-15, NLRP-3, TLR-3, TLR-7 and TLR-9 were determined.
Results: Monocytes of individuals with PSD produced significantly higher viral loads (p=0.01) and cytokines (IL-10=p
Conclusion: Monocytes from PSD appear to show marked differences in viral sensing and production of inflammatory mediators in response to the DENV, when compared to those who experienced PAD, suggesting that initial innate immune responses may influence the subsequent disease outcome.

Presentation type: Poster presentation

P22

Eigallocatechin-3-gallate as a potent inhibitor of ATPase site of Zika virus NS3 helicase

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Zika virus (ZIKV) disease is one of the neglected tropical diseases that has transformed into a major issue of global health concern in present scenario. ZIKV is a flavivirus that threatens the world with recent outbreaks. The extent of infection and the modes of infection have led to the risk of spread worldwide. ZIKV infection has found to be implicated with severe diseases such as microcephaly and Guillain Barre's syndrome. Moreover reports have suggested that ZIKV can transmit sexually and found in seminal and vaginal fluids. Similarly, till date there is not a single specific drug or vaccine present in market against ZIKV. ZIKV helicase protein is an attractive drug target for search of antivirals. Viral helicases shows the unwinding activity of double stranded polynucleotides by utilizing energy released from ATP hydrolysis. ATPase activity of viruses is directly related to helicase activity which is further important to complete the replication process. In a previous study green tea molecule EGCG has been reported to inhibit the ZIKV entry into cells. However EGCG mechanism of action has not been known yet. In our study, we have first investigated the EGCG affinity for ATPase site of helicase through molecular docking and simulations. Computational findings were further verified through in-vitro ATPase assays on ZIKV helicase protein. EGCG is showing effective competitive inhibition of ATPase site with Ki value of 0.6uM. Further inhibition of helicase activity has been seen with molecular beacon based assays. These results suggest that EGCG could be considered as lead molecule of further cell based viral assays.

Presentation type: Poster presentation

P23

Evolutionary prospective of Japanese Encephalitis Virus

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Rationale: Japanese Encephalitis Virus (JEV) is a vector borne flavivirus where human are the dead end host. JEV is responsible for around 10,000 deaths per year round the world specific to the South-East Asia and Oceania region.

Methodology: We have taken epidemiological data from the first reported JEV and consecutive outbreaks in Japan, Korea, and India. Statistical analysis was carried to correlate time and region gaps, age factor, and evolutionary aspects based parameter for the JEV outbreaks incidents from the early Japanese and Korean studies as well as Indian reported studies.

Results: A near arithmetical increment time gap between the JEV outbreaks was found in earlier Japanese study based data. Korean and Indian based JEV reported studies are diverse, not only on time gap but also on region based, inside the country especially in India. Among Indian states, reported JEV outbreak data, based on VRDL laboratory, the median age of deaths occurred in Assam- 35 years, Tripura- 17 years, Uttar Pradesh -12 years, and Odisha – 8.5 years were found.

Discussion: JEV outbreaks are annual, especially in India which has a huge population with substandard life style. In India, most of the tropical zones are affected and carry a diversity of age. The same virus is affecting different age group people in different region. There might be a traceable evolutionary linkage between the same virus of different region or different viruses of same family in same or different region.

Presentation type: Poster presentation

P24

Cyclooxygenase-1 (Cox-1) transcript level in Peripheral blood mononuclear cells (PBMC's) of dengue patients

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Cyclooxygenase (COX) is a key enzyme in arachidonic acid metabolism which mediates inflammation during disease conditions. There are two well known isomers of COX in which Cox-1 is constitutively expressed in many tissues and had long been considered the housekeeping function, whereas Cox-2 is strongly induced by pro-inflammatory challenges. The role of COX enzymes in the pathogenesis of dengue viral infection in not known previously until a recent report stated an increased expression of Cox-2 in dengue cases. The study suggested that COX could be a potential therapeutic target for treating dengue. Though Cox-1 is a known constitutive gene, the status of its expression during dengue infection is not explored. To fill this gap, in the present study we quantified the expression of Cox-1 transcripts in the PBMC's obtained from dengue cases (N=34) compared to other febrile illness (OFI) (N=20) and healthy controls (HC) (N=24) using SYBR green Real time PCR. The results shows that there is no shift in mean Ct between OFI and healthy controls, whereas a highly significant variation in Ct between control and dengue cases. Compared to healthy controls, we observed a nearly 50% reduction in the fold change of Cox-1 expression in dengue cases, whereas no change in OFI group. This result along with previous report suggests that both Cox-1 and Cox-2 isomers are involved in the pathogenesis of dengue virulence. However a detailed study on the protein expression of Cox-1 and the mechanism that alters Cox-1 needs to be done.

