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ट्रांसलेशनल स्वास्थ्य विज्ञान
एवं प्रौद्योगिकी संस्थान

TRANSLATIONAL HEALTH SCIENCE
AND TECHNOLOGY INSTITUTE

ANNUAL REPORT

2014 - 2015

Our Mission

By integrating the fields of medicine, science, engineering and technology into translational knowledge and making the resulting biomedical innovations accessible to public health, to improve the health of the most disadvantaged people in India and throughout the world.

Our Vision

As a networked organization linking many centers of excellence, THSTI is envisaged as a collective of scientists, engineers and physicians that will effectively enhance the quality of human life through integrating a culture of shared excellence in research, education and translational knowledge with the developing cohorts and studying the pathogenesis and the molecular mechanisms of disease to generate knowledge to complement the processes of designing interventions and technology development.

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- Vaccine & Infectious Disease Research Centre (VIDRC)
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- Centre for Biodesign & Diagnostics (CBD)
- Centre for Human Microbial Ecology (CHME)
- Policy Centre for Biomedical Research (PCBR)
- Drug Discovery Research Centre (DDRC)

PARTNERSHIP CENTRE

- Population Science Partnership Centre (PSPC)

EXTRAMURAL CENTRE

- Clinical Development Services Agency (CDSA)

THSTI Society

1. **Dr. G. Padmanaban**
   Distinguished Professor,
   IISc Bangalore
   President

2. **Dr. K. VijayRaghavan**
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   Department of Biotechnology
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   New Delhi
   Member Ex-officio

3. **Dr. V.M. Katoch**
   Secretary, DHR and DG ICMR,
   New Delhi
   Member Ex-officio

4. **Mrs. Anuradha Mitra**
   Joint Secretary and Financial Advisor,
   Department of Biotechnology,
   New Delhi
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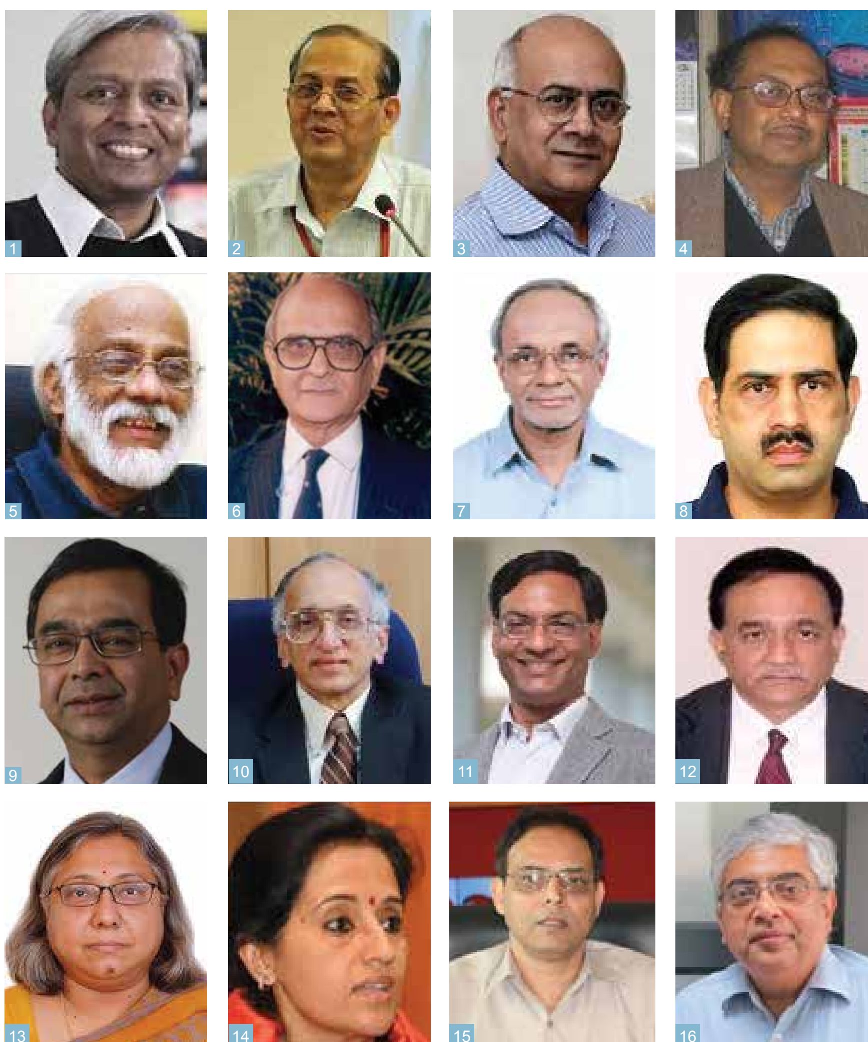
12. **Dr. G.B.Nair**
    Executive Director, Translational Health Science and Technology Institute, Gurgaon
    Member-Secretary

