ICGEB-IUBMB Workshop
on

Human RNA Viruses

10–12 February, 2010

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II Workshop "Human RNA Viruses"
10th February – 12th February 2010
ICGEB New Delhi

PROGRAMME

Wednesday 10th February

08:00 – Departure from Hotels
08:30 – Registration
09:30 – Inaugural session (Director, ICGEB, New Delhi and Workshop coordinators)

Session 1

10:00 – 10:45

Dr. Takashi Fujita	Mechanism of RNA recognition by the RIG-I-like receptors and activation of antiviral program

10:45 – Break

Session 2

11:15 – 12:00

Dr. R. Holland Cheng	Design of theranostic capsule based on modular arms and hinges of protein structural elements (MANHOPE)

12:00 – 13:00

Dr. Scott B. Halstead	Antibodies determine virulence of Dengue viruses

13:00 – Lunch

14:00 – 15:00 Poster Session 1 All posters presenters will be in attendance.

Session 3

15:00 – 15:45 Student talks (10 min each + 5 min questions)

Boguslaw Szewczyk	Fast detection of small changes in genomes of recent Influenza A isolates by multi-temperature single-strand conformational polymorphism (MSSCP) technique (P-37)
Grehete Gonzalez	Improvement of molecular diagnostic for surveillance of respiratory viruses in Cuba (P-17)
Arindam Mondal	Involvement of an intracellular phosphatase activity upon Chandipura virus life cycle: A possible transcription to replication switch (P-24)

15:45 – 16.30

Dr. Navin Khanna	Dengue vaccines - current progress and challenges

16:30 – 17:15

Dr. Alessandro Marcello	Spatial and temporal correlates of RNA virus replication

17.30 – Return to Hotels

Thursday 11th February

8:30 – Departure from Hotels

Session 4

09:30 – 10:15

Dr. Adolfo Garcia-Sastre	H1N1 pandemic influenza

10:15 – 11:00

Dr. Sunil K. Lal	The nucleocapsid protein of influenza virus downregulates the PKR pathway through the host chaperone protein, hsp40; a unique strategy to keep host translation "ON"

11:00 – Break

Session 5

11:30 – 12:15

Dr. Monsef Benkirane	Reactivation of latent HIV-1: what can we do with what we know?

12:15 – 13:00

Dr. Sven Thamm	The challenge of HIV diversity and its implications for diagnostic assay performance

13.00 - Lunch

Session 6

14:00 – 14:45 Student talks (10 min each + 5 min questions)

Maryana V. Bardina	How cardioviruses alter nucleocytoplasmic transport of the host cell (P-7)
Lavina Gharu	Genetic and biological properties of HIV-1 Indian clade-C envelopes with extended co-receptor tropism (P-16)
Teppo Salminen	Homogeneous and heterogeneous Hepatitis C antibody assay based on multiepitope proteins and fluorescent lanthanide chelates (P-33)

14:45 – 15:30

Dr. Oscar Burrone	Replication and assembly of Rotavirus

15:30 – 17:00 Poster Session 2 All posters presenters will be in attendance.

Tea/Coffee will be served at posters.

Session 7

17:00 – 17:45

Dr. C. Durga Rao	Wider scale analysis reveals a lack of correspondence between the virulence of rotavirus strain and the diarrheagenic property of the cognate enterotoxigenic protein NSP4: Is there a structural diversity among NSP4s?

17:45 – 18:30

Dr. Luis Enjuanes	Gene expression and virus-host interaction in Coronavirus

18:30 – Reception and Dinner at ICGEB

20:00 – Return to Hotels

Friday 12th February

8:30 – Departure from Hotels

Session 8

09:30 – 10:15

Dr. Luis Enjuanes	Mechanism of transcription in Coronavirus and protection against SARS-CoV

10:15 – 11:00

Dr. John McLauchlan	Interactions between viral and host factors that are needed to promote replication and assembly of Hepatitis C virus

11:00 – Break

Session 9

11:30 – 12:15

Dr. Saumitra Das	The interplay between host factor and viral protein regulates translation and replication of Hepatitis C virus

12:15 – 13:00

Dr. Shahid Jameel	Multi-tasking by viral proteins

13:00 – Lunch

Session 10

14:00 – 14:45 Student talks (10 min each + 5 min questions)

Manjula Kalia	Heparan sulfate proteoglycans are required for cellular binding of the Hepatitis E virus ORF2 capsid protein and for viral infection (P-19)
Upasana Ray	Determinant within NS3 protease critical for RNA binding to mediate the switch from translation to replication of Hepatitis C virus RNA (P-31)
Marycelin M. Baba	Arbovirus infections in febrile patients suspected of malaria and typhoid in Nigeria (P-4)

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Dr. Tariq Rana	RNAi pathways and host-virus interactions

15:30 – 16:00

Concluding remarks and announcement of poster prizes.

16:15 – Return to Hotels

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POSTER ABSTRACTS
(ARRANGED ACCORDING TO LAST NAME OF PRESENTING AUTHOR)

Speaker Abstracts

Mechanism of RNA Recognition by the RIG-I-like Receptors and Activation of Antiviral Program

Takashi Fujita
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Cytoplasmic non-self RNAs such as those generated by invading viruses are recognized by a family of sensor molecules termed RIG-I-like Receptor (RLR). Here I discuss the mechanism of sensing non-self RNA by the RLRs. Biochemical and structural studies have shown that one of the RLR, RIG-I, specifically recognizes distinct features of non-self RNA: 5'ppp-RNA and short double stranded-RNA. In addition, a domain at the C-terminus with basic concave surface is involved in recognition of these substrate RNA species. Specific RLR, RIG-I and MDA5 show distinct specificity such as preference of 5'-phosphate group or length of RNA. These findings define three functional domains of RLR and provide insights on how it functions as a molecular switch through interaction with virus-specific RNA ligands.

Design of Theranostic Capsule Based on Modular Arms & Hinges of Protein-Structural Elements (MANHOPE)

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Based on the success of sorting the structural heterogeneity, the feasibility of observing assembly intermediates can now provide the essential framework to observe the local modularities of subunit and to rationalize the overall presentation of the consequent macromolecules. The multivalence of these higher ordered assembly will be exemplified as platform technology for nanomedicine design, whereas the protein domains are to be the modular units in directing the pathway of self-organizations. Single-molecule tomography and cryoEM image reconstructions are implemented to analyze how the weak-force associations are transformed based on the interactions of the involved subunits. Technical advancement and heterogeneity sorting algorithms played the keys in featuring the discrete states of protein conformation with characteristic assembly intermediates.

Antibodies Determine Virulence of Dengue Viruses

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Four dengue viruses (DENV) cause syndromes that are self-limited or severe. The severe syndrome, dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), characterized by sudden vascular permeability has consistently been observed to accompany dengue infections in individuals circulating heterotypic dengue antibodies at infection-enhancing concentrations**.** In humans, dengue infections target monocytes/macrophages where, absent neutralization, heterotypic antibodies, perhaps directed at domain I-II of the envelope protein form immune complexes, attach to Fc receptors, suppress innate immunity and increase productive infection. Among the wide spectrum of micro-organisms that replicate in macrophages, cell entry via non-neutralizing IgG antibody complexes may result in increased infection due to idiosyncratic Fcγ-receptor signaling. This phenomenon, intrinsic antibody-dependent enhancement of infection (iADE), modulates the severity of diseases as disparate as dengue and *Leishmaniasis*. Paradoxically, the ligation of monocyte/macrophage Fcγ receptors by immune complexes suppresses innate immunity, liberates IL-10 and biases Th 1 to Th 2 responses. Evidence for iADE in dengue viral infections, insight into intracellular mechanisms and implications for enhanced pathogenesis following ligation of macrophage Fcγ receptors by infectious immune complexes are reviewed. Dengue antibodies, actively or passively acquired, modify dengue infections from mild to severe; severe dengue is not caused by inherent viral virulence.

Dengue Vaccine-Current Progress and Challenges

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Dengue is a flaviviral disease that is currently a public health problem of global proportions. Vaccines for other flaviviral disease have been successfully made. But a dengue vaccine has been elusive despite decades of effort. Several factors such as the existence of four antigenically distinct viruses that cause disease, the immune enhancement phenomenon underlying disease pathogenesis and the lack of a good animal model of the disease have collectively contributed to making the task of developing a dengue vaccine a formidable one. Global awareness of dengue in recent years has kindled renewed interest in developing dengue vaccines. This presentation will provide a brief overview of dengue disease, the rationale that drives and the hurdles that confront vaccine development efforts, the various approaches to dengue vaccines, current status of clinical trials and conclude with the challenges that must be addressed successfully before a dengue vaccine can become available for human use.

Spatial and Temporal Correlates of RNA Virus Replication

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Viral RNA biogenesis is a crucial step in the replication of RNA viruses and retroviruses that require both the production of genomic RNAs and of translation templates. Cellular and viral factors concur in the biogenesis of RNA at the specific sub-cellular site where the reaction takes place. The possibility of tracking viral RNA in living cells gives the unique possibility of measuring the kinetic parameters of RNA biogenesis as well as defining the dynamic recruitment of host and viral factors to the site of replication.

Retroviruses integrate their genome into the chromatin of the host cell and are subject to the same control mechanisms governing transcription in the nucleus. There is increasing evidence that the spatial position of a gene within the nucleus in time affects its activity. Therefore it becomes important to study the chromatin environment in space and time of the HIV-1 provirus, particularly in cells where a tight transcriptional control allows the virus to hide away from antiviral treatment and immune response. We recently showed that the HIV-1 provirus is found at the nuclear periphery of latently infected lymphocytes associated *in trans* with centromeric heterochromatin (Dieudonné, et al. EMBO Journal, 2009). Our results reveal a novel mechanism of transcriptional silencing involved in HIV-1 post-transcriptional latency and open wider perspectives for the general organization of chromatin in the nucleus. Furthermore, by engineering a tagged HIV-1 RNA we could characterize the HIV-1 transcription cycle allowing precise kinetic measurements of RNA polymerase elongation rates as well as initiation, splicing and termination steps (Boireau, et al. JCB 2007). We also analyzed the dynamic of the TAR:Tat:pTEFb complex at the site of HIV-1 transcription in living cells (Molle, et al. Retrovirology 2007). Our data suggest that this complex dissociates from the polymerase following transcription initiation, and may undergo subsequent cycles of association/dissociation.

We extended this approach to the tick-borne encephalitis virus (TBEV). Flaviviruses are positive RNA viruses that assemble the replication complex in the cytoplasm of the infected cells (Miorin, et al. Virology, 2008). The modified TBEV replicons were competent for RNA replication and allowed the visualization of replicated genomic RNA that accumulated in cytoplasmic structures with a distinct sub-cellular localization. A dynamic view of TBEV RNA biogenesis in living cells can be seen at ftp://ftp.icgeb.org/pub/tmp/BHKA3movie.avi.

Our work provides an experimental framework to analyze viral RNA biogenesis and RNA/protein dynamics in four dimensions.

H1N1 Pandemic Influenza

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The recent influenza A pandemic has been caused by an H1N1 influenza virus of swine origin. We have characterized the phenotypic properties of this new human influenza A virus with respect to transmissibility and pathogenesis in the guinea pig and mouse animal models. In guinea pigs, the virus, as opposed to previous swine influenza viruses, demonstrated efficient aerosol transmissibility. In mice, severity of disease varied between different viral isolates. Interestingly, vaccination of mice with old human H1N1 strains or with classical swine H1N1 strains provided protection against lethal challenge with the new pandemic H1N1 virus. This was mediated by induction of cross-reactive neutralizing antibodies and implies that individuals exposed to old H1N1 viruses (before 1950) are likely to be protected against disease induced by the new virus. The role of the NS1 gene of this virus in its virulence was evaluated by reverse genetics. Finally, a wide genome siRNA screen in tissue culture revealed cellular genes required for efficient influenza virus (including the new pandemic H1N1 virus) replication. A subset of these cellular genes might represent attractive targets for the development of novel antivirals for the therapeutic treatment of influenza.

The Nucleocapsid Protein of Influenza Virus Downregulates the PKR Pathway through the Host Chaperone Protein, hsp40; a Unique Strategy to Keep Host Translation "ON"

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Influenza A viruses encode a 498 amino acid nucleocapsid (NP) protein which is required for viral encapsidation. In addition, NP has also been postulated to be involved in nuclear import and export of the genome, interactions with viral polymerase, matrix protein, or CRM-1 protein. We have identified the chaperon heat shock protein 40 (hsp40) as an interaction partner for NP. The interaction was confirmed by immunoprecipitation, and colocalization studies using NP transfected and influenza A virus-infected cells. In addition, the levels of both phospho-PKR (p-PKR) and phospho-eIF2α (p-eIF2α) were reduced and silencing NP restored p-eIF2α to near normal levels.

During the course of viral infection, the host cell, by balancing the levels of phospho-eIF2α controls the translation during normal or during viral infection. One of the important molecules for transmitting the stress signal to eIF2α is a serine-threonine kinase, PKR which is one of the pivotal keys of host defense against viral infection. As one of anti-viral strategies, host cells modulate hsp40 which finally leads to PKR phosphorylation. A direct target of phospho-PKR is eIF2α leading to phosphorylation of eIF2α that halts the translation machinery. All these changes, finally lead to triggering apoptosis of the infected cell. In order to avoid translation arrest as a result of host antiviral response, animal viruses have developed various strategies to downregulate the activity of PKR to prevent phosphorylation of eIF2α so that translation of viral proteins are continued. Our findings suggest that besides NS1, NP also modulates host translational machinery in influenza virus infected-cells to keep the host translation going.

Reactivation of Latent HIV-1: what can we do with what we know?