Presentation type: Poster presentation

P25

Detection and Characterization of Saffold virus, an Emerging Cardiovirus, in Acute Flaccid Paralysis Patients from Uttar Pradesh, India

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Saffold viruses (SAFV), a recently discovered human cardioviruses are correlated with neurological symptoms. Increased rate of non-polio acute flaccid paralysis (NP-AFP) is a serious health concern in India. Few studies, have detected SAFV in NP-AFP children but no studies yet have been done in India. Thus, SAFV is also likely to be present in NP-AFP cases in India. Therefore, we investigated the presence, prevalence and distribution of SAFV in NP-AFP cases in India. Initially, the culture supernatant of one of the NP-AFP stool sample inoculated in green monkey kidney cells with uncharacterized cytopathic effects was subjected to sequence independent single primer amplification (SISPA) analysis; the resultant amplicons were cloned and sequenced. Sequence analysis revealed presence of SAFV encoded proteins (sequence similarity 78-85%). Next, AFP stool samples (virus negative; WHO algorithm), collected from Uttar Pradesh (UP), India (May, 2015 – June, 2017), subjected to SAFV screening targeting the 5′ untranslated region (UTR) of the virus. We detected SAFV in 9.6% (n=108) of 1,116 samples tested. For further characterization, we genotyped and sequenced the VP1 gene in the positive samples and found 70 different SAFV strains belonging to seven different genotypes (SAFV 1 – 7). We also observed SAFV co-infection with viruses such as enterovirus and cosavirus in (n = 8) of cases. Finally, the SAFV infection appeared to be prevalent (67.1%) in younger children.

Presentation type: Poster presentation

P26

In the absence of IFN-I signaling, the TNFα orchestrates robust innate immune response in the virally infected central nervous system.

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The central nervous system (CNS) is a relatively immune-privileged compartment but can elicit an elaborate innate and inflammatory immune response through the sentinel cells. We previously demonstrated that type-I IFN (IFN-I) signaling is central to innate immune response during noncytopathic virus infection (Lymphocytic Choriomeningitis Virus) and plays a crucial role in the myeloid cell recruitment to the CNS. To gain an insight into the function of IFN-I following cytopathic virus infection, we looked at the CNS gene response using vesicular stomatitis virus (VSV) encephalitis model.Here we studied differential gene expression profiling to identify the host cellular factors associated with CNS pathogenesis. The data reveal that in the absence of IFN-I signaling (IFNR-/-), a surprising higher number (n=1357) number of genes were differentially expressed compares to WT mice(n=281). The genes involved in immune response were enriched and tallied against the protein database to generate meta-network of the proteins (interactome) and ranked by using degree centrality. The results demonstrate that in the absence of IFN-I signaling, TNF-α orchestrates innate response as it is connected maximally to networked candidates. It is shown to be a key regulator in the immunomodulation of CNS defense against cytopathic virus infection. Based on these result, we propose that in the absence of IFN-I, the TNF-α signaling drives the expression of cytokines such as CXCL10 and CCL5 to recruit myeloid cells to the site of infection. Further, IFN-γ produced by myeloid cells along with TNF-α synergistically activate IRF1-dependent and IFN-I independent pathways that drive CNS anti-viral immune response.