THSTI Governing Body



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15. **Dr. Sudhanshu Vrati**
    Dean, Academics,
    Translational Health Science and Technology Institute, Gurgaon
    Member

16. **Dr. G.B.Nair**
    Executive Director,
    Translational Health Science and Technology Institute, Gurgaon
    Member-Secretary

THSTI Leadership

G. Balakrish Nair is the Executive Director of THSTI. His prior assignment, spanning over two decades, was at the National Institute of Cholera and Enteric Diseases (NICED), Kolkata, culminating as the Institute's Director. The tenure at NICED was bridged with a seven year stint as Director, Laboratory Sciences Division, International Centre for Diarrhoeal Diseases Research, Dhaka, Bangladesh. His contributions to the discovery of *Vibrio cholerae* O139 Bengal, earned him the prestigious Shanti Swarup Bhatnagar award for Medical Sciences in 1998. In his role as the head of the organization, he has brought a unique culture of gentle reshaping of scientific minds towards translational research at THSTI. He also leads the Centre for Human Microbial Ecology at THSTI.

Dr. Sudhanshu Vrati did his MSc in Microbiology from G. B. Pant University of Agriculture and Technology in Pantnagar, DIIT in Biochemical Engineering from Indian Institute of Technology Delhi, and PhD in Biochemistry from the Australian National University in Canberra. He did his postdoctoral research at CSIRO Molecular Sciences in Sydney. Dr. Vrati is the Dean of THSTI responsible for its academic program and the Head of its Vaccine and Infectious Disease Research Centre. He is a virologist with research interests spanning the RNA virus replication, antivirals, and vaccine development. Dr. Vrati has won several distinctions including the National Bioscience Award, the NASI-Reliance Industries Platinum Jubilee Award, the Tata Innovation Fellowship Award, and the fellowship of the Indian Academy of Sciences and the National Science Academy of India.

Professor Shinjini Bhatnagar is the Dean of Clinical Research at THSTI. She served as Senior Research Scientist and Pediatric Gastroenterologist at the Department of Pediatrics, AIIMS. She is the Head of Pediatric Biology Centre and also the co-ordinator of Centre for Biodesign and Diagnostics and the National Biodesign Alliance. In her role as the Clinical research Dean, she has brought in an innovative approach of amalgamating clinical research into biological sciences to enhance translational capacity of THSTI.

Dr. Kanury Rao is the Head of Drug Discovery research Centre at THSTI. He is a faculty in the International Centre for Genetic Engineering and Biotechnology (ICGEB). A Fellow of several academies, Dr. Rao was awarded The Shanti Swarup Bhatnagar Award for Biological Sciences, in 1997. As an Adjunct Faculty in THSTI and the head of a focused product and pipeline development team of scientists, he leads the major drug discovery research program at THSTI.

Dr. Sudhakar Bangera joined CDSA with experience of 21 years in diverse areas of healthcare (Hospitals and Medical Schools), global Contract Research Organisations (CRO), Academic Research Organisation (ARO), Site Management Organisation (SMO), Medical Imaging, Clinical Bioavailability and Bioequivalence (BA-BE), and a global pharmaceutical company. He is a multifaceted, dynamic healthcare and clinical research professional leading the CDSA team at THSTI.