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Human Immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS, infects primarily cells of the immune system. The outcome of HIV-1 infection results from complex interactions between viral proteins and host cell factors. In most cases, HIV-1 successfully hijacks cellular pathways and bypasses cellular restriction factors for optimal replication leading to continuous rounds of infection, replication and cell death. Continuous viral replication causes the loss of CD4+T cells and progression to immunodeficiency in infected individuals. However, in certain situations virus replication can be successfully controlled. First, HAART (Highly Active AntiRetroviral Therapy) treatment revealed the existence of a pool of resting memory CD4+ T cells harbouring integrated but silent HIV-1 provirus. Although this situation occurs in a small number of cells, it suggests that intracellular defence mechanisms can be effective against HIV. This long lived viral reservoir is believed to be the major obstacle against HIV-1 eradication by HAART. Second, HIV-infected individuals who are able to control their virus to undetectable levels for many years in absence of any treatment have been identified and referred to as Elite HIV controllers "EC". Again, this is a rare situation observed in 0.5% of infected patients. Still, it demonstrates that it is possible to naturally and effectively control HIV replication and disease progression. A common feature of these two situations is that virus replication is controlled at the gene expression level. A major challenge in the HIV field is to understand how these naturally occurring situations where intracellular defence and/or immune response win the battle against HIV. We will discuss the interplay between HIV and its host.

The Challenge of HIV Diversity and its Implications for Diagnostic Assay Performance

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The underlying sequence diversity between HIV-1 groups and subtypes, resulting in distinct phylogenetic classifications, can be considerable. For example, envelope gene sequences may differ by 20–30% between subtypes and up to 50% between groups. This high level of HIV genetic diversity has important implications for screening, diagnostic testing and patient monitoring. Continued diversification and global redistribution of HIV groups, subtypes and recombinants make it imperative that serological and molecular assays be designed and evaluated to ensure reliable performance on all HIV infections. Recognizing the importance of this issue, Abbott initiated a comprehensive program to monitor global diversification of HIV, search for newly emerging variants, assemble large volume panels of genetically and geographically diverse strains, and develop strategies to determine the impact of HIV diversity on assays used for detecting and monitoring HIV infection. Efforts to identify and characterize rare and emerging HIV strains have lead to the identification of HIV-1 group O, group N, and dual infections of groups M and O. A panel of >1.000 plasma specimens was established that includes specimens collected from several countries in Africa, Asia, Europe, and South America; the panel comprises infections due to HIV-1 group M subtypes A, B, C, D, F, and G, as well as CRF01, CRF02, and unique recombinant forms, group N, and group O. Our recent efforts also led to the discovery of a new provisional group P strain (publication pending). Altogether, serological and molecular characterization of this unique panel has provided vital sequence data to support assay development and an invaluable source of well-defined specimens to evaluate and compare assay performance. The ability to address the challenge posed by ongoing evolution of HIV and the emergence of new variants requires continued surveillance of global HIV strain diversity, a sound scientific foundation for assay development, and suitable panels to evaluate and validate assay performance.

Replication and Assembly of Rotavirus

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Rotaviruses are non-enveloped viruses with a segmented double-stranded RNA genome that infect a number of different mammalian species (including humans) and birds provoking diarrhoea. The infective virion is a triple-layered particle (TLP) with an internal core containing the viral genome (11 dsRNA segments), the RNA-dependent RNA polymerase VP1 and the RNA capping enzyme VP3, surrounded by an inner-most layer formed by pentamers of VP2. A second middle layer covering the core consists of trimers of VP6 giving rise to an intermediate double-layered particle (DLP), which upon acquiring the third outer-most layer of VP7 and VP4 forms the fully assembled viral particle (TLP). The infective particle encodes overall six structural (VP1, VP2, VP3, VP4, VP6, VP7) and five non-structural proteins (NSP1, NSP2, NSP3, NSP4, NSP5).

Rotavirus genome replication and the first steps of virus morphogenesis take place in viroplasms, cytoplasmic viral factories containing four structural (VP1, VP2, VP3, VP6) and two non-structural (NSP2, NSP5) proteins. NSP2 and NSP5, the only non-structural proteins found in viroplasms, are essential for viroplasm formation and for viral replication. When co-expressed in uninfected cells form viroplasm-like structures (VLS). Viroplasms are the sites of transcription of plus-strand RNAs. A central role of NSP5 in orchestrating the formation VLS was shown upon co-expression of NSP5 with either the core protein VP2 or with the non-structural protein NSP2. Both interactors induce also hyperphosphorylation of NSP5, yet this modification appears no to be involved in determining the ability of NSP5 to induce VLS assembly. The ability of each viroplasmic protein to be recruited was investigated and shown to be in all cases dependent on NSP5, resembling their distribution in viroplasms. We have recently found that rotavirus replication is totally dependent on the activity of the proteasome. Using proteasome inhibitors and molecular analysis of different steps of the virus replication cycle we found that the assembly of viroplasms is highly dependent on an active proteasome, suggesting the existence of a mechanism in which a defined cellular protein(s) needs to be degraded to permit infection to proceed.

Wider Scale Analysis Reveals a Lack of Correspondence between the Virulence of Rotavirus Strain and the Diarrheagenic Property of the Cognate Enterotoxigenic Protein NSP4: is There a Structural Diversity Among NSP4s?

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Rotavirus nonstructural protein, NSP4, has been shown to function as viral enterotoxin in newborn mouse model system. Although the sequence of NSP4 from several symptomatic and asymptomatic strains has been determined, no sequence pattern or motif that distinguishes the virulent and avirulent phenotypes could be identified. Our recent studies using NSP4ΔN72 peptides from SA11 and Hg18 strains suggested that only the highly diarrheagenic peptides bound thioflavin T (ThT) efficiently and that the functions of NSP4 are dependent on a unique conformation that is specifically recognized by thioflavinT. Though NSP4 has been recognized as rotaviral enterotoxin, a few observations did to support the diarrheagenic activity associated with NSP4 and reported a lack of correlation between virulence of the strain and the diarrheagenic activity of the cognate NSP4. Due to the very limited number of studies, the controversy on the enterotoxigenic property of NSP4 remained unresolved. To attempt resolve the reported inconsistency in the relationship between the virus virulence and diarrheagenic property of NSP4, we carried out comparative analysis of the biophysical, biochemical and diarrheagenic properties of ΔN72 peptides from a large number of rotavirus strains. Further, we determined the 3-dimensional structure of ΔN94ΔC29 peptides from a few strains. These studies revealed that different NSP4 ΔN72 peptides differed widely in their biophysical, biochemical and diarrhea-inducing properties. No correlation between virulence property of the virus and the diarrheagenic property of NSP4 was observed. Our results suggest a conformational/structural basis for the lack of correlation between virus virulence and diarrheagenic activity of recombinant purified proteins. The naturally occurring amino acid substitutions in the flexible C-terminal region appear to significantly influence the conformational and/or three-dimensional structure of the peptides and their biological properties. We provide evidences for the ability of different NSP4ΔN94ΔC29 peptides to form various oligomeric structures that could be of significance to the biological function.

Mechanism of Transcription in Coronavirus and Protection Against SARS-CoV

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Coronavirus (CoV) transcription is regulated at two levels. One, by transcription regulating sequences (TRSs) that control the transcription of all viral genes and, a second one, by a transcription regulating motif (TRM) that, in the native viruses, has only been observed in genus alpha CoVs regulating the transcription of nucleoprotein (N) mRNA. CoV transcription implies a discontinuous mechanism by which the 5'-terminal leader sequence is fused to the 5' end of every mRNA coding sequence (body). TRSs preceding each gene (TRS-B) include a conserved core sequence (CS), also found at the 3'-end of the leader (TRS-L), and variable 5' and 3' flanking sequences. In transmissible gastroenteritis CoV, a general mechanism regulating transcription of mRNAs levels, driven by the ΔG of TRS-L and cTRS-B duplex formation, has been described. In addition, a novel TRM, that specifically regulates the expression of N mRNA, has been described by our laboratory. We have shown that this mechanism is based on a long distance RNA-RNA interaction mediated by a 9-nt sequence (dE, distal element) located 448 nt upstream of the CS-N. This sequence interacts with a complementary sequence (pE, proximal element) located 7nt upstream of the CS-N. Characterization of minimal requirements of the TRM in mutant replicon genomes has revealed that 5' dE flanking sequences are required to maintain optimum N mRNA transcription, and that the distance between dE and pE influences TRM activity, as reduction in this distance led to a significant increase in N mRNA accumulation. In addition, it was shown that TRM activity is independent of the gene and the position in the viral genome. A combination of the optimization of the TRS and of the TRM can be used to considerably improve expression of mRNAs in CoV derived vectors. In a complementary approach, the modification of CoV genomes has led to the engineering of attenuated viral phenotypes that are good candidates in vaccine development to protect against the severe and acute respiratory syndrome (SARS). SARS-CoV appeared in 2002, infected 8000 people and killed about 10 percent of them. In order to identify virus genes involved in virulence, and to generate attenuated phenotypes, a collection of deletion mutants have been constructed. A gene relevant in virus virulence has been identified and the possible mechanisms of attenuation have been studied. Deletion of gene E led to a significant reduction in virus titers. Interestingly, the ΔE mutants provided protection to challenge with homologous and heterologous pathogenic SARS-CoV strains in hamsters and Tg mice. Furthermore, the E protein deletion mutants provided complete protection against a virulent mouse adapted

SARS-CoV in conventional mice, indicating that the ΔE mutants are promising vaccine candidates. To identify the mechanism of action of gene E, the effect of this gene on host cell expression was analyzed. The expression of a large group of heat shock genes, which influence inflammatory and immune responses, was increased in ΔE in relation to *wt* virus-infected cells. This effect might cause a decrease on the inflammation and an increase of the B, T and NK cell responses in ΔE-infected animals, resulting in a SARS-CoV with attenuated phenotype. These data reinforce the notion that E protein is a virulence factor.

Interactions between Viral and Host Factors that are Needed to Promote Replication and Assembly of Hepatitis C Virus

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It is predicted that 2.2% of the world's population has been infected with hepatitis C virus (HCV), an estimate that equates to approximately 130 million people. Infection typically occurs through percutaneous exposure to infected blood. Acute infection is often symptom-free but, thereafter, up to 85% of individuals develop a chronic infection in the liver. Over a period of decades, chronic infection can lead to severe liver disease, including decompensated cirrhosis and hepatocellular carcinoma, which are end-stage conditions.

HCV has a positive-sense, single-stranded RNA genome of some 9.6 kilobases that encodes a polyprotein of about 3000 amino acids. Flanking the open reading frame for the polyprotein are 5' and 3' untranlsated regions (UTRs), which contain elements that regulate translation and replication. The polyprotein is generated by the host cell translation machinery and cleaved co- and post-translationally by viral and cellular proteases to yield the mature viral proteins. The N-terminal segment of the polyprotein encodes the structural components of the virus, which are incorporated into virions. The C-terminal component of the polyprotein contains non-structural proteins that provide a variety of functions, including those necessary for synthesis of viral RNA and assembly of infectious particles.

Until recently, a description of the virus life cycle has been hindered by the lack of tissue culture systems capable of producing infectious progeny. Consequently, most of our insight into the different stages of HCV infection has relied on surrogate models and cell-based systems that reproduce discrete phases of the virus growth cycle. Such approaches have enabled functional characterisation of the viral proteins but not in the context of production of infectious virions. The recent emergence of a system that generates infectious HCV in tissue culture cells has opened the opportunity to validate the knowledge obtained with previous surrogates systems and represents a landmark in determining the processes, which govern productive growth of HCV.

In this lecture, I will describe some of the basic characteristics of the virus and the cell-based systems that have been developed to study HCV RNA replication and virion assembly. To date, Huh-7 human hepatocellular carcinoma cells are the only cells capable of producing infectious HCV, illustrating the strict dependence of the virus on host cell factors and processes. To illustrate the importance of the host cell environment, I will outline studies on the contribution of lipid storage organelles and lipid metabolism to the virus life cycle. Such analysis is important to understand the mechanisms that govern HCV infection and also could lead to new classes of drugs for eradicating the virus from chronically infected patients.

The Interplay between Host Factor and Viral Protein Regulates Translation and Replication of Hepatitis C Virus

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The translation of the positive strand genomic RNA of the hepatitis C virus (HCV) is an early obligatory step of the infection process. Human La protein has been shown to be required for the Internal Ribosome Entry Site (IRES) mediated translation of the HCV RNA. Earlier, we have shown that La protein binds to 5'UTR near initiator AUG (iAUG) and induces a conformational alteration in the IRES to facilitate the ribosome assembly. We have also shown that a small peptide derived from the RNA binding domain of La protein interferes with the assembly of 48S complexes and inhibit translation and replication of HCV RNA. Recently, we have observed that the protease domain of the HCV-NS3 protein ($NS3^{pro}$) also binds to HCV-IRES. More importantly, $NS3^{pro}$ binding was found to dislodge the human La protein binding near the iAUG resulting inhibition of HCV-IRES function. Interestingly, over expression of $NS3^{pro}$ decreased the rate of HCV-IRES mediated translation, however, replication of HCV replicon RNA enhanced significantly compared to control. Earlier, La protein has been shown to help in replication of HCV RNA. Our results suggest that the $NS3^{pro}$ binding to HCV IRES reduces translation in favor of RNA replication. Since in plus strand RNA viruses, translation precedes genome replication, so to prevent collision of the ribosomes with the replication complex the translation must be switched off. We have provided an intriguing mechanism for HCV and demonstrated that the competition between the host factor (La) and the viral protein ($NS3^{pro}$) for binding to HCV-IRES might contribute to the molecular switch from translation to replication of the HCV-RNA.