Presentation type: Poster presentation

P27

Inhibition of dengue virus RNA-dependent RNA polymerase by binding of a novel inhibitor at the dsRNA exit site

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Dengue is one the most prevalent diseases in the world as the incidence of the spread of its infection has increased dramatically in the recent years. However, there are still no effective drugs or vaccine available against it. Dengue Virus (DENV) non-structural protein 5 (NS5) has RNA Dependent RNA Polymerase (RdRp) at its c-terminal through which the viral genome i.e. single-stranded RNA (ssRNA) replicates in the host cell. The RdRp is one of the attractive targets for antiviral drugs discovery as it is absent in the human cells and other organisms.

A pipeline encompasses computational chemistry, in-silico studies, chemical synthesis and biological evaluation in an iterative manner to identify hits and hit-to-lead conversion.

We identified a novel indole class of compound against DENV RdRp which possesses anti-dengue activity at low micromolar range in cell-based assay. The most likely binding site and binding mode of this compound were identified through in-silico, in ligand-dependent and -independent manner. The different allosteric sites are identified as possible binding sites, furthermore, through different filtering criteria and molecular dynamics simulations, we identified a novel allosteric site for this compound.

From ligand and structure-guided approach, a new chemical moiety was designed which potentially able to block the RNA replication. The binding site of the compound is localized at the junction of thumb-finger domain, it probably blocks the exit site of dsRNA. Therefore, we propose that the exit site nearby priming loop might serve as a promising site in the DENV RdRp for the antiviral drug discovery.

Presentation type: Poster presentation

P28

Expression of complement receptor 3 and regulatory protein CD46 on dendritic cells of antiretroviral naïve and treated HIV-1 infected individuals and its correlation with immune activation status

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During infection and budding, human immunodeficiency virus-1 (HIV-1) acquires regulators of Complement Activation (RCAs) along with the host cell membrane on the viral envelope. Activation of host complement system results in opsonization of virus by complement fragments, however the virus evades complement mediated lysis (CoML) by virtue of the RCAs on the viral envelope. The RCAs on HIV-1 envelope process complement protein C3 into various fragments that promote viral entry and infection of cells through different complement receptors. Complement opsonized HIV-1 has been shown in vitro to infect dendritic cells (DCs) in a CR3 dependent manner, although the role of CR3 and CD46 in natural HIV-1 infection is not clear. Surface expression of CR3 and CD46 on DC subsets of 30 antiretroviral naïve, 31 treated(cART) HIV-1 infected individuals and 30 seronegative controls was measured by flow cytometry and plasma levels of cytokines and complement activity(C3c levels) were quantitated by sandwich ELISA. Significantly lower surface expression of CR3 and CD46 was observed on DC subsets in naïve and treated HIV-1 infected individuals compared to controls. Significantly higher complement activation and plasma levels of IL-4, IL-8, IL-10 and IFN-γ were observed in treatment naïve HIV-1 infected individuals than controls. Significantly lower plasma levels of IL-4, IL-6, IL-8 and IL-10 were observed in treated vs. naïve HIV-1 infected individuals. Our findings suggest that alterations in expression of CR3 and CD46 on DCs along with complement activity could be factors that influence viral persistence and HIV-1 disease progression and need to be further evaluated.

Presentation type: Poster presentation

P29

Papain-like cysteine protease of hepatitis E virus interacts with IPS-1 and inhibits RIG-I signaling pathway

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Rationale:
Hepatitis E virus (HEV) is an etiological agent for acute and chronic viral hepatitis. Host innate immune response against RNA viruses involves recognition by specific pattern recognition receptors (PRRs), such as retinoic-acid inducible gene-I (RIG-I) leading to type I interferon production. Viruses have evolved multiple strategies to evade host immune responses, including interference of innate immune signaling by viral encoded proteins. RIG-I was shown to be one of the primary PRR responsible for HEV detection. We hypothesized that HEV proteins may modulate RIG-I signaling.

Methods:
Cell-based reporter assays were performed to determine the effect of HEV proteins on RIG-I activation/signaling. Interplay between the viral proteins and host signaling proteins were assessed by immunoprecipitation. Effect of PCP on RIG-I adapter, IPS-1 aggregation or ubiquitination was evaluated by western blot analysis and immunofluorescence assay.