Mr. M.V. Santo is a HR and IR professional with a substantial blend of public and private enterprise experience. A specialist in organization building, his contributions to THSTI are in the multiple facets of administrative functions. He has built a team which contributes significantly to ensure seamless support to all aspects of scientific and academic activities at the Institute.

From the Executive Director's Desk

G. B. Nair

The core constitution of the Institute is an amalgam of disciplines and among our research staff we have biologists of a variety of kinds, physician researchers, engineers and mathematicians. In addition, we have a clinical partnership with the civil hospital in Gurgaon where some of our major studies are ongoing.

During the year under report, several interesting developments have taken place at THSTI. Early in February 2015, the Institute shifted en masse from its interim location in Gurgaon to the brand new magnificent campus in Faridabad. The shift is now completed and THSTI and the Regional Centre for Biotechnology are functioning from the new campus. The laboratories and all associated equipment have been installed and we are back to the bench and to our area of work.

THSTI has six intramural centres, one partnership centre and an extramural centre. Two of the centres of THSTI namely Vaccine and Infectious Diseases Research Centre (VIDRC) and Pediatric Biology Centre (PBC) have completed five years and after rigorous DBT conducted evaluation followed the path as chartered by the Governing body at its 8th meeting on 18th June, 2013. One more centre namely the Centre for Biodesign and in vitro Diagnostics (CBD) will be evaluated by a DBT conducted review in the course of a year. Two other centres namely Drug Discovery Research Centre (DDRC) and Centre for Human Microbial Ecology (CHME) have just completed 2 years and their performance is being reviewed. The sixth Centre of THSTI, the Policy Centre for Biomedical Research (PCBR) has been recently reviewed by the Central Advisory Board and continues to be part of THSTI. Collaborations with the Partnership Centre are escalating and the effectiveness of the extramural Clinical Development Services Agency (CDSA) is now becoming apparent in a variety of ways.

Despite the disruption of work due to shifting, there has been considerable progress at the Research front. I will not dwell on the scientific achievements because much of this annual report is about the Science done at THSTI during the ensuing year. However, what I would like to highlight is the complexities that are inherently there in a multidisciplinary setting such as THSTI. The core constitution of the Institute is an amalgam of disciplines and among our research staff we have biologists of a variety of kinds, physician researchers, engineers and mathematicians. In addition, we have a clinical

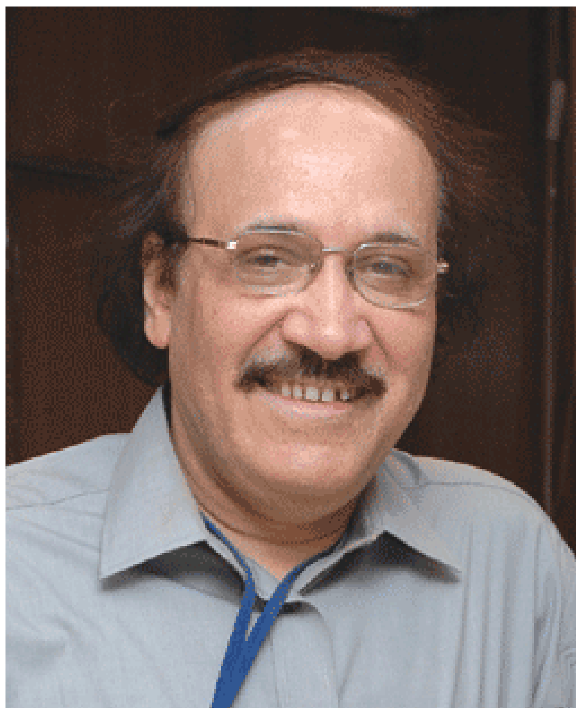
partnership with the civil hospital in Gurgaon where some of our major studies are ongoing. These partnerships need to be judiciously nurtured. To find this kind of diversity under one roof is exceptional and such diversity is the very character of THSTI. The expectations of discliplines working together within and between each other is what will accomplish the translational mandate of THSTI. This is a theoretically attractive, conceptually noble and an achievable target but the devil lies in the detail. Each of these disclipines, independently have very different course work for their students, different emoluments and allowances had they been employed in their parent discipline, different benchmarks of administration and assessment of performance and many other differences. In fact THSTI revels in differences more than similarity. The Senior Management at THSTI alongwith the SAGE led by Dr. M.K. Bhan has attempted to ameliorate and amend these differences and pave a path of consistency. Lots more needs to be done at the higher echelons of management if an exceptional Institute like THSTI is to emerge. These are serious challenges but they need to be met upfront and solved.