Multi-Tasking by Viral Proteins

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The outcome of a viral infection depends upon virus-host interactions, particularly the modulation of critical host responses by the virus. These include the host immune response, programmed cell death and signal transduction.Vertebrates have developed an elaborate immune system to counter pathogens and respond to infection through innate (pathogen non-specific) as well as adaptive (pathogen-specific) immune responses. Pathogens, viruses included, establish infection and replicate in hosts despite a robust immune response through a variety of evasion mechanisms that target innate immunity as well as the humoral and cellular compartments of adaptive immunity. One innate response of cells to virus infection (or unscheduled nucleic acid synthesis) is Programmed Cell Death or Apoptosis. Consequently, there are multiple examples of viruses inhibiting apoptosis of infected cells. The immune response and apoptosis are both dependent upon elaborate signal transduction networks in host cells.Viruses exert these effects on the host through proteins encoded by their genomes. Since viral genomes are small, the conservation of sequence space demands that viral proteins perform multiple tasks. In this lecture, I will present results from three such systems that are researched within our group.The human immunodeficiency virus (HIV) is an excellent example of a persistent virus that slowly disables the host immune system. The persistence of HIV depends critically on a few viral proteins, the most important of which is an accessory protein called Nef, which is expressed early in infection. Nef optimizes the cellular environment for HIV replication by promoting the survival of infected cells and by signaling the apoptosis of HIV-specific cytotoxic cells. A widely studied property of Nef is to downregulate the surface expression of immunologically important proteins on the surface of infected antigen-presenting cells (APCs; macrophages, dendritic cells) and T cells. This includes CD4, MHC I, MHC II and CD28. We studied the effects of Nef on the surface expression of the B7 family of costimulatory molecules, CD80 and CD86 in APCs. Nef directly binds the cytoplasmic tails of CD80 and CD86, and through a mechanism that involves Src, protein kinase C and actin polymerization, recruits it into transitional vesicles that eventually fuse with the Golgi. In addition to its effects on MHC I downregulation, this leads to attenuated antigen presentation and escape of infected cells from HIV-specific cytotoxic T cells. Our results also show effects of HIV-1 Vpu, another viral accessory protein on the MHC II pathway through binding to the MHC II invariant chain. The hepatitis E virus (HEV) causes an acute, self-limited infection. Our results over the past few years suggest that the HEV open reading frame 3 (ORF3) protein modulates multiple host cell signaling pathways towards the promotion of cell survival. These include activation of the Erk/MAPK

signaling pathway, attenuation of the mitochondrial death pathway through increased expression and oligomerization of the outer mitochondrial membrane porin, VDAC and hexokinase, and by prolonging endomembrane growth factor signaling. This protein also regulates energy homeostasis in the cells and regulates the inflammatory acute phase response. I will show mechanistic details of these to highlight the multiple roles of a viral protein in regulating cell signaling and survival.

RNAi Pathways and Host-Virus Interactions

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RNA-mediated gene silencing (RNAi) is initiated by double stranded RNA helix that can be exogenously introduced or endogenously created from small non-coding RNAs called micro RNAs. Understanding the structure and function of small interfering RNAs (siRNAs) that trigger RNAi has illuminated broad functions of the ancient RNAi machinery in animals and plants and has provided guidelines to achieve efficient gene silencing for biological and therapeutic applications of RNAi. MicroRNAs (miRNAs), ~22-nucleotide RNAs, assemble into RNA-induced silencing complexes (RISC) and localize to cytoplasmic substructures called P-bodies. Dictated by base-pair complementarity between miRNA and a target mRNA, miRNAs specifically repress posttranscriptional expression of several mRNAs. Recent studies have illuminated broad functions of the ancient RNAi machinery in animals and plants viruses. I will discuss our current understanding of various gene silencing mechanisms and a few examples of RNA-mediated regulation of host-pathogen interactions.

Poster Abstracts

(Arranged According to Last Name of Presenting Author)

P1

Surveillance and Genetic Characterization of Circulating Influenza-A Virus Strains in Kolkata During 2005-2008

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BACKGROUND

Influenza-A viruses continuously acquire changes both genetically and antigenically due to high frequency of mutations for overcoming immune responses of host.

METHODS

Initial screening for influenza-A viruses in outpatients, diagnosed with acute respiratory tract illness was done by real time PCR between September 2005 and September 2008. The full length hemagglutinin (HA) and neuraminidase (NA) genes of influenza positive samples were amplified and sequenced. Nucleotide and antigenic changes were determined by comparing local strains with the vaccine strains or strains reported from other countries.

RESULT

A total of 1986 nasal and/or throat swab were obtained out of that 185 samples (9.31%) were found positive for human influenza virus infection. Among these positive isolates, 51 (2.56%) and 134 (6.74%) were subtyped as H1N1 and H3N2 respectively. The influenza A showed distinct seasonality in Kolkata as in all three years, the increased prevalence was observed during June-Sept correlating with rains and high humidity. Phylogenetic analysis of individual gene segments revealed that the HA and the NA gene of both H1N1 and H3N2 isolated subtypes clustered with the current vaccine strain for the Northern Hemisphere 2008–2009. HA gene of all the strains possesses conserved sequences at the receptor-binding site. Changes in N-linked glycosylation site or antigenic sites of HA1 due to amino acid substitutions were observed in some strains, indicating that circulating strains are in continuous process of evolution. As for the NA, amino acid substitutions were observed in N1

and N2, but not at the catalytic or the framework sites. All viruses sequenced showed no mutations conferring resistance to oseltamavir.

CONCLUSION

The three year surveillance study in Kolkata confirms; (i) prevalence of influenza A among ARTI patients (9-10%), (ii) correlation of infection with rainy season and (iii) concurrence of circulating strains with the WHO designated vaccine strains. Since influenza-A strains undergo continuous genetic drift, the study also highlights the importance of continuing surveillance for early recognition of major antigenic changes and timely interventions during epidemics.

P2

Interaction of HIV-1 Nef with CD80/CD86 Immune Co-Stimulatory Receptors

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The HIV accessory protein Nef is one of the earliest viral proteins to express following infection and is suggested to be a virulence factor. It down modulates the surface expression of several important membrane receptors, including CD4, MHC class I and class II. This causes attenuation of the adaptive immune response and promotes viral replication. Our collaborators and we have shown that Nef also promotes the removal of costimulatory proteins CD80 and CD86 from the surface of infected antigen-presenting cells. Our mechanistic studies support a two-pronged model in which Nef directly binds the cytoplasmic tails of CD80 and CD86, and promotes the organization of dynamic actin through the activation of protein kinase C and Src. This leads to an endocytic event that sequesters the surface molecules into transitional vesicles, which eventually fuse with the Golgi. With the objective of further understanding the details of Nef-mediated down modulation, we have studied the binding of Nef proteins to peptides representing the cytoplasmic tails of CD80 and CD86. Being highly positive charged, these peptides suggested electrostatic interaction with negatively charged residues of Nef. In cell based assays, functional studies of Nef by mutating negatively charged residues did not affect receptor down-regulation. To get further mechanistic details, we carried out interaction studies with purified Nef and custom synthesized peptides from CD80 and CD86 receptors. Recombinant Nef proteins were expressed in *E. coli*, purified and used to set up *in vitro* binding assays with 20 amino acid long synthetic peptides. Circular dichroism and tryptophan fluorescence measurements showed distinct changes in the protein secondary structure upon peptide binding. Dynamic Light Scattering experiments have indicated CD80/86 cytoplasmic domain peptide mediated aggregation of Nef. Experiments using Surface Plasmon Resonance have shown high affinity binding of Nef with the CD86 peptide. Mutation of Asp^{123}, which is required for dimerization of Nef preserved its interaction with CD86 peptide, suggesting dimerization independent interaction. Also the residues Glu^{108} and Asp^{111}, making a negatively charged pocket for dimerization, when mutated along with Asp^{123} still preserved Nef interaction with CD86 peptide. In the light of all these studies we infer that Nef might employ a promiscuous approach to interact with cell surface receptors for their down-regulation. This becomes even more convincing with the rise in the literature reports citing Nef mediated down-regulation of

newer receptors. The conserved structure of Nef core might adapt as a highly flexible scaffold for these interactions.

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P3

Development of Recombinant Proteins as Diagnostic Intermediates for Chikungunya Infection

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JUSTIFICATION

Chikungunya is an important disease with explosive outbreaks occurring in many South East Asian countries. Since many clinicians as well as general practitioners find it difficult to confirm diagnosis of this infection only on clinical grounds, particularly when it co exits during outbreaks with dengue. Therefore laboratory confirmation of chikungunya infection is important for proper management of patients. Laboratory diagnosis of chikungunya is hindered by the unavailability of reliable commercial diagnostic kits and inaccessibility of reagents. There is a need to develop an assay that can confirm chikungunya, produced at low cost and easily standardized for use in field settings. Further, differential rapid, optimized diagnostic assay with high sensitivity and specificity is essential for clinical management and epidemiological studies. Currently available laboratory diagnostic kits depend on Enzyme-Linked Immunosorbent Assay (ELISA) based on whole viral antigens which cause biohazard risk, high production cost and cross reactivity with other organisms of the same genus/family. Therefore, a diagnostic intermediate using a single recombinant protein antigen to overcome problems associated with whole viral antigen/lysate is important. **Objective:** To assist confirmation of chikungunya outbreaks through developing a rapid laboratory diagnostic assay.

METHODOLOGY

In the present study, expression and purification of Envelope proteins (E1 and E2) of chikungunya virus and evaluation of their potential use as diagnostic intermediates in IgM ELISA for the detection of anti-chikungunya IgM antibodies using a panel of serum samples were performed. These serum samples (n=15) were collected from clinically confirmed chikungunya patients. These samples were laboratory confirmed by a currently available IgM ELISA produced by National Institute of Virology, Pune, India.

RESULTS

Both recombinant proteins, E1 cloned to pET32a (Molecular Weight 55 KDa) and the E2 cloned to pET 28a (Molecular Weight 43 KDa) were purified under denatured condition. IgM ELISAs developed using E1 and E2 proteins have confirmed chikungunya infection in 9 (9/15) and 10 (10/15) samples respectively.

DISCUSSION

Analysis of the sensitivity and specificity of IgM ELISA developed using these two recombinant protein antigens should be performed using a large panel of well characterized serum samples.

ACKNOWLEDGEMENT

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P4

Arbovirus Infections in Febrile Patients Suspected of Malaria and Typhoid in Nigeria

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INTRODUCTION

Arbovirus infections are clinically difficult to diagnose at the prodromal stage because symptoms and signs of such cases are often indistinguishable from other febrile illnesses such as malaria or influenza as few examples. In Nigeria febrile illnesses have been restricted to malaria/typhoid investigations and treatment with complete neglect for arboviruses. The study was designed to determine the role of arboviruses in febrile cases ion Nigeria.

MATERIALS

1948 sera from malaria/ typhoid suspected cases in six ecological zones were tested for Dengue viruses (DEN). Seasonal variation in the prevalence of some arboviruses viruses on 973 patients was studied. In addition, 59 pools of Aedes spp and 52 of Culex quinquefasciatus/Mansonia spp were tested for DEN and WNV by RT-PCR and cultured for virus isolation. All the sera were screened for both IgM and IgG by MAC-ELISA. All the IgM positive sera were further analysed by RT-PCR and plaque reduction neutralization test (PRNT) for confirmation.

Another 500 sera from the same group of patients were tested for the presence of Complement Fixing (CF) antibody to Lassa fever virus (LFV) and Salmonella typhi.

RESULTS

Thirteen (0.67%) of 1948 sera were positive for 1 and 2 IgM from 4 zones. I.2 % of 973 sera had WNV IgM antibodies. DEN infections were more during the rainy season while WNV peaked during the cold harmattan season. Mixed infections of DEN-2 and WNV observed in two samples, eventually had neutralizing antibodies for WNV. Overall, PRNT and ELISA results for both DEN and WNV were in concordance. Dengue IgG antibodies in Zahel

savanna (81.7%), Rain forest (69.0%) and Wooded savanna (69.0%) were significantly different from Grass (38.15%) Sudan (32.6%) savanna. The high prevalence of WNV IgG (80.16%) obtained in this study is an indication of the endemicity of Flaviviruses in the environment. Fourteen of the 59 pools of Aedes and 40 of 52 pools of Culex quinquefasciatus tested showed viral RNA to DEN 1-4 and WNV respectively. While one IgM positive serum had detectable RNA to DEN, 33.3% had WNV RNA. However, negative and border line sera of WNV IgM showed detectable WN viral RNA. Neither DEN nor WNV was isolated from the mosquitoes. Fifty-seven (11.4%) of 500 sera had antibodies to LFV while 41.4% tested positive for S, typhi and 29 (5.8%) for both S. typhi and LFV. Recently 37.5% of malaria/typhoid suspected cases had DEN IgM/IgG antibodies. 25 of the cases were DEN IgM (possible primary infections) while 18 were IgG, an indication of past infection and 17 were both IgM and IgG implying possible secondary dengue infections. 11.7% of these patients had antibodies to both malaria and DEN while 36.7% tested positive for both DEN and S. typhi suggesting possible concurrent infections. Virus isolates from these cases have been obtained but are yet to be identified.

CONCLUSION

Arbovirus infections have been detected in patients suspected and treated for malaria/typhoid. Concurrent infections between arboviruses and malaria/ S. typhi have been demonstrated.

KEYWORD

Arbovirus, febrile illness, malaria typhoid, Nigeria,

P5

Studies on Gene Expression Systems for the Production of Virus Like Particles (VLPs) of the Bird Flu Virus H5N1

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Influenza A viruses possess a segmented negative-strand RNA genome which encodes the 10 polypeptides required for effective execution of the virus life cycle. These 10 proteins are encoded within eight genomic RNA segments, each of which is encapsidated by multiple subunits of the nucleoprotein (NP) and is associated with a few molecules of the trimeric polymerase (PB1, PB2, and PA subunits) forming the functional ribonucleoprotein complex (RNP). Surrounding these structures is a layer of the matrix protein M1 that appears to serve as a nexus between the core and the viral envelope. This host-cell-derived envelope is studded with the two virally encoded major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), and a minor amount of the small nonglycosylated integral membrane protein M2.

In this project HA, NA and M1 genes of H5N1 are cloned into various expression vectors (Bacterial, Mammalian, and Yeast) with appropriate tag for purification. Given that double HA and NA cytoplasmic tail deletions appear to affect the efficiency of budding and morphology of the virus particles without completely abrogating assembly and exit of virion particles, it seems likely that M1 protein alone may be able to direct viral assembly and budding. Cells expressing M1 in the absence of any other viral protein have been found to localize matrix protein to the plasma membrane. Whether this matrix protein is completely embedded into the plasma membrane or merely attached by electrostatic interaction is uncertain. Recent studies on influenza Virus Like Particle (VLP) formation clearly demonstrate the significance of M1 protein as well as the other structural components in the assembly and budding process, which can be driven by the single expression of the M1 protein.