Results:
HEV encoded papain-like cysteine protease (PCP) significantly inhibited IFNβ production through RIG-I signaling. Further analyses indicated that the observed inhibition is not due to the proteolytic activity of PCP, but due to its physical interaction with IPS-1, a known adapter for RIG-I. Association of PCP severely restricted IPS-1 aggregation and ubiquitination, which further prevented its interaction with downstream signaling partners (such as TBK1) essential for interferon production. Lastly, HEV PCP was unable to inhibit toll-like receptor signaling, which is not dependent on IPS-1.

Discussion:
PCP probably plays a major role in establishing HEV infection by interfering with host immune response and a PCP-mutant HEV could be a potential live-attenuated vaccine candidate.

Presentation type: Poster presentation

P30

Human Immunity to chikungunya infection

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Chikungunyna virus is expanding globally and continue to cause major public health threat to Indian populations. Vaccine efforts are underway, and it is hoped that these will eventually progress to human evaluation. However, currently we have little understanding of the phenotypes and functions of the human T cells in chikungunya patients, a knowledge that is essential for improving vaccine design/ testing and evaluation efforts. Here, we provide a detailed analysis of the CD8 T cell responses in chikungunya patients from India. We found that $CD38^{+}HLADR\text{-}38^{+}$ CD8 T cell subset expanded dramatically in chikungunya febrile patients with frequencies averaging about 20% of the total CD8 T cells, and reaching as high as 50% of the CD8 T cells in some patients. The frequencies of these activated CD8 T cells were substantially low and barely above background levels in afebrile patients reporting to the clinic with persistent arthralgia/ arthritis that was lasting for more than 30 days. These massively expanding CD8 T cells observed in the acute febrile patients were highly proliferating ($ki67^{+}$), robustly expressing markers indicative strong Th1 differentiation ($T\text{-}bet^{+}$), cytotoxic functions ($Perforin^{+}$) and inflammatory/ synovial tissue homing characteristics (CX3CR1 and CXCR4). Interestingly, antigen-stimulation mediated IFN-g producing functions of these cells was highly compromized, reminiscent of the “cytokine stunned” phenotype seen in other situations such as human dengue or hepatitis C infections. Taken together, these results suggest that these highly differentiated effectorCD8 T cell that were massively expanding during acute chikungunya febrile infection might be involved in protection by homing to infected tissues and eliminating infected targets rather than causing inflammation.

Presentation type: Poster presentation

P31

Role of exosome associated microRNAs in HCV induced pathogenesis

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Hepatitis C virus is a liver specific virus, which causes chronic hepatitis. It has been reported that apart from the classical receptor mediated transmission, HCV is transmitted via. Non-receptor mediated exosomal pathway as well. Exosomes are small multi-vesicular bodies which have a major role in cell communication and signaling. Exosomes are composed of proteins, mRNA and miRNAs whose composition may varies depending on the cell type. To study the changes in the level of exosome associated miRNAs under HCV infection we isolated exosomes from the serum of HCV infected cirrhosis and hepatocellular patients sample and performed RNA-seq. We identifed and validated a number of miRNAs that are differentially expressed under different stages of HCV infection compared to healthy control. Further, we would like to understand if the increased secretions of these miRNAs have any role in the progression of HCV infection to carcinogenesis. We extended our study with miR-375, which was found to be significantly up-regulated in serum exosomes of patient's samples. Similar results were found in the cell culture system as well. Increased intracellular miR-375 found to specifically target IGFBP4, causing a decrease in IGFBP4 expression in cell, resulting in the malignant phenotype such as cell proliferation and cell growth. Conversely, with antimiR-375 treatment we could observe the rescue of the phenotype and IGFBP4 expression ex- vivo. Hence, we hypothesize that miRNAs and proteins associated with exosomes will have a significant role in the disease progression after HCV infection via activating the cell signaling cascade in neighboring cells.

Presentation type: Poster presentation

P32

The correlation of pre-existing immunity against JEV with severe dengue disease as measured by ELISA with JEV specific peptides

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Rationale
We sought to identify non-cross reactive, Japanese encephalitis virus (JEV) specific antibody epitopes, in order to determine if the presence of pre-existing immunity to JEV associated with dengue disease severity.