After serving for four years at THSTI and on the eve of my superannuation, I leave THSTI with lots of fond memories and many smiling faces. I am sure that this Institute will grow in leaps and bounds and the smiles will cascade into a tsumani of success. The country needs the produce of the research that stems from Institutes like this.

I would like to thank the SAGE, Professor M.K. Bhan, in particular, the Chair of the Governing Body, Professor VijayRaghavan and Members of the governing body and the scientists and the staff of the institute. I also wish to express my gratitude to Dr. T.S. Rao, our past nodal officer and to our very efficient present nodal officer Dr. Alka Sharma and above all to Professor VijayRaghavan, Secretary DBT for all the help and assistance. To the staff at THSTI, I can only say that THSTI is where it is because of your efforts and your teamwork.

Scientific Strategy and Advisory Group of Experts (SAGE)

Message from the SAGE Chair



Dr. M K. Bhan

Translational Health Science and Technology Institute (THSTI) completes another of its formative years. The challenges of establishing new institutes and clusters in our country in terms of resources, processes, people and the right type of infrastructure, in this case for translation science are well known to many of us.

THSTI has these and many other challenges to cope with. To begin with, the mandate is translation and the struggle is to define what that implies in the setting of a public institution. It has a centre / program based design with fear that this may create silos. What could be measures of productivity and the relative weight to publication quality, intellectual property, and pursuing mission and team based long term projects or contributing to development of new tools, platforms or technologies and products. Finding faculty with strong scientific background and translational experience and quality technical resource managers was and continues to be a challenge. The debate about policy and direction has been interesting but sustaining the discourse with strong faculty engagement without fatigue has not been easy.

As Chair, SAGE, I nevertheless look back at progress with reasonable satisfaction. THSTI adopted a distributed leadership model from the outset with a Director and several Deans. The collegiality among seniors has been exceptionally good.

There is a growing consensus, in term science and translation emphasis just as there are gray areas. The scientific and translational programs initiated so far include disease biology of high burden diseases (prematurity, neonatal, immunity), infection science, drug discovery, microbiome and disease and health, and affordable innovation through biodesign. Very notable is the fact that many of these programs represent collaboration across centres and institutions with effective coordination of large teams. There already is a flow of publications and THSTI made a significant contribution to the development of the very affordable, now commercialized rotavirus vaccine.

The scientific and translational programs initiated so far include disease biology of high burden diseases (prematurity, neonatal, immunity), infection science, drug discovery, microbiome and disease and health, and affordable innovation through biodesign.