After expression and purification of these proteins we plan to check the possibility of formation of M1 VLPs. If VLPs are formed, HA and NA which are expressed separately will be adhered to the VLPs and the immune response against them will be assessed in mice by measuring the levels of IgG and IgM secreted. If VLPs are not formed, the immune response to the individual proteins, M1, HA and NA will be assessed. Following this, we aim to test for immune protection using VLPs or individual proteins against H5N1 challenge in poultry animals.

P6

Differential Transactivation and Apoptotic abilities of Vpr C variants: their Implication in HIV Pathogenesis

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For the development of effective vaccines and therapeutics we need to understand the biology of HIV-1 pathogenesis with respect to different subtypes and recombinant strains. Vpr, an accessory HIV-1 protein influences variety of cellular functions such as transactivation of the long terminal repeat and certain heterologous promoters, nuclear import of preintegration complexes, induction of cell cycle arrest in G2 phase, and induction of apoptosis in infected cells. Most of these studies have been carried out with respect to the subtype B genes, while the predominant HIV-1 strain circulating in India is subtype C. Information related to subtype C Vpr is missing which is relavent in Indian context. Therefore, we carried out studies regarding the transactivation potential, cellular viability and apoptotic abilities of Subtype C Vpr (93In905, Indian isolate) gene. Vpr-C was able to cause transactivation moderately in comparison to subtype B but effective laddering was observed in DNA isolated from HEK 293 cells transfected with Vpr C. The enhanced capability of Vpr C to induce apoptotic laddering and cell death is being investigated further by computational biology approach. Recently there have been reports of HIV-1 B/C recombinants from India. Thus a genetic and functional analysis of the Vpr gene from HIV-1 infected individual from North India was carried out. Vpr gene from 6 out of the 8 samples had an L64P mutation (located at the predicted 3rd alpha helix) while the other two were novel recombinants with genetic architecture B/D-C-D-C and B/D-C-D-B. The recombinants were competent in causing transactivation but the L64P mutants activated LTR promoter poorly. Also, the two recombinants and the L64P mutants were able to cause a decrease in cell viability. Most of our samples (7/8) possessed Q at position 77 which was earlier reported important for long term non-progression and impaired induction of apoptosis for subtype B. This was clearly not the case with all our infected individuals who showed normal progression to HIV/ AIDS. We also engineered L64P mutation in subtype C background which adversely affected its ability to transactivate. In contrast, L22A mutation in subtype C resulted in augmented LTR transactivation-an observation common with subtype B. Thus our study highlights the importance of studying Vpr biology with reference to evolving variants to understand the pathophysiological outcome of this rapidly evolving virus.

P7

How Cardioviruses Alter Nucleocytoplasmic Transport of the Host Cell

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The picornavirus family is composed of non-enveloped, positive-sense RNA viruses and includes important human and animal pathogens such as poliovirus, rhinoviruses, foot-and-mouth disease virus, hepatitis A virus, and many others. Some picornaviruses, for example, poliovirus, dramatically alter nucleocytoplasmic traffic in the host cells by disrupting selective permeability barrier of the nuclear envelope and suppressing active transport. This effect is assigned to the function of viral protease 2A, which demolishes the nuclear pore complex by degrading some nucleoporins.

In the present studies, we assessed nucleocytoplasmic traffic in cells infected by cardioviruses – representatives of the picornaviruses, which lack poliovirus 2A-related protease. We showed that cardioviruses similarly increase permeability of the nuclear envelope, but achieve this by strikingly different mechanism. The key role is played by small viral leader (L) protein, devoid of enzymatic activity; its Zn-finger domain and phosphorylation site are relevant for this function. By contrast to poliovirus, cardiovirus infection is not accompanied by degradation of the examined nucleoporins; the overall structure of the nuclear pore is preserved, yet disorganized. Interestingly enough, at least one component of the pore complex – Nup62 - is hyperphosphorylated in cardiovirus-infected cells. Taking into account localization of Nup62 in the central transport channel of the pore, we proposed that phosphorylation of Nup62 (possibly along with other nucleoporins) is a key factor in the permeabilization of the nuclear pores. By making use of cardioviruses with mutated L, we ascertained that level of Nup62 phosphorylation correlates with transport disorder. Inhibitory assay *in vitro* suggests the involvement of cellular protein kinases (probably, mitotic cyclin-dependent kinases) in cardiovirus-elicited permeabilization of the nuclear envelope. We hypothesize that L protein triggers transport alteration by hijacking some components of the phosphorylation pathway involved in the control of the nucleocytoplasmic exchange during cell division.

Thus, two groups of picornaviruses target nuclear pore complexes and disrupt nucleocytoplasmic transport, yet utilize fundamentally different mechanisms. The biological significance of transport disorder for picornaviruses largely remains uninvestigated. One can speculate that viruses benefit from relocalization of stimulatory nuclear factor to the cytoplasm, enhanced access to the nucleus for viral products, and abolished antiviral response.

P8

Identification of Inhibitors of Early Steps in HCV Life Cycle

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INTRODUCTION

Approximately 3 % of the world's population is infected with hepatitis C virus (HCV). HCV infection causes chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. The current treatment strategy is pegylated-interferon and ribavirin. Its success is limited, depends on HCV genotype, and has severe side effects. As a result, new therapeutic options are urgently needed. Targeting early steps in HCV lifecycle might be crucial to inhibit infection. For this aim, I have developed a screening system for HCV infection based on immunofluorescence technique. I have used a chemical library with known roles of chemicals. I have screened 240 different compounds and looked for their effects on HCV infection.

MATERIALS & METHODS

Cell line and infectious virus. Huh-7.5 cell line (propagated in DMEM; grown in 37 °C in 5 % CO_2) and J6/JFH-1 chimeric infectious virus were used.

Chemicals and antibodies. Two hundred and forty chemicals with known activities of ICCB library of Harvard University were tested. Mouse anti-HCV NS5A antibody, mouse AlexaFluor-488 nm secondary antibody, and DAPI were used. HCV Infection assay. 2 x 10^3 cells/well were seeded on a 96-well plate. Next day, DMEM was changed with Opti-MEM. Chemicals were injected with a Robotics system (80 compounds for each plate). After 2-hour incubation, cells were infected with J6/JFH-1 for 2 hours. Then, medium was changed with a fresh medium and cells were incubated for 60 hours. Positive (IFN-α) and negative (untreated) controls were used in the experiment. Cell fixation and IF. Cells were fixed by 4% Para formaldehyde. Then, cells were washed with PBS. 1:500 diluted mouse anti-HCV NS5A antibody, and 1:5000 diluted DAPI were incubated for 1 h. Cells were washed again with PBS. 1:500 diluted mouse AlexaFluor-488 nm secondary antibody was incubated for 30 min. Lastly, cells were washed with PBS and kept in 4°C until screening with microscopy.

Screening and analysis. Plates were screened under the automated fluorescence microscopy. Two pictures were taken for every well. Acuity Express was used to analyze the pictures for counting total cell number, and % of infected cells. Every plate was analyzed individually. Average value of positive and negative control wells was calculated before analysis. Mean of total cell number was calculated for all plates individually. Having cell number in a well with less than the half of the mean value was considered as toxic and it is not counted for efficiency of HCV infectivity. Inhibition value of infection was calculated by taking ratio between average value of positive control and a specific well.

RESULTS

Two hundred and forty compounds (3 plates) were screened in this experiment. According to the results, 134 of them found as non effective, 82 of them were toxic, and 24 of them had an at least 3 times or more effects on HCV infection. After a PubMed search, I have noticed that 7 chemicals (cyclosporin A, lovastatin, tamoxifen, two cyclooxygenase inhibitors, and two protein kinase A inhibitors) have already been confirmed by other researchers. Then, I have selected 4 chemicals (EHNA, L-NAME, chelerythrine, and splitomicin) for the further tests. They were tested at four different concentrations. I was unable to conclude the research because my time finished.

CONCLUSION

In conclusion, four hours incubation of the chemicals and two hours virus incubation are optimal for my study since viral entry and translation of HCV RNA occur within first six hours. Using Opti-MEM is a vital step for efficient infection. However, it should be replaced by DMEM after 4 hours. Sixty hours incubation time after infection is important. In general, screening and analyzing systems worked well. Actually, I found some effective chemicals, which are previously confirmed by others. It indicated that my screening and analyzing system worked well. Because, my time finished, I could not screen the selected chemicals again and I believe that it should be performed again.

P9

The HEV ORF3 Protein Modulates Growth Factor Receptor Trafficking, STAT3 Translocation and the Acute Phase Response

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Hepatitis E, an acute self-limiting viral disease, is endemic to developing countries where it is responsible for large outbreaks and rampant sporadic cases. Earlier studies suggest that the viral ORF3 protein (pORF3) is essential for infection in vivo and is likely to modulate the host response. Here we report that pORF3 localizes to early and recycling endosomes and causes a delay in the post-internalization trafficking of growth factor receptors like epidermal growth factor receptor (EGFR) and hepatocyte growth factor receptor(c-Met) to late endosomes/lysosomes. Interaction of pORF3 with CIN85, a multidomain adaptor protein implicated in Cbl-mediated down-regulation of receptor tyrosine kinases was found to be responsible for delayed growth factor receptor degradation. The phosphorylated cytoplasmic signal transducer and activator of transcription 3 (pSTAT3) protein requires growth factor receptor endocytosis for its translocation from the cytoplasm to nucleus. Consequently, lower levels of pSTAT3 were found in the nuclei of ORF3-expressing Huh7 human hepatoma cells. This results in downregulation of the acute phase response, a major determinant of inflammation in the host. We propose that through its effects on EGFR and c-Met receptor trafficking, pORF3 prolongs endomembrane growth factor signaling and promotes cell survival. The effects on STAT3 translocation would result in a reduced inflammatory response. Both of these events would contribute positively to viral replication.

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P10

Studies on Chikungunya Virus Infection: Role of Non-Structural Protein-2 and Cellular Proteins

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Chikungunya fever (CHIK F) is an acute mosquito-borne febrile arthritis caused by an alphavirus belonging to family *Togaviridae.* The disease is characterized by abrupt onset of high fever, arthralgia, myalgia, headache and rash. The typical clinical sign of the disease is the poly-arthralgia which is very painful and may persist for several months in some cases.

CHIKV is an enveloped, positive-strand RNA virus with around 11.5 kb long genome which encodes four non-structural proteins (nsP1-4) and three structural proteins (C, E1-2). The non-structural proteins are the essential components of the viral replicase and transcriptase. Analogy to other alphaviruses, nsP1 is responsible for methylation and capping of viral mRNAs, the function of nsP3 is poorly characterized yet, nsP4 is the catalytic subunit of RNA dependent RNA polymerase. Non-structural protein-2 is the key replication protein with RNA helicase, RNA triphosphatase activity and this is the auto-protease responsible for cleavage of the non-structural polyprotein. Our understanding about the functional characteristics of nsP2 of CHIKV is yet incomplete and the structure–function relationship of nsP2 is in preliminary stage only.

Thus, the main focus of the present study will be to characterize the function of nsp2 during infection and identify small functional motifs of nsP2 through genetic and biochemical approaches to understand the structure-function relationship of this protein. Along with that cellular proteins involved in CHIKV infection in mosquito and mammalian cells will be identified by mass spectrometry. Taken together this information will be extremely important to understand the role of nsP2 and other cellular proteins during infection in mammalian as well as in mosquito cells and this in long run will help to use existing drugs or design new drugs to control the disease caused by CHIKV.

We have just started our work by isolating Chikungunya viral RNA from the prototype strain S-27 and cDNA was generated using random hexamers. This cDNA was used as template for the amplification of whole NSP2 (1 to 798 aa) and the N- (1-420 aa) and C-terminus (421-798aa) of this gene. Primers were designed by us and PCR was standardized to

get the amplification of these genes. These PCR products were cloned in pBiEx-1 vector using Bam HI and Xho I restriction enzymes. Moreover, the PCR products were also cloned in pCDNA3 vector to study the localization of these proteins in mammalian system as well as to identify the interacting cellular proteins. The 293T cells were transfected with plasmids containing NSP2 genes and the expression of the proteins were checked by Western Blot analysis using His-antibody. At present we are working on the localization of the fragments of nsp2 in mammalian cells and expression of the proteins in insect cells for biochemical study.

P11

Inhibition of HIV-1 with Peptide Conjugates of Polyamide Nucleic Acids

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The two most commonly used anti-HIV-1 drugs target two key enzymes, protease and reverse transcriptase. These drugs have been of great success so far in reducing the viral load in AIDS patients. However emergence of resistant strains to these drugs has made the management of this disease more difficult. Drug resistant strains are usually recognized when the viral load does not fall even after the administration of the combination therapies. Alternative strategies to establish a stable drug line to combat the HIV-1 menace is the thrust of the ongoing antiviral research. The major plunge in this direction has been the accessory and regulatory proteins of HIV-1 as the new antiviral drug targets. However, the biggest drawback with these targets is that they are not sufficiently explored and their role inside the cell and the mode of action in virus production and maturation is still not well understood at the molecular level. Another major strategy, extensively worked upon, to inhibit the HIV-1 replication is by targeting regulatory conserved and non-mutable sequences on the viral genome using antisense technology. In the last one decade with the advancement in the field of antisense technology and gene silencing polyamide nucleic acid (PNA) has emerged as a very potential antisense therapeutic molecule to inhibit specific cellular messages. The potential of sequence specific PNAs conjugated with cell penetrating peptides (CPP) has been demonstrated as effective antiviral and virucidal agents. Using this technology we have successfully targeted transactivating response (TAR) element and conserved primer binding site (PBS) on the HIV-1 RNA genome to block the viral replication and infection. The anti-HIV-1 PNA-CPP conjugates are not only antiviral but are also strong virucidal agents that has potential for external topical formulations designed to block HIV-1 infection or as a prophylactic agent for inactivation of HIV-1 in the circulating plasma prior to attachment and entry.

P12

Chikungunya Virus - Protein-Protein Interaction Among Viral Proteins

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Chikungunya is an acute illness comprising of fever, skin rash and severe arthralgia caused by chikungunya virus (CHIKV). CHIKV had not gained much attention until 2006 when it caused a major epidemic in southern and central Indian states resulting in around 1.4 million patients. In the absence of research data many of the scientific queries related to CHIKV mediated disease mechanism have remained unsolved. Hence, there is an urgent need to study the interaction of CHIKV proteins among themselves. This will help us identify possible targets that might be involved in disease progression.