Methodology
We identified 36 JEV specific, highly conserved peptide sequences, 20 amino acids long, by using Clustal W. Using ELISA assays, we assessed the antibody responses to these peptides in DENV and JEV seronegative individuals (n=20) DENV seronegatives who were vaccinated for JEV (n=30), DENV seropositives who were vaccinated for JEV (n=30) and DENV seropositives who were not vaccinated for JEV (n=30). We further evaluated if presence of JEV specific antibody responses associate with severe dengue by assessing the JEV antibody responses in aged matched, 172 individuals with past asymptomatic dengue (AD), and 172 individuals with past severe dengue (SD).

Results
7/36 peptides elicited responses only in those who had received the JEV vaccine, while none of those who were DENV seropositive and did not receive the vaccine responded. 99 (57.5%) of those with SD and 51 (29.6%) of those with AD had JEV specific antibody responses. The presence of JEV-specific antibody responses significantly associated with SD (p

Discussion
Presence of JEV-specific antibodies appear to associate with SD, which should be further investigated due to implication in JEV vaccination programs.

Key words: JEV, ELISA, Dengue, JE vaccination

Presentation type: Poster presentation

P33

Relocalisation of nuclear factors to regulate HCV RNA replication in cytoplasm

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Hepatitis C virus is a positive sense, single stranded RNA virus which completes its life cycle in the cytoplasm of host hepatocytes. Viral ORF codes for structural and non-structural proteins and is flanked by 5'UTR and 3'UTR. The IRES element present in 5'UTR is responsible for initiation of translation of uncapped viral RNA. Translated viral proteins are involved in initiation of replication on the 3'UTR to increase viral copy number. Various cellular factors are required for regulation of translation and replication of viral RNA. Most of these factors are nuclear residents and relocalize to cytoplasm upon HCV infection. From our laboratory, it has been shown that HuR, La and PTB have differential effect on viral replication. The function of these factors is largely governed by their availability for binding to viral RNA. In turn, their availability can be guided either by time kinetics of their relocalisation to the cytoplasm, or by certain post-translational modifications which assist RNA binding activity of the proteins. Our study focuses on the role of various host factors including lncRNAs, miRNAs and proteins in orchestrating the relocalisation. Results suggest that HuR, La and PTB come out to cytoplasm almost same time post infection. It appears that specific PTMs could influence their functional availability for HCV RNA. Taken together, results suggest the 'tug-of-war' between the host response and viral strategies helps to regulate the abundance of nuclear factors to establish chronic infection and ultimately contribute to cellular pathogenesis.

Presentation type: Poster presentation

P34

Clinical and molecular trends of rotavirus among neonates from Chennai, South India

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Rationale:
Rotavirus predominantly causes mild or asymptomatic infections among neonates worldwide. Very few studies analyze the risk factors and documents the strains of rotavirus among neonates. This study focuses on the detection of circulating strains, risk factors of RV infection among neonates.

Methodology:
A total of 336 stool samples were collected from the neonates who were admitted >48 hours in the neonatal intensive care unit (NICU) after obtaining their parental consent. Rotavirus antigen detection was done using commercial enzyme immunoassay Rotaclone (Meridian Bioscience INC.,). The samples tested positive by ELISA were subjected to conventional VP7 & VP4 RT-PCR to identify genotype.

Results:
Rotavirus antigen was detected in 29.5% of neonates. Rotavirus detection was significantly higher (36.3%) among late preterm and term neonates (>34 weeks) than in moderate and very preterm babies (2kg had a significantly higher shedding of rotavirus (36.6%) when compared to low birth weight babies < 2kg (24.1%) (P=0.01). Gender, breast feeding and mode of delivery did not influence rotavirus infection. Among the neonates who had gastro intestinal symptoms such as diarrhea, vomiting, feeding intolerance, necrotizing enterocolitis, 40% had rotavirus infection in contrast to asymptomatic neonates it was 29.1%. A single genotype G10P [11] was identified in our NICU.