For the interactome analysis, all nine CHIKV genes will be cloned in BD (binding domain) and AD (activation domain) vectors of yeast two hybrid system. The clones will be verified by sequencing and protein expression. A total of 81 interacting will be generated and assayed for protein-2 interactions. The interactions will be validated by GST pulldown/immuno-precipitation assays. The data generated by this study should help in understanding the CHIKV viral life cycle and disease progression and should lead to hypothesis and mechanisms to explore such inhibitors and peptides which could stop the viral progression.

P13

Effect of Parallel Feeding of Oxidizing Agent and Protein on Fed-Batch Refolding Process of Recombinant Interferon Beta

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There is an increasingly need for production of genetically engineered therapeutic proteins to fulfill the worldwide demand for biopharmaceuticals. Recombinant production in *Escherichia coli* is efficient except that expressed proteins aggregates and form insoluble inclusion bodies and need to undergo renaturation. Oxidative agents such as iodosobenzoic acid (IBA) are usually used in the renaturation process of the proteins with disulfide bounds. The ratio of oxidizing agent and protein is a critical factor which affects proper refolding of such recombinant proteins. Two different feeding strategies were studied. Initially only the protein was feed to the reactor and the protein refolding yield was assessed using SDS-PAGE electrophoresis of recovered protein and scanning the thickness of the bands using a densitometer. IBA concentration of 10-15 μM resulted in a recovery of more than 95% of refolded interferon beta (IFNβ), when 8 μg/ml of protein were applied to the reaction. On the other hand, we tried feeding protein and oxidizing agent simultaneously. Using a factorial design of experiments different concentrations of IBA and IFNβ were simultaneously applied to the refolding buffer using a fed-batch process. The Reverse-Phase HPLC method was utilized to differentiate the native interferonβ-1b from the non-native proteins e.g. oxidized and related proteins formed during the refolding process. Concentrations of IBA significantly influenced native to non-native protein ratio hence affecting the protein quality and yield. The oxidized and related proteins were affected reversely. High concentrations of IBA increased the formation of oxidized protein while at low concentrations there was a slight increase in related proteins. The optimized condition of 40 μg ml^{-1} of IFNβ to 8 μM of the oxidizing agent was found to favor the refolding process.

KEYWORDS

Parallel feeding, Refolding, Recombinant interferon β, Fed batch, Factorial design of experiments, Oxidized agent.

P14

Rotavirus Infection: Clinical Case Studies in Children below 5 Years of Age at Kolkata, Eastern India During January 2008 to June 2009

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Rotaviruses are important enteric pathogens causing acute watery dehydrating diarrhoea in various host species, including birds and mammals. Rotavirus infections heavily impact on health of humans and livestocks worldwide. In humans, Group A Rotaviruses (GARVs) are detected in up to 50-60% of all childhood hospitalizations due to acute gastroenteritis each year. They cause annually an estimated 130 million primary infection among children <5 years of age. Of these, every 5th and 65th cases require medical visits and hospital admissions, respectively, and 1 in 293 cases is fatal (~ 611,000 per year). More than 80% of fatal rotavirus infections occur in developing countries, where poor hygiene and sanitation, malnutrition, feeding practices, maternal immunity, and higher incidence of infection seriously affecting the immune status have detrimental effect on the outcome of rotavirus infections.

The epidemiology of rotavirus infection is complicated. As with other enteric infections, GARVs are transmitted mainly by faecal-oral route. The stability of GARVs in the environment accounts for the possibility of water- or food-borne outbreaks. GARVs show year around activity in tropical areas, but show marked seasonal activity in countries with temperate climate, where virus activity peaks during winter and spring.

In our present study, we discuss about the rotavirus epidemiology in children below 5 years of age, hospitalized for acute gastroenteritis in the ID & BG Hospital, Kolkata during

January 2008 to June 2009. From a total of 1899 children admitted with diarrhoea in the ID & BG hospital, 1720 children were enrolled in the study and 1360 diarrhoeic faecal specimens were collected and screened for rotavirus by PAGE and ELISA. A total of 503 (37%) shown to be positive for rotavirus.

The rotavirus detection rate was highest amongst 06-23 month age group cases (approx.82%); detection rate declines there after. These data highlights the fact that rotavirus vaccines must be administered within the first three months of age to get the maximum preventive benefit in the at risk group to reduce the huge burden of rotavirus morbidity and mortality in Kolkata, India.

The global recommendation for rotavirus vaccination marks a major step towards reducing the contribution of rotavirus to child mortality. Further work is needed, however, to maximize the efficacy of the vaccine in developing countries that have a high burden of rotavirus diseases, to minimize the barriers to vaccine implementation, and to provide a sustainable, low-cost supply of rotavirus vaccine to achieve the full benefit for children throughout the world.

P15

Structural Aspect of Leader RNA and its Role in Interaction with P Protein

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Chandipura virus (CHPV) is a human pathogen that specifically infects the child to cause encephalitis and subsequent death. In order to develop successful drug against this virus, knowledge of the molecular mechanism of viral life cycle will be of much help. My lab in collaboration with Prof Chattopadhyay's lab is studying the aspect of viral replication-transcription mechanism for quite a long time. It was established that the viral protein P which can be phosphorylated plays a crucial role in determining the viral replication-transcription pattern through its interaction with viral polymerase L. L is a master polymerase performing both the process. P can also interact with viral nucleocapsid protein N as well as a small RNA of viral origin which gets transcribed first without being translated into proteins called Leader RNA (l). There have been a lot of speculations regarding the role of leader RNA in viral life cycle. It was reported that leader RNA can go to nucleus igniting the possibility to act as micro RNA.

In this study we are interested to decipher the structure of the leader RNA. We are also interested to find out the point of interactions of leader RNA in "leader RNA-P" interactions. In order to determine the structure of the leader RNA we are using different chemical foot-printing techniques along with the NMR techniques. To study the interactions with P protein we are creating point mutants of leader RNA and then checking their ability to interact with P protein by electrophoresis mobility shift assay (EMSA). RNase foot-printing techniques are also used to determine the interacting bases with the protein.

P16

Genetic and Biological Properties of HIV-1 Indian Clade-C Envelopes with Extended Co-receptor Tropism

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INTRODUCTION

The emergence of envelopes with extended tropism will help in providing insights towards characterization of HIV-1 subtype C circulating in countries like India where CCR5 variants are predominant and will also examine their correlation with other clade C envelopes that are strictly CCR5 using. In the present study we examined genetic and biological properties of Indian clade C envelope genes amplified from two late stage patients with special reference to their multiple co-receptor utilization, usage of cell surface CD4 and CCR5 and sensitivities to chemokine ligands.

AIMS AND OBJECTIVES

To study the genetic properties of envelope variants modulating extended co-receptor tropism and quantum of interactions with cell surface CD4 and CCR5.

To correlate the differential CD4 and CCR5 usages by envelopes with their sensitivity to entry inhibitors.

METHODOLOGY

Complete *env* genes were amplified from 2 HIV+ PBMCs and cloned into pCDNA 3.1 TOPO or pSVIII env vector. Phylogenetic analysis of gp160 was done using neighbor-joining method. Pseudovirions capable of single round of replication were produced in HEK-293- T cells. Co-receptor usages were determined in CD4 + GHOST and NP2 cell lines expressing different co-receptors . Envelope affinity to cell surface CD4 and CCR5 were tested in engineered Hela cell lines expressing varying CD4 and CCR5. Entry inhibition assays were carried out in TZM-bl cells with TAK-779 (CCR5 inhibitor) and AMD3100 (CXCR4 inhibitor).

RESULTS

Three and fournfunctional envelope clones were obtained from NARI-VB105 and NARI-VB52 patients respectively. *Env* clones of NARI-VB105 were found to use 6 while NARI-VB52 used 2 different co-receptors in addition to CCR5. Genetic properties revealed that all three envelope clones of NARI-VB105 contained 'GPGR' motif instead of 'GPGQ' motif and key substitutions uniquely distributed at 5 positions compared to other reference clade C envelope V3 sequences .Modeling data indicated that NARI-VB105 envelopes V3 loop contained more basic amino acid residue contributing to net positive charge of +8. However, NARI-VB52 *env* clones contained low V3 loop net charge +3 with conserved GPGQ motif typical of clade-C, these *env* clones were found to contain longer V1 loop and additional PNGTKS and EESN sequences in V4 and V5 loop respectively .

A wide spectrum of variation in infectivity of all the *env* was found in different cell lines which express varying CD4 and CCR5 receptor. Some envelopes which have higher affinity to CD4, showed strong association with their capacity to infect PBMC and CD4 +T cells maximally over others. However, no significant correlation was found between CD4 and CCR5 dependence with the sensitivity to TAK-779 and AMD3100.

CONCLUSION

Our data showed important insights in entry properties of HIV-1 clade C envelopes obtained from terminal patients with extended tropism and provide new information that may be relevant towards transmission and disease progression. The multitropic *env* with low CD4 dependence might enhance the ability of variants to exploit different cell types thereby facilitate enhanced replication and subsequent disease progression by increasing local viral load and transmission to T- lymphocytes.

P17

Improvement of Molecular Diagnostic for Surveillance of Respiratory Viruses in Cuba

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INTRODUCTION

Influenza, the most contagious of the Acute Respiratory Infections (ARI) is considered an emergent and re- emergent disease due to a wide range of variants among the world population.

OBJECTIVES

To show the advance in the diagnosis and characterization of the influenza virus, including pandemic flu, in Cuba.

MATERIALS AND METHODS

A total de 309 samples from patients with clinical diagnosis of ARI were processed during 2005-2006 using a scheme of molecular diagnosis. Four RT-PCR assays were implemented for the virological diagnosis of ARI caused by influenza virus.

RESULTS

Sample distribution was, 65.6% of the samples belonged to patients under 18 years old, 21.35%were from ambulatory patients, 18.4 % from hospitalized patients and 60.1% from units belongings to the sentinel networks. Of the total of the processed samples, 13 (4.2%) were positive to influenza virus type B, 6 (1.9%) to influenza virus type A and 4 (1.2%) to influenza c virus. Three (50%) of the positive samples to influenza type A were H3/N2 subtype virus and three could not be subtype (50%).

DISCUSSION

The advances in the molecular biology have revolutionized the biological sciences. At present, those tools are applied in studies of influenza virus to try to identify the frequent

causes of lethal influenza epidemics. Reference laboratories worldwide have implemented the powerful tools of molecular biology for surveillance and control strategies, our laboratory is one to them.

The results of this study are an example how the National Influenza Centre has advanced diagnostic tools to cope with the surveillance of the ARIs at current conditions and is prepared to give response to the threatening influenza pandemic. However; it is necessary to continue improve the national surveillance system in order to increase the knowledge of the circulating virus in tropical countries.

P18

Presence of Distinct HIV Variants in Blood and Spermatozoa

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BACKGROUND

Sexual transmission though an inefficient but is the most common route of HIV infection. Although free virus and seminal leukocytes were suspected to be the source for sexual transmission, Human immunodeficiency virus (HIV) is genetically extremely variable. Analysis of the V3 loop sequence of HIV *env* region indicated presence of different viral variants in peripheral blood mononuclear cells (PBMC), lymph node, spleen, brain and lung in a same individual. The viral isolates present in the genital secretions and cells including vaginal epithelial cells, seminal leukocytes and sperm represent the initial virus responsible for sexual transmission of HIV into partner. It has been demonstrated that sperm bound virus can also be directly transmitted into urogenital cells and in turn facilitate HIV transmission. Sperm bound virus but not the free virus has been shown to gain entry into the oocyte. Animal studies using the rhesus macaque model indicated the possibility that the highly acidic vaginal pH may not allow the survival of free virus and seminal leukocytes. It is therefore likely that spermatozoa may act as a reservoir for HIV and facilitate sexual transmission of HIV as well as possibly transmit the virus to fetus at the time of fertilization.

METHODS

Genotypic characterization is performed by heteroduplex mobility assay (HMA) with Viral RNA /DNA isolated from PBMCs, seminal leukocytes, spermatozoa of HIV Positive individuals. The phenotypic characterization evaluated by CD4 count by flow cytometry and viral load by Real time PCR.

RESULTS

Distinct pattern of HMA observed in spermatozoa and PBMCs of the same individual. Sequence analysis showed 96% homology of C2/V3 region of HIV1 C *env* from spermatozoa while that with the PBMCs of the same individual showed 93% homology.

CONCLUSION

Presence of HIV variants in blood and semen play an important role in sexual transmission of HIV. Presence of virus in the sperm shows that the spermatozoa are also another factor responsible for sexual transmission of HIV.

P19

Heparan Sulfate Proteoglycans are Required for Cellular Binding of the Hepatitis E Virus ORF2 Capsid Protein and for Viral Infection

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The hepatitis E virus (HEV), a non-enveloped RNA virus is a major public health concern. The mode of attachment and HEV entry into target cells for productive infection remains unidentified. For many viruses, the initial stage of the entry process is the binding of a viral attachment protein to a generalized receptor, followed by interaction with a more specific host cell receptor. These generalized receptors, more commonly known as attachment factors, concentrate virus particles on the host cell and create conditions favourable for receptor binding. The major capsid protein of HEV is encoded by its open reading frame 2 (ORF2). We have used a ~56 kDa recombinant pORF2 that can self-assemble as virus like particles (VLPs), to show that cell surface heparan sulphate proteoglycans (HSPGs), specifically syndecans, play a crucial role in the binding of pORF2 to Huh-7 liver cells. Removal of cell surface heparan sulphate by enzymatic (heparinase) or chemical (sodium chlorate) treatment of cells, or competition with heparin, heparan sulphate and their over-sulphated derivatives, caused a marked reduction in pORF2 binding to the cells. Specificity is likely to be dictated by well defined sulfation patterns on syndecans. We show that pORF2 binds syndecans predominantly to highly sulphated HSPGs via 6-O-sulfation. Using an *in vitro* infection system, we also show a marked reduction in HEV infection of heparinase treated cells. Our results indicate that HSPGs might play an important role in determining the tissue tropism of HEV.

P20

The *Severe Acute Respiratory Syndrome Coronavirus* (SARS-CoV)-7a Protein is a RNA Silencing Suppressor (RSS)

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RNA silencing is a novel eukaryotic gene regulatory mechanism that operates at the level of RNA. It serves as a natural antiviral defense mechanism in plants, invertebrate and vertebrate animals, to restrict viral proliferation and disease manifestation. The viruses, as a counter defense, have evolved a suppression strategy, using which the viral protein interferes with the RNA silencing pathway. These proteins are called RNA silencing suppressors (RSS) and almost all the plant viruses are known to encode RSSs. Though a few RSSs are reported from insect and animal viruses, these have not yet been screened from the majority of animal viruses. Considering the various recent reports highlighting the importance of RNA silencing as antiviral defense in animal system, screening for the animal viral ORFs for RSS activity has become essential, which eventually help in developing antiviral strategies.

Severe acute respiratory syndrome coronavirus (SARS-CoV) is one of the highly infectious positive-stranded RNA viruses that was spread worldwide and killed hundreds of people in early 2003. The virus has been reported to generate abundant dsRNA as replicative and transcriptive intermediates that can induce RNA interference against the virus. Therefore, it becomes imperative to identify whether the virus encodes any RSS or not. Considering, the fact that RSSs act cross kingdom, we analyzed three different ORFs of SARS-CoV, *viz.* 3a, 7a and N for the RSS activity using *in planta* assay systems. SARS-7a had been identified as an RSS among the three ORFs, tested using two different assays, *viz.,* reversal of silencing assay and viral amplicon based assay. The analysis of *in vitro* generated nonsense mutant of SARS-7a indicated that the suppression factor was a protein and not RNA. The domain mapping of the protein has further elucidated that the central region of about 57amino acid was indispensable for the RSS activity. As the viral pathogenecity is known to be tightly linked with the RSS activity, SARS-7a could thus be predicted to be a pathogenecity factor. Hence SARS-7a could be an effective target for antiviral therapy.

P21

A Histidine Switch in Hemagglutinin–Neuraminidase Triggers Paramyxovirus-Cell Membrane Fusion

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Most paramyxovirus fusion proteins require coexpression of and activation by a homotypic attachment protein, hemagglutinin-neuraminidase (HN), to promote membrane fusion. However, the molecular mechanism of the activation remains unknown. We previously showed that the incorporation of a monohistidylated lipid into F-virosome (Sendai viral envelope containing only fusion protein) enhanced its fusion to hepatocytes, suggesting that the histidine residue in the lipid accelerated membrane fusion. Therefore, we explored whether a histidine moiety in HN could similarly direct activation of the fusion protein**.** In membrane fusion assays, the histidine substitution mutants of HN (H247A of Sendai virus and H245A of human parainfluenza virus 3) had impaired membrane fusion promotion activity without significant changes in other biological activities. Synthetic 30-mer peptides corresponding to regions of the two HN proteins containing these histidine residues rescued the fusion promoting activity of the mutants, whereas peptides with histidine residues substituted by alanine did not. These histidine-containing peptides also activated F-virosome fusion with hepatocytes both in the presence and in the absence of mutant HN in the virosome. We provide evidence that the HN-mimicking peptides promote membrane fusion, revealing a specific histidine "switch" in HN that triggers fusion.

P22

Inhibition of Hepatitis E Virus Replication using Short Hairpin RNAs

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Hepatitis E virus (HEV) is a non-enveloped, icosahedral, single-stranded, positive-sense RNA virus, which is the major cause of water borne hepatitis. Till date there is no licensed therapy of vaccine against HEV. Protein based Vaccine which in its Phase III trials has its own demerits. In order to find new approaches for development of new therapeutic strategies against HEV infection, we looked forward to exploit RNAi mechanism. RNA interference (RNAi) is a cellular, sequence-specific system induced by double-stranded RNA which we used to silence several genes and cis-acting element (CAE) of HEV. In the present study, short-hairpin RNAs (shRNAs) were developed against helicase, replicase and 3′CAE of HEV. Processing of shRNA into siRNA was verified by northern hybridization. Possibility of stimulation of innate immune system due to shRNA expression was checked by transcript analysis for Interferon–β and 2′, 5′-Oligoadenylate synthetase genes. We have carefully optimized the concentration of shRNA in such as way that it should not affect cellular microRNA machinery to larger extent. Initially, the selected shRNAs were tested for their antiviral potential against the respective genes/3′CAE using inhibition of fused viral gene-Renilla Luciferase reporter constructs. The effective shRNA were tested for their silencing potential on HEV replication in HepG2 cells using HEV replicon (AF076239) and its derivative reporter replicon using transient transfection system. RNAi mediated silencing was demonstrated by luciferase assay and real time PCR in reporter fused and native replicon transfected cells respectively. Tested shRNAs were found to be effective in inhibiting virus replication by varying extent (45-93%).

P23

Current Research on Immune Responses to the Dengue Virus

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Dengue viral infections are the most important mosquito borne viral infections in the world and the number one killer mosquito borne infection in Sri Lanka. Sri Lanka has been affected with epidemics of Dengue Hemorrhagic Fever (DHF) for over 2 decades, and is currently experiencing the worst ever dengue outbreak this year.

Most dengue viral infections are thought to give rise to asymptomatic infection or mild clinical disease in a majority of individuals and usually severe dengue infections are usually associated with secondary dengue infections. However, we and others have found that severe disease may occur even during primary dengue infections especially in infants and pregnant women.

In order to investigate the possible factors that lead to severe clinical disease, we initially set out investigate dengue virus NS3 protein specific immune responses in individuals with varying severity of past dengue infections. This would be important to understand the changes and the status of the dengue virus specific T cells in the host with time. In addition, we thought that this information would help us to answer important questions such as: how long dengue specific protective immune responses last, when serotype cross-reactive T cells appear/disappear and if these changes are the same in all individuals or the host factors which influence these.

We have started to investigate dengue specific functional T cell responses in healthy individuals with varying severity of past infection. We recruited healthy individuals from Sri Lanka with past dengue infection who had a dengue infection 3-5 years earlier. Individuals who had asymptomatic dengue infections were also recruited. Ethics for the study was granted from the local ethics committee.

Peripheral blood mononuclear cells (PBMC) were obtained from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation. They were then resuspended in RPMI 1640 plus 10% fetal calf serum (FCS). *Ex vivo* interferon gamma ELISpots were used to determine dengue specific functional T cell responses to dengue virus NS3 protein. NS3 was chosen because it is one of the most immunodominant proteins of the dengue virus and

multiple epitopes have been defined within this protein. The peptides used in the ELISpots are synthetic 20 mer peptides overlapping by 10 amino acids which spanned the whole length of the NS3 protein of DEN2 and DEN3. These were synthesized in house in an automated synthesizer using F-MOC chemistry.

We have found that the majority of individuals react to dengue NS3 overlapping peptides. We are in the process of carrying out more experiments in this area to obtain more data.

P24

Involvement of an Intracellular Phosphatase Activity Upon Chandipura Virus Life Cycle: A Possible Transcription to Replication Switch

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Chandipura virus, a member of the rhabdoviridae family and vesiculovirus genera, has recently emerged as human pathogen that is associated with a number of outbreaks in different parts of India. Although, the virus closely resembles with the prototype vesiculovirus, Vesicular Stomatitis Virus, it can be readily distinguished by its ability to infect humans. Study of different parts of viral life cycle will shed light upon the transcription replication machinery of RNA viruses. Being a human pathogen this virus acts as an important model system for the study of host-pathogen interaction. In RNA viruses the most crucial event of viral life cycle is the switching between the transcription and replication mode of the single RNA dependent RNA polymerase. The viral Phosphoprotein P, a co-factor of the polymerase, is believed to play important role in modulating the dual mode of the polymerase depending upon its phosphorylation status.

P protein in the host cell undergoes post-translational modification by cellular CKII, being phosphoryated at Ser 62 residue. This phosphorylated P protein acts as a transcriptional activator of the viral RdRp complex. However the source of unphosphorylated P protein in the infected cells (necessary for replication) still remains unclear. Our search into the means of availability of unphosphorylated P protein leads us to a novel phosphatase activity that can dephosphorylate P protein and may switch the polymerase from transcriptase to replicase mode. We have screened a number of cell lines for such phosphates activity. Bacterially expressed P protein was in-vitro phosphorylated with ^{32}P labeled by recombinant CKII and used as a substrate. Some selective cell lines like A549 or HeLa have shown measurable phosphates activity towards P, however cell lines like BHK21 or Vero cells (which are the established host for Chandipura) failed to do so. This phosphatase activity also seemed to affect the viral infectivity in cell lines as well. The viral titre is lower with cells showing phosphatase activity than those which are not showing the same extent of activity. In this work we have tried to identify the unknown phosphates and to decipher its role in viral life cycle.

P25

Evaluation of a New Recombinant Hepatitis B Vaccine Potency in Mice Formulated with Two Conventional Aluminum Adjuvants

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OBJECTIVE

To investigate enhancement of a new recombinant hepatitis B vaccine potency in mice, two aluminum adjuvant gels, commercially named as Alhydrogel and Adju-phos adjuvants (Superfos, Denmark), were tested in BALB/c mice with various formulations.

MATERIAL AND METHOD

Different concentrations of a new r-HBsAg ranging from 0.16, 0.32, 0625, 1.25 and 5ug were formulated with a constant amount of each adjuvant (500 ug aluminum per ml). Each vaccine was administrated on one dose and with the same schedule in a group consists of 15 mice. Mice were bled after 28 days of immunization and their sera were tested for total anti-HBs antibody by Elisa.

Anti-HBs antibody titers in mIu/ml were calculated by using a standard curve and data were reported as geometric mean titer (GMT). The GMT values were accompanied by 95% confidence intervals.

Also, the GMT differences between groups were determined by Mann-Whiteny and Kruskal-Wallis statistical methods.

RESULT

Results showed that 100% of mice were seroconverted and seroprotected by vaccines which contained 0.625 ug or higher amounts of HBsAg and formulated with Adju-phos adjuvant. In contrast, the 0.625 ug dose of vaccine which formulated with Alhydrogel was only seroconverted 53% and seroprotected 33% of mice. Only the 5 ug dose of such vaccine was seroconverted 100% of mice.

Also, in various formulations of vaccine higher amount of r-HBsAg was bounded to Alhydrogel than Adju-phos adjuvant.

DISCUSSION

Mice immunized with Adju-phos formulated vaccines showed 20-80 folds higher anti-HBs antibody titers compared to those immunized with Alhydrogel formulated vaccines. Apparently, these adjuvants had different effects on the immunogenicity of the HBsAg vaccine.

CONCLUSION

adju-phos adjuvant has an excellent adjuvanticity and compability with this r-HBsAg and was primed a strong anti-HBs response after primary immunization of mice.

P26

Molecular Evolution of Overlapping Genes of HIV – 1

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INTRODUCTION

The phenomenon of viral proteins encoded by overlapping reading frames has attracted the attention of evolutionary biologists since its discovery. Overlaps have been hypothesized to be involved in genome size minimization and as a regulatory mechanism of gene expression. One question of evolutionary interest raised by this phenomenon is how natural selection can act simultaneously on two different reading frames by the same DNA sequence.

OBJECTIVE

1) To study the rate and pattern of evolution of HIV-1 within and among the individual through long term follow up. 2) To study the effect of evolution on viral proteins encoded by overlapping reading frames.

METHODOLOGY

Near full length genome was amplified from HIV+ve PBMCs or Plasma and cloned into pCR2.1 vector. Positive clones were sequenced by primer walking method. All the four overlapping regions (*p6gag/pro*, *tat1/rev1, tat2/rev2/gp41, nef/LTR*) were studied by aligning them with the help of Clustal W and Phylogenetic distance were calculated using maximum likelyhood model using Kimura-2 parameter.

RESULTS & CONCLUSION

Among all four overlapping regions p6/pro were found the most variable and the motif encoded is responsible for Vpr binding, and virus budding by interacting with two host proteins Tsg 101 and ESCRT-I (AIP1). Functional study of these variations will reveal the effect of this kind of variation on viral life cycle.

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Cloning, Expression, Purification and Characterization of Dengue Virus Serotype 2 Envelope Protein Antigen as a Chimera with Hepatitis B Surface Antigen in *Pichia Pastoris*

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Dengue viruses are mosquito (*Adese aegypti, Adese albopictus*) borne, positive-stranded RNA viruses of the genus Flavivirus within the family Flaviviridae. There are four antigenically distinct serotypes of dengue viruses (DEN-1, 2, 3 and 4), which can cause a broad spectrum of diseases like dengue fever, dengue hemorrhagic fever & dengue shock syndrome. Infection with any one dengue serotype provides lifelong homologous immunity. Sequential infection with different serotypes, in areas where multiple serotypes co-circulate, has the potential to trigger life-threatening diseases like dengue hemorrhagic fever & dengue shock syndrome which is supposed to be mediated by antibody dependent enhancement mechanism (ADE). Dengue is becoming a major public health problem in the world. Each year, there are an estimated 50–100 million cases of dengue fever, including 250,000–500,000 cases of dengue hemorrhagic fever & dengue shock syndrome (DHF/DSS), and nearly 30,000 dengue related deaths. More than one-half of the world's population now lives in areas at risk for the disease. Hence there is a need for an effective vaccine for Dengue. There are several candidate dengue vaccines which are in different phases of development. In our lab we had previously shown that Dengue-2 envelope protein fused at the N terminus of HBsAg (*Den2E-HBsAg*) makes virus like particles (VLPs). Since the expression levels of *Den2E-HBsAg* construct was low we decided to work on the smaller antigenic version of the same construct, *Den2.Ag-PreS2-S* (Dengue-2 envelope protein antigen as an N terminal fusion with Pres2-S, Hepatitis B "M" antigen). We have cloned the gene into methylotrophic yeast *Pichia pastoris.* The fusion protein has been purified using conventional chromatographic techniques, which involve strategies like membrane extraction, diafiltration, hydrophobic & gel filtration chromatographies. We have also purified this fusion protein using immunoaffinity chromatography. The electron microscopy of the purified protein samples shows the presence of VLPs. The immunization of these VLPs in BALB/c mice has shown good titers of neutralizing antibodies. Our current efforts are underway to develop these VLPs into a candidate vaccine for dengue.