Conclusion:
A single strain G10 P[11] was found to circulate in our tertiary care centre. Rotavirus infection was significantly higher in Late preterm, term, higher birth weight and symptomatic neonates.

Presentation type: Poster presentation

P35

Translation to replication switching by resource segregation during Flavivirus life cycle

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Single-stranded RNA (ssRNA) virus infection cycle progresses by resource (viral RNA) allocation to segregated macromolecular complexes: host ribosomes (translation), viral RNA polymerase (replication) and viral structural proteins (packaging). Many positive-sense ssRNA viruses from Flaviviridae family further compartmentalize replication process in membranous vesicles, which is proposed to help in selective resource allocation to vRNA polymerase. Currently, detailed and quantitative understanding of resource allocation during viral life cycle is missing. We hypothesized that spatial segregation of replication imparts replicative advantage for the virus by optimizing the viral protein to nucleic acid ratio. Using Japanese encephalitis virus infection in neuro2a cells, we measured vRNA dynamics in the context of fraction of vRNA undergoing translation, replication and packaging during virus life cycle. We observed high levels of vRNA translation during early stages of infection as expected for a (+) ssRNA virus. However, as infection progressed, vRNA transitions from a translation dominant phase to a replication phase while the total vRNA continued to increase. Additionally, we mathematically modeled the kinetics of resource distribution in a well-mixed versus a compartmentalized cell infection model and compared this to experimental findings. Our model predicts that this translation to replication switch is inherent to the lifecycle architecture with replication compartmentalization in vesicular membrane structures. Perturbation of the modeled kinetic parameters demonstrates drastic changes in viral burst sizes beyond critical switching times, observed in experiments, suggesting multiple steady states for the virus lifecycle. We posit that analysis of intracellular viral dynamics using a combination of experimental and modeling efforts can provide novel insights into viral life cycle.

Presentation type: Poster presentation

P36

Genetic Variations of the Hemagglutinin gene of Pandemic Influenza A (H1N1) Viruses in Assam, India during 2016-2017

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Rationale: Sudden re-emergence of influenza A/H1N1pdm09 in 2015 affected over 39,000 persons with more than 2,500 deaths in India making it the largest epidemic since 2009. This necessitates a continuous surveillance and monitoring of influenza activity in Assam for disease prevention and formulating effective vaccination strategies.

Methodology: 3708 Nasopharyngeal/Throat swab samples were collected from Dibrugarh district of Assam in 2016-2017 who presented with Influenza like illness. The influenza A/H1N1pdm09 virus was detected using TaqMan assay and further sequence and phylogenetic analysis was carried out using MEGA6.06 and structural analysis using I-TASSER server targeting the hemagglutinin (HA) gene.

Results: Twenty samples in 2016 and 208 samples in 2017 were found to be positive for influenza A/H1N1pdm09 virus. In 2016, HA gene showed 97.1- 97.5% identity with A/California/04/2009 vaccine strain with three mutations (K180Q, S202T and S220T) at antigenic sites along with other reported mutations. Further in 2017, HA gene showed 99.5% identity with A/Michigan/45/2015 vaccine strain with two conserved mutations at I312V and R240Q along with two novel substitutions V190I and I527T in two of the isolates. Phylogenetic analysis of the studied samples formed a monophyletic clade with 6B genogroup. The 3D structural analysis of HA protein revealed significant conformational change which may affect the structure of receptor binding pocket and in antibody recognition.

Discussion: The study revealed the circulation of distinct influenza A/H1N1pdm09 strains in 2017 compared to 2016 with marked genetic variations. This may further turn into a more virulent strain escaping viral neutralization thereby increasing disease severity.

Presentation type: Poster presentation

P37

Use of DNAzyme to inhibit Dengue virus replication

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Rationale: DNAzymes are DNA oligonucleotides capable of cleaving RNA in a sequence-specific manner. We aim to use the DNAzyme technology to develop synthetic DNA molecules that can cleave the RNA genome of Dengue virus (DENV) in a specific and effective manner, thus potentially inhibiting the virus replication. We hypothesize that DNAzymes targeted against the DENV genome could reduce the viremia level in patients potentially saving them from the severe form of disease.