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Higher Prevalence of Rotavirus G and P Untypables in Kolkata and Usage of Multiplex PCR Method for Genotyping–Probable Indication of Evolution in Genotypes

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World wide, acute gastroenteric illnesses continue to be one of the most important causes of illness and death, mostly amongst young children and infants in developing countries. It accounts for nearly 2 million deaths per year among children under five, making it the second most common cause of child death worldwide.The main infectious agents responsible for human enteric infectious include several viruses(rotaviruses, Human Caliciviruses(HuCV), astroviruses, enteric adenoviruses and several bacterial agents, out of which Rotavirus is the single most important cause of infantile gastroenteritis worldwide, affecting an estimated 130 million infants and causing 873,000 deaths every year.

During the present surveillance at The I.D. and B.G. Hospital, Kolkata the various viral agents responsible for diarrhoea were detected We used Vikia Rota-Adeno kit for primary detection of rotavirus and adenovirus according to the manufacturers' instructions (bioMerieux).Rotavirus groups confirmation were performed by PAGE method. The Rotavirus is the most prevalent (18.91%) causative agent of diarrhoea in Kolkata. Next, The multiplex PCR method was used for the genotyping of Rotavirus on the basis of the nature of the VP4 and VP7 genes. The primers used for the VP7 genotypes were G1(618 bp),G2(521 bp),G3(682 bp),G4(452 bp),G8(754 bp),G9(179 bp),G10(266 bp) and G12(450 bp) which showed the highest prevalence of G1(23%) followed by G9(20%), G12(19%),G2(12%) and G10(4%). 18% of the samples were untypable for the VP7 genotype which is very significant. Primers used for the VP4 genotypes were P[4] (483 bp), P[6] (267 bp), P[8] (345bp), P[9] (391 bp), P[10] (583 bp), P[11] (312 bp).Among the P types, P[8] was the most prevalent (28%) followed by P[4] (23%) and P[6] (9%).Here also 38% of the VP4 genotypes were untypable with the available typing primers.

The increase in the number of untypable G and P types indicates the prevalence of unknown genotypes circulating in this locality in Kolkata.

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Development of a Common Vaccine for Influenza A H1N1 with a Coverage for Newly Emerging Viral Strains with Herd Immunity Effect

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INTRODUCTION

In April 2009, a novel strain of H1N1 (Swine Origin [SO]) came to world attention as the cause of many illnesses and deaths in Mexico. Air travel contributed to the spread of H1N1. Cases now span the globe, and pandemic response plans are being activated to meet a threat to public health that is of uncertain magnitude and severity but is increasingly of pandemic potential. Although much has changed since the 1918 pandemic, the current outbreak rightly raises concern and prompts action. As of 29th November 2009, worldwide more than 207 countries and overseas territories or communities have reported laboratory confirmed cases of more than 300,000 of pandemic influenza H1N1 2009, including at least 10,000 deaths. As many countries have stopped counting individual cases, particularly of milder illness, the case count is likely to be significantly lower than the actual number of cases that have occurred. The virus is a novel strain of influenza for which extant vaccines against seasonal flu provide little protection.

The Hemagglutinin and Nuraminidase molecules are the antigenically active parts on the Influenza A surface. They exhibit a considerable polymorphy due to a high mutation rate in the viral genome. Especially the gene coding for the Hemagglutinin mutates so rapidly. They undergo two kinds of mutations; antigenic shift and drift. In shift, a new virus with antigenically distinct Hemagglutinin or Nuraminidase will appear. Drifts consist in point mutations, continuously changing the composition of the antigenic sites giving rise to new virus strains. This variation in the surface molecules contribute to antigenic variation and hence give the new viral strains to escape from the immunity.

Of the cases reported, 64% are in 5- to 24-year-olds and just 1% are in individuals older than 65 years, an unusual pattern compared with seasonal influenza. This has led to speculation that older individuals have at least some degree of pre-existing immunity, possibly from years of immunization with seasonal flu vaccines. They contained different H1N1 viruses than the current outbreak strain, or previous infection.

OBJECTIVES

To develop an effective vaccination with the effect of herd immunity against the Influenza A virus including the newly emerging viral strains with blanket coverage to all such future outbreaks of Influenza A pathogens.

METHODOLOGY

The whole genome of the influenza A virus will be studied and serological studies will be carried out in the immune individuals. An identified recombinant H1N1 envelope protein is encapsulated to a harmless virus strain in order to make the H1N1 vaccine. Human models could be used to test the immunity of this vaccine against H1N1.

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Stable Expression of Envelope Glycoproteins of HCV Genotype 3a in Huh-7 Cell Line

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BACKGROUND

Characterization of antibodies targeting the attachment and entry of the viral particles into host cells is important for studding antibody mediated neutralization. Antibodies against the envelope glycoproteins have neutralizing capacity and can prevent HCV infections.

AIM

The aim of the present study was the development of a system for screening of HCV anti envelope neutralizing antibodies in the serum of HCV patients during acute and chronic HCV infections.

RESULTS

We cloned the envelope glycoprotein coding genes from local HCV isolates in mammalian expression vector and studied stable expression of local envelope genes in continuous cell lines.

CONCLUSION

Stable cell lines developed can provide a useful tool to detect anti-HCV envelope antibodies in the serum of HCV infected patients and to test binding of potential antiviral molecules to HCV envelope glycoproteins of genotype 3a.

KEYWORDS

Hepatitis C virus, glycoprotein, attachment, entry, vaccine, mammalian cell lines

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Determinant within NS3 Protease Critical for RNA Binding to Mediate the Switch from Translation to Replication of Hepatitis C Virus RNA

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Hepatitis C virus (HCV) non structural protein 3 (NS3) plays pivotal role in polyprotein processing and viral RNA replication. HCV NS3 protein has two domains: the protease and the helicase. The N terminal domain of NS3 has a chymotrypsin like fold which exhibits a robust serine protease activity. In contrast, the helicase domain was shown to bind the viral RNA and help in replication. We have shown that the protease domain on its own can bind HCV-IRES at the SLIV region near the initiator AUG. The RNA binding leads to inhibition of IRES mediated translation and enhancement of HCV RNA replication. We have mapped the putative contact points of the protease on the HCV IRES and also predicted the RNA binding residues of the protease. Using computational approach, we have demonstrated that the RNA binding residues are mostly in the C terminal half of the protease domain. Also, deletion analysis clearly showed that the RNA binding residues primarily reside in the C terminal half. Interestingly, a 30-mer peptide derived from the putative RNA binding region showed significant RNA binding ability and successfully inhibited IRES mediated translation. Structure prediction studies suggest that this peptide might assume a turn like structure both in isolation and as well as in the context of full length NS3 protein. Finally, mutation of predicted RNA binding residue showed reduced RNA binding ability of the protease domain and appears to loose the ability to inhibit HCV IRES function efficiently, reconfirming above observation. The structural determinant within the NS3 protease necessary for the RNA binding activity and the consequent inhibition of IRES function to switch over to replication of the viral RNA is currently being investigated.

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Understanding the Interaction between the Leader RNA and Phosphoprotein of the Chandipura Virus—A Step Towards a Novel Peptidomimetic Antiviral

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Chandipura Virus (CHPV), a prototype non segmented, negative stranded RNA virus has been inflicting severe health hazards that has often augmented to epidemic proportions, in parts of the Indian sub-continent over the last few decades. The absence of a specific anti-viral treatment, together with the incubation period being as low as 48 hours, demands the development of novel antiviral approaches targeting specific macromolecular interaction indispensible to the viral life cycle. Here we present the development of a novel peptidomimetic approach that blocks the interaction between the phosphoprotein P and the leader RNA l, resulting in a strong inhibition of viral replication.

Previous reports by our laboratory have shown for the first time that the unphosphorylated P protein interacts with the 49nt long Leader RNA, and this interaction is indispensible for genome replication. The phosphorylated form of the P protein (P1) has been established to function as a cofactor for the functional RdRp complex during transcription. However, it now appears that the unphosphorylated form of the P protein (P0) in conjunction with the leader RNA has a role in modulating the Viral RdRp into the replication mode. Attempts to locate the leader RNA binding domain of the P protein by truncation mutagenesis suggested the role of the C-terminal conserved domain. In this study we have identified the specific amino acid residues involved in this interaction, and site directed mutagenesis of these residues abrogates the leader RNA binding of the P protein, as ascertained by Electrophoretic mobility shift assay, ligand induced fluorescence quenching, fluorescence anisotropy, etc. However, the major confirmation of the role of these RNA binding residues came when a synthetic 36mer peptide encompassing these residues managed to strongly inhibit the viral replication in Vero cells. This paved the way to use this peptide; C-terminally fused to 6-D Arg residues to facilitate cellular uptake; as a novel peptidomimetic agent with a strong and rapid inhibitory potential on the viral replication, by acting as a competitive inhibitor of the full length viral P

protein in binding the leader RNA. A mutant peptide in which the 217-219 AA has been mutated to alanine failed to show detectable anti-viral effect, confirming the involvement of these residues in the P protein-leader RNA interaction. The replication inhibitory potential of the peptidomimetic has been assessed through viral plaque reduction assay, qRT-PCR of the +ve stranded replication intermediate, and immunofluorescent detection of nascent viral proteins resulting from either primary or secondary viral transcription. On the other hand, purified mutant and wild type proteins were instrumental in estimating the K_d of RNA bindings among other things. Therefore, in the absence of any specific treatment to the Chandipura virus, peptidomimetic approaches may be one of the probable groups of antiviral of the future.

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Homogeneous and Heterogeneous Hepatitis C Antibody Assay Based on Multiepitope Proteins and Fluorescent Lanthanide Chelates

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Hepatitis C virus diagnostics is generally based on an immunoassay detecting anti-HCV antibodies or molecular assay detecting HCV RNA. Immunoassays are the simplest and most inexpensive method of diagnosis, but conventional immunoassays require several capture polypeptides with different epitopes for reliable detection of geographically distributed genotypes and rare genotypes can still remain undetected.

A recombinant multiepitope protein (MEP) has been developed earlier incorporating epitopes four HCV antigens recognizing different genotypes and optionally a biotin acceptor peptide for *in vivo* biotinylation. All these epitopes are linear, immunodominant and many are phylogenetically conserved. Using MEP as an antibody binder in an immunoassay eliminates the need for several separate antigens. Also, MEP is easy and cheap to produce in *E. coli* system.

We have developed a heterogeneous and a homogeneous immunoassay for anti-HCV antibodies based on the multiepitope protein and time-resolved fluorometry. The homogeneous fluorescence resonance energy transfer (FRET) assay was based on MEP conjugated either with a long-lifetime fluorescence terbium chelate donor or an AlexaFluor 680 acceptor. FRET occurs when both donor and acceptor are brought to proximity by differently labeled MEP conjugates attaching to different binding sites on an anti-HCV antibody. Background is minimized because the donor has very little crosstalk in the measured acceptor emission range.

In the heterogeneous assay, *in vivo* biotinylated MEP immobilized on streptavidin plates was used as a capture and MEP or a secondary antihuman antibody conjugated to a europium chelate as tracer. Both assay formats were tested with a worldwide HCV performance panel and the MEP recognized all tested genotypes. In addition, the assays were tested with an in-house panel of patient sera and compared with Innotest HCV Ab IV and Ortho HCV 3.0 assays.

When the MEP-conjugate was used as tracer, the binding of the antibody on the solid-phase MEP and the binding of the tracer MEP to the antibody were both HCV antibody specific and background originating from high excess of non-virus specific antibodies was eliminated. However, the assay using MEP both as capture and tracer was strongly affected by the avidity of the antibody response, with low signals from recent infections that have an unmatured antibody response. The homogeneous assay, which uses two MEP antigens as binders, is similarly affected. Using a secondary antihuman antibody conjugate as tracer did increase the background from negative samples, but the assay was less affected by the avidity of the antibody response.

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Cloning and Expression of NS-1 and prM Proteins for the Diagnosis of Dengue Infection

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Diagnosis of Dengue fever (DF)/ Dengue haemorrhagic fever (DHF)/Dengue shock syndrome (DSS) has been done by HI/MAC-ELISA/NT tests. Among this MAC-ELISA has been routinely used throughout the globe for detection of dengue viral infections. The needed dengue virus antigens for the assays are prepared from suckling mouse brain and from cultured cells under *in vitro*. The former method is biohazardous while handling the dengue infected mouse colonies and the latter is costly in maintaining cell lines and they limit the wide spread use of this method for obtaining dengue antigens for diagnosis. In addition, this method gives whole viral antigens. They generally cross react with other closely related flaviviral antibodies giving false positives, thus they are disadvantageous. Hence, we propose to develop an alternative method for dengue viral detection based on proteins of prM and NS-1 produced through recombinant DNA technology using bacteria as an expression system.

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Evolutionary Forces Acting on DDX3X Potentially Affects HIV-1 Replication

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HIV-1 Rev protein is an important regulator of viral transcription and nucleo-cytoplasmic transport. Rev achieves this activity through a series of interaction and association with a number of host factors. In the present study, we extensively analyzed the known protein interactions using a combination of computational and evolutionary approaches. We studied the patterns of evolution of all well known HIV-1 Rev interacting proteins (obtained from HIV-1 protein interaction database) by correlating their structural and sequential divergence parameters across different mammalian species. Protein sequence cluster for each protein was obtained from Uniref (at both 50% and 90% sequence homology) while coding sequences (CDS) were collected from NCBI and Ensemble databases. All the sequences were aligned by Clustal X2 Gonnet PAM250 matrix. Based on criteria such as protein redundancy, sequence conservation and antiviral activity, we shortlisted DDX3X as a potential host factor that can be employed for therapeutic interventions. Using likelihood based statistical analysis of sequence divergence (F61 model), we further showed that evolutionary forces are responsible for demarking functionally important residue cluster (conservation scores) on the surface of DDX3X and these regions can regulate the interaction of DDX3X with Exportin-1 at a specific interfacial region (using global and local docking simulations). All the conservation scores were plotted on the RCSB PDB derived crystal structure (e<3e60) of DDX3X using Chimera Extensible Molecular Analysis System. The role of amino acid conservations, inter atomic bond angles and energies were obtained by a combination of Force Field algorithms (FFE, ArgusLab, FoldX). Using *in silico* and statistical evolutionary analyses, we found that this cluster overlaps with key phosphorylation sites important for DDX3X function as well as are targets for nucleoside analogues of ATP (Local docking by Hex) that have been shown to down-modulate HIV-1 replication.