Methodology: Genome sequences of the various DENV serotypes, derived from the Viprbrc database, were aligned to identify the conserved regions containing the AU or GU sequences that constitute the preferred cleavage sites for the DNAzyme. DNAzymes targeting these conserved sequences were synthesized and their effect on DENV replication in Huh7 cells was checked by Real Time-PCR for intra-cellular viral RNA level and Focus Forming Assay (FFA) to estimate the extra cellular viral titre.

Result: Serotype-specific as well as cross-reactive DNAzymes were designed and synthesised. During the primary screening against DENV2, DNAzymes which showed a significant reduction in the intra-cellular virus RNA as well as extra cellular virus levels were selected for secondary screening. Thus, DNAzymes 9, 10, 11 and 12 showed a significant reduction in DENV2 viral titre as well as intra-cellular viral RNA levels in Huh7 cells.

Discussion: Various modifications will be tested to improve the DNAzyme activity and stability. The DNAzyme-based therapy offers several advantages as they are easy to produce, cost-effective, non-immunogenic, highly stable, and unlikely to show any side-effects.

Presentation type: Poster presentation

P38

Molecular characterization and clinical correlates of Norovirus from Chennai, South India

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Background:
Norovirus is one of the predominant causes of acute gastroenteritis worldwide. Very few studies have documented the burden of norovirus in India. This study aims to determine the prevalence, genotype distribution and clinical severity of norovirus infection.

Methodology:
A total of 163 stool samples were collected from children (< 5 years) who were admitted with diarrhoea in a tertiary care centre after obtaining informed consent. Clinical details were documented in a proforma and the severity of acute gastroenteritis was assessed using the modified Vesikari scale (0-20). Samples were screened for rotavirus antigen using commercial ELISA. Multiplex real time Taq Man RT-PCR was used to detect human norovirus GI and GII. Positive samples were amplified by conventional RT-PCR spanning both partial polymerase and capsid gene fragments and RT-PCR positive products were sequenced and genotyped by comparing to norovirus reference sequences.

Results:
Rotavirus antigen was detected in 31.3% of stool samples. Norovirus was detected in 9.8% of the stool samples of which 15 (94%) were GII and 1(6%) was GI. GII.4Yerseke (18.2%) and GII.3 (18.2%) were the most prevalent genotypes. GII.P21-GII.13, GII.P7-GII.7, GII.Pe-GII.4Yerseke, GII.P16-GII.4Yerseke, GII.P16-GII.4Sydney, GII.P7-GII.P6, GII.P16-GII.3, GII.P21-GII.21, GII.P7-GII.9, GII.P16-GII.3 and GI.P1-GI.1 genotypes were identified. The mean Vesikari score among norovirus infected and un-infected children was 12.81+ 2.73 and 10.23+ 2.74 (p=0.005).

Conclusion:
Rotavirus was observed in 31.3% and norovirus in 9.8% of the children with acute gastroenteritis. GII.P16-GII.4 and GII.P16-GII.3 were the most predominant circulating genotypes. The severity of acute gastroenteritis was higher in norovirus infected children.

Presentation type: Poster presentation

P39

Ex Vivo Generation and Single-Cell Analysis of Human Monoclonal Antibodies from Dengue Virus Infected Patients

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Antibodies have been implicated in both protection and pathology of dengue virus infections. However, much of this data is gathered from serum/plasma responses that is a cumulative of historical and ongoing infection. To precisely understand the role of antibodies with respect to the ongoing dengue virus infection, we employed the cutting edge approach of generating of human monoclonal antibodies from individual plasmablasts from peripheral blood of dengue patients that allows us to probe for answers at a single cell level.
This method involves ex vivo single cell sorting of plasmablasts from peripheral blood of well-characterized dengue infected patient followed by single cell molecular cloning of immunoglobulin heavy- and light- variable regions into expression vectors containing the defined constant region followed by transient co-transfection of HEK 293A cells with the heavy and light chain expression vectors made from genes arising from the same cell.
Thus far, using this powerful technology, for the first time in India, we have made 73 number of human monoclonals, of which 36 are specific to dengue and 12 neutralize dengue and zika virus at various concentrations. All the neutralizing antibodies are dengue-envelope specific and bind the highly conserved fusion loop of the dengue virus envelope.
Together, with the ongoing comprehensive analysis of the B cell repertoire and somatic hypermutations, these studies provide a detailed understanding of the dengue-specific plasmablast cell response at a single cell level and create a platform for testing these antibodies for basic research, diagnostic, prophylatic and as well as therapeutic applications.

Presentation type: Poster presentation

P40

Autophagy induction effectively controls opportunistic mycobacterial infections in macrophages co-infected with HIV

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RATIONALE:
Opportunistic bacterial infections amongst HIV-infected individuals pose a serious health challenge. While immediate control of bacterial pathogens is typically attributed to innate defense mechanism, whether HIV-mediated modulation of innate mechanisms like autophagy contributes to opportunistic infections, remains obscure. So, we aim to study host pathogen interactions at cellular level which would open more avenues to find therapeutics in HIV co-infection.

METHODOLOGY: CFU,Confocal

RESULTS AND DISCUSSION
Using U1.1 and U937 macrophages, we show, HIV activation or infection inhibits autophagy and helps survival of pathogenic Mycobacterium tuberculosis and non-pathogenic non-tubercular mycobacterial strains (NTMs) like Mycobacterium avium complex and Mycobacterium fortuitum. HIV achieves so by blocking xenophagy flux. HIV induced inhibition of xenophagy flux could be reversed by trehalose, a natural disaccharide known for its pro-autophagy activity. Also, treatment with trehalose results in a substantial decrease in intracellular Mtb and NTMs during single infection or when co-infected with HIV. Trehalose, we show for the first-time, acts as a PI(3,5)P2 agonist to activate TRPML-1, a calcium ion channel present on lysosomal membrane and activates autophagy. Remarkably, trehalose treatment significantly reduced HIV-p24 levels in PBMCs infected with clinical HIV strains or in PBMCs derived from treatment-naïve HIV patients.

CONCLUSION:
Present study deciphers a novel mechanism in which trehalose acts as a PI(3,5)P2 agonist to activate TRPML-1 to regulate autophagy induction. Trehalose mediated autophagy induction was sufficient to compromise bacterial survival during HIV co-infection and HIV survival in PBMCs.

SIGNIFICANCE:
Our study highlights the immense potential of autophagy regulators in therapeutic intervention of HIV and associated opportunistic infection.

Presentation type: Poster presentation

P41

Development of a Magnetic Nanoparticle based rapid immunoassay for early dengue diagnosis

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Rationale: Acute febrile illness due to dengue is common in the tropics and majority of patients present to primary health centres. However dengue diagnostics are mostly performed at tertiary care centres. Application of Magnetic nanoparticles in diagnostics is less explored. We aimed at developing MNP based immunoassay to address early diagnosis suitable even in resource limited settlings.

Methodology: Institutional Ethics Committee approval (IEC-NI/12/OCT/30/50) was obtained. Written informed consent was obtained from all the participants A MNP based immunoassay was optimised with rabbit polyclonal dengue antibody immobilised to MNPs as the capture antibody, mouse monoclonal dengue antibody as secondary antibody followed by antibody conjugated Qdots Fluorescence of the immune complex was detected using a table top transilluminator. The entire procedure was completed in < 2 hours. Serum samples from patients with acute febrile illness was used to evaluate the MNP assay. Suitable quality controls were included. The performance of the assay was compared with Real time dengue PCR. Results & Discussion: Of the 97 participants, 83 were clinical dengue (WHO2009); probable dengue (n=47), dengue with warning signs (n=36) and severe dengue (n=7. About (n=75) were positive for Real time dengue RT-PCR. The MNP assay had a sensitivity of 93.7% and a specifity of 70%. Positive predictive value of the MNP assay and positive likelihood ratio was 80 % and 2.06 respectively. This MNP assay will be useful in ruling in diagnosis of dengue. Conclusion: This bench top MNP based immunoassay is simple qualitative assay for early detection of dengue in resource limited settings.

Presentation type: Poster presentation

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