We conclude that any natural or induced perturbation at the observed functional region would potentially modulate HIV-1 replication and would constitute an attractive target for intervention.

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HIV-1 Virion Infectivity Factor (VIF) is a Potent Activator of NF-κB Signaling Pathway

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HIV-1 Vif is a basic protein that is essential for the pathogenesis of the virus in T Lymphocytes. The role of Vif was dissected when it was observed that some cells were permissive for Vif deficient HIV-1 replication whereas some cells required Vif for efficient virus replication. Further studies gained insights into the role of Vif in neutralizing a host protein, APOBEC3G which introduces hyper-mutations in HIV-1 cDNA. Further, Vif is known to be a part of cytoplasmic Ribonuclease complex as well as binds to HIV-1 RNA preferentially. During our study we found that Vif activated HIV-1 LTR independently as well as in the presence of Tat. Vif was also able to activate NF-κB promoter. Since activity of NF-κB is tightly coupled to IκBα, it was observed that Vif repressed IκBα promoter as well as degraded IκBα when present under CMV promoter thereby pointing towards dual strategy adopted by Vif to activate NF-κB. It was observed that Vif induces phosphorylation of IκBα thereby leading to its degradation through ubiquitination. It was also observed that Vif induced IKK Beta and Gamma but not IKK epsilon. All these evidences clearly points towards the role of Vif in reprogramming the host's proteins to degrade IκBα.

Our studies reveal a novel role of Vif in regulating NF-κB signaling. Since this is a central pathway in immune system, hence the study opens new vista to study the role of Vif in immune evasion.

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Fast Detection of Small Changes in Genomes of Recent Inluenza A Isolates by Multitemperature Single-Strand Conformational Polymorphism (MSSCP) Technique

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Single-stranded DNA fragments attain a number of conformational forms which may be separated by electrophoresis in native polyacrylamide gels giving a characteristic pattern of electrophoretic bands. Minute sequence changes (e.g. point mutations) may have significant effect on electrophoretic pattern of single-stranded DNA. Changes of gel temperature during electrophoresis increase the sensitivity of mutation detection in PCR products; this technique, a modification of SSCP technique, was named MSSCP (where M stands for "multitemperature"). This method modified in our laboratories was applied for characterization of influenza A cDNA fragments. A series of primers were synthesized after the comparison of the hemagglutinin, neuraminidase and PB2 gene sequences of different origin. PCR reactions were run using these primers and the products were denatured. Single-stranded DNA fragments were subjected to MSSCP electrophoresis where, after silver staining, they gave characteristic ssDNA band patterns. This technique was applied to analyse hemagglutinin, neuraminidase and PB2 gene fragments from avian flu and recent swine flu isolates. Minor differences within a serotype were detected which makes the MSSCP technique a valuable tool for quick preliminary characterization of influenza variants.

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Neutralization of Hepatitis C Virus by Monoclonal and Polyclonal Antibodies as a Way for Vaccine Development

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BACKGROUND/AIM

Hepatitis C Virus is a global health problem that infects not less than 12% of the Egyptian population. The high rate of mutatins in the E1 and E2 proteins hampers attempts to develop effective immunological products for treatment or prophylaxis of HCV infection. We utilized large scale multiple sequence alignment of E1 and E2 to design genetically conserved peptides from viral envelop proteins (particularly among type 4 isolates). The aim of this study is to develop monospecific polyclonal Abs in goats and mouse monoclonal antibody against 4 conserved peptides derived from E1 (single peptide) and E2 (3 peptides) and to test viral neutralization of each Ab using cellular and non cellular neutralization assays.

MATERIALS AND METHODS

One synthetic peptide from HCV E1 (p35) and three synthetic peptides from E2 region (p36, p37 and p38) were used in this study. We generated novel caprine anti-HCV polyclonal antibodies targeting conserved amino acid sequences from E1 and E2 proteins as well as mouse monoclonal antibody targeting HCV E1. We employed immuno-capture-RT-PCR and viral infection in vitro using Huh7 cells in viral blocking experiments to examine the neutralizing activity of the generated antibodies. To test whether similar antibodies are generated in chronic HCV patients, the designed synthetic peptides were used to screen for the presence of corresponding antibodies in 100 patients positive for HCV RNA and 100 un-infected subjects as controls.

RESULTS

Synthetic peptides detected corresponding human IgGs in all HCV positive patients (100%) and non of the controls (0%) as shown by ELISA and dot-ELISA methods Caprine Abs against p35, p37 and p38 but not p36 displayed viral neutralization properties using immuno-

capture RT-PCR of virus bound to Ab/ CNBr- activated Sepharose 4B columns and to Ab coated on PCR tubes. Cellular assays confirmed immunocapture RT-PCR results therefore suggesting that p36 although immunogenic but its antibody is non neutralizing. Immunization of Swiss mice with E1 and E2 specific epitopes can elicit polyclonal antibody responses capable of neutralizing HCV virions in HCV positive sera as determined by immuno-capture HCV RT- PCR.

CONCLUSION

Selection of highly conserved peptide immunogenic epitopes from E1 and E2 proteins is a promising strategy for long term efficacy in fighting HCV infection. At minimum, there is much that HCV specific antibodies can teach us about vaccine design.

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Proteomics and Metabolomics Study of Plasma and Urine of Acute Hepatitis E Patients

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BACKGROUND

Hepatitis E, caused by the hepatitis E virus (HEV), is endemic to developing countries where it manifests as large waterborne outbreaks and sporadic cases. Though generally self-limited with a low mortality rate, some cases progress to fulminant hepatic failure with high mortality. Thus it is believed that the host response plays an important role in determining the progression and outcome of hepatitis E. With no identified predictive or diagnostic markers, the events leading to disease exacerbation are not known, and thus results in poor management of fulminant cases.

METHODS

For proteomics analysis of plasma and urine, a differential imaging (DIGE) based two dimensional gel electrophoresis platform was optimized, wherein the differentially expressed protein spots were selected and identified by mass spectrometry. The levels of one plasma protein, transthyretin, and one urine protein, alpha-1-microglobulin (α1m), were validated by enzyme linked immunosorbent assay (ELISA) on a larger set of samples.

For metabolomics analysis of plasma and urine, these samples were processed and analyzed by ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy, followed by statistical analysis and annotation, using the web-based metabolomic software 'MetaboAnalyst'.

RESULTS

Protein profiling of these biofluids of hepatitis E patients and healthy controls by DIGE and mass spectrometry have helped us identify over 30 unique proteins to be differentially expressed during acute infection. ELISA results showed a significant decrease in plasma

transthyretin concentration ($p<0.005$) and increase in urine α1m levels ($p<0.001$) in acute hepatitis E patients as compared to controls. Metabolomic analysis revealed a number of metabolites to be differentially regulated in acute infection. These metabolites could segregate the diseased samples from the healthy controls with an accuracy of 78.2% in case of plasma and 82.5% in case of urine. These studies also reveal the potential involvement of metabolic pathways in acute hepatitis E.

CONCLUSION AND SIGNIFICANCE

Our results demonstrate the utility of characterizing plasma and urine proteomes and metabolomes for signatures of the host response to HEV infection. We predict that plasma transthyretin and urine α1m could be reliable biomarkers of acute hepatitis E. Besides the utility of this approach for biomarker discovery, analysis of the metabolic and proteomic changes in human biofluids has increased our understanding of the host-virus interaction and hepatitis E disease.

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Influenza Nucleocapsid Protein Interacts with Hsp40 and Mitigates PKR Mediated Host Cellular Response: A Mechanism Conserved in H5N1 Bird Flu and H1N1 Swine Flu Viruses

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Virus infection in mammalian cells triggers activation of host responses targeted towards blocking entry, replication and spread of the virus. One common host response to all DNA and RNA viruses is activation of PKR which phosphorylates translation initiation factor eIF2Alpha resulting in translation 'Shut Off'. To overcome this hurdle Influenza virus employs NS1 protein which blocks activation of PKR, however even in absence of NS1 the Influenza virus is able to replicate inside mammalian cells, indicating presence of other mechanisms which prevent activation of PKR. We report here for the first time that Influenza Nucleoprotein also participates in mitigating PKR mediated host response. In a yeast two hybrid screen, we identified a host cellular factor which is Heat Shock protein 40 (Hsp40), interacts with Influenza Nucleoprotein. This interaction was found to be very strong in Liquid beta gal assay and was confirmed in mammalian cells transfected with H5N1 NP expressing plasmid as well as infected with H1N1 PR8 virus. This interaction was found to be conserved across different subtypes of Influenza viruses, including the seasonal H3N2 Human flu, pandemic H5N1 Bird Flu and recent H1N1 Swine Flu viruses.

Activation of PKR pathway happens in many ways, one of which is by upregulation of Hsp40, which blocks activity of p58IPK, which in turn is cellular inhibitor of PKR. We have shown that KNK437 drug mediated downregulation of Hsp40 leads to increased viral mRNA translation. Further we have shown that through interaction with Hsp40, influenza virus Nucleoprotein downregulates the phosphorylation of PKR and eIF2A. Also siRNA mediated depletion of NP in Influenza virus infected cells leads to normalized phosphorylation levels of PKR and eIF2A. siRNA mediated silencing of both NS1 and NP has more cumulative effect on upregulation of PKR, indicating both proteins contribute to the pathway in negative manner. Thus we report that seemingly innocuous Nucleoprotein, whose primary function is incapsidation of the RNA genome, is actually another shot in the armour of Influenza virus which acts against cellular host response.

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The 3b Accessory Protein of Severe Acute Respiratory Syndrome-CoV Interacts with RUNX1/Aml1b and Modulates its Activity

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The causative agent of Severe Acute Respiratory Syndrome was found to be a novel coronavirus whose genome is predicted to encode 14 ORFs expressing structural and non-structural preoteins. SARS-CoV genome also encodes several group specific unique accessory proteins which are found to be dispensable for viral replication. Our study aims at identifying putative host interacting partners of accessory protein ORF3b (154 amino acid) and to decipher the physiological relevance of the interaction.

Lung library screening with 3b as a bait led to identification of RUNX1 (Runt related transcription factor)/Aml1 (Acute myeloid leukaemia) as an interacting partner. RUNX1 is known to be crucially required for normal haematopoiesis and leukemogenesis. It regulates various myeloid and lymphoid promoters and enhancers. Co-immunoprecipitation and colocalization studies validated RUNX1 and 3b interaction. From pull down assays and electrophoretic mobility shift assays we propose that 3b complexes with RUNX1-CBFβ transcription complex. Further, we showed recruitment of 3b to RUNX1 binding elements on promoter regions, by chromatin immunoprecipitation assay. Besides, functional studies unveiled an increase in the transcriptional activity of RUNX1 in the presence of 3b in HEK293 and Jurkat cells. Data procured from kinase assay and pharmacological inhibitors treatment implied that 3b regulates RUNX1 activity by regulating its phosphorylation levels. Eventually, we conclude that 3b interacts and enhances RUNX1 transcriptional activity most likely by regulating its phosphorylation levels.

From the data obtained, we hypothesize that SARS CoV with its accessory protein 3b may modulate RUNX1, a component of host transcriptional machinery in developing a pathological state during the course of infection.

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The SARS Coronavirus 3a Protein Causes Endoplasmic Reticulum Stress and Induces Ligand-Independent Downregulation of the Type 1 Interferon Receptor

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The Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) is a positive strand RNA virus with an ~ 30 kb genome. Compared to other human and animal coronaviruses, the SARS-CoV genome contains 9 unique open reading frames (orf). Of these, orf3a is the largest and encodes a protein of 274 amino acids. The 3a protein is part of the virus particle, is expressed abundantly in infected as well as transfected cells, and localizes to intracellular and plasma membranes. We have earlier shown the 3a protein induces apoptosis through increased Bax oligomerization, p53 expression and activation of p38 MAP kinase. We show here that the 3a protein induces endoplasmic reticulum stress and the unfolded protein response (UPR). The 3a protein differentially modulates UPR with transcriptional activation of the ER chaperones GRP78 and GRP94. There is increased phosphorylation of eIF2α and transcriptional activation of the C/EBP homologous protein (CHOP) in 3a-expressing cells. However, the 3a protein had no effects on the ATF6 and XBP1 arms of the UPR. Activation of the PERK arm of UPR regulates innate immunity through the interferon (IFN) pathway. The 3a protein was found to induce serine phosphorylation within the IFN alpha-receptor subunit 1 (IFNAR1) degradation motif and to increase IFNAR1 ubiquitination. Confocal microscopic analysis showed increased translocation of IFNAR1 into the lysosomal compartment and flow cytometry showed reduced levels of IFNAR1 in 3a-expressing cells. These results provide further mechanistic details of the pro-apoptotic effects of the SARS-CoV 3a protein, and suggest a potential role for it in attenuating interferon responses. The 3a protein induced serine phosphorylation within the IFN alpha receptor 1 (IFNAR1) degron, which is a signal for receptor degradation and would make 3a-expressing cells insensitive to

IFN. Interestingly, IFN was not found to be clinically useful in treating SARS patients. It is tempting to speculate that the 3a protein of SARS-CoV may be partly responsible for this IFN resistance.

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Author Index

Important Telephone Numbers

ICGEB Reception	2674 1358 /61, 2674 2357 /60
NIPGR Reception	2673 5170, 2674 1751
NII Reception	2671 7121-45, 2671 7010-19 (9 lines)
Chintan Guest House	2671 7116 /17 /18 (three lines)
India Habitat Centre	4366 3333
India International Centre	2461 9431

CITY TAXI SERVICES

Meru Taxi	4422 4422 (Radio Taxi)
Easy Cabs	4343 4343 (Radio Taxi)
Kamal Taxi Service	26184353, 26191543
(Located at ICGEB main gate)	

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