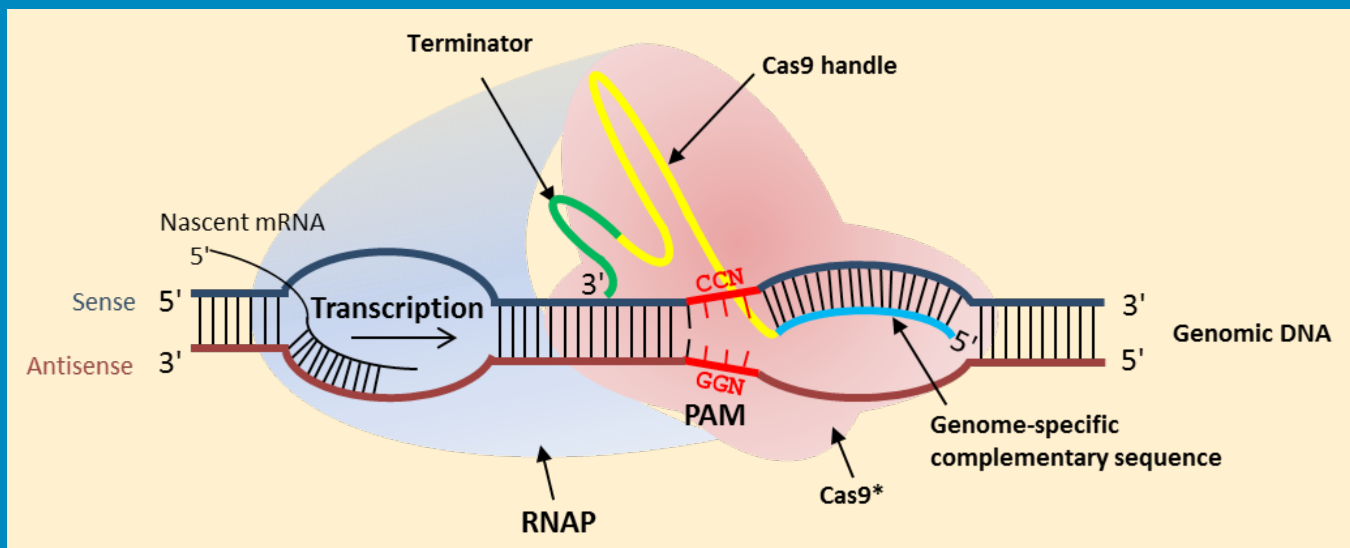
Respect for local relevance is reflected in several projects related to nutrition disorders.

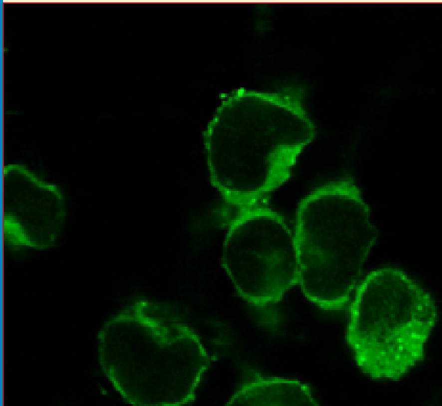
The concept of extramural units is proving to be useful with good progress on clinical development support for products developed by Indian scientist and companies and regulatory clinical trial training across the country by Clinical Development Service Agency (CDSA). The goat surfactant project of CDSA, AIIMS and Cadila laboratories is a good example of affordable innovation. The population science collaboration of THSTI with Society for Applied studies is engaged in elucidating role of gut and systemic inflammation and the gut microbiome in limiting efficacy of clinical rehabilitation of malnourished children. THSTI through its pediatric biology centre established an excellent clinical facility in a district hospital to support its interinstitutional program on genomic epidemiology and biomarker discovery related to premature birth, in renal biology and neonatal immunology with AIIMS and NII . Overall, many of these are interesting developments.

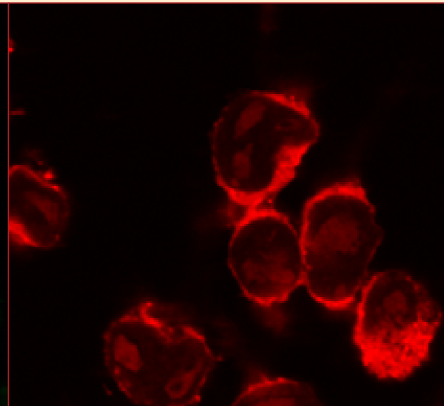
In the ultimate, translation is more about how problem informed our scientific questions and programs are more than phase I and phase II trials. Identifying targets for diagnostics, biomarkers or therapies, science driven development of difficult to make vaccines against big killers, using science to predict emerging pathogens and prepare response to outbreaks are the kind of endeavours that suits a translationally oriented institution. For a small institution like THSTI, all these are big tasks; that require bold steps and large resource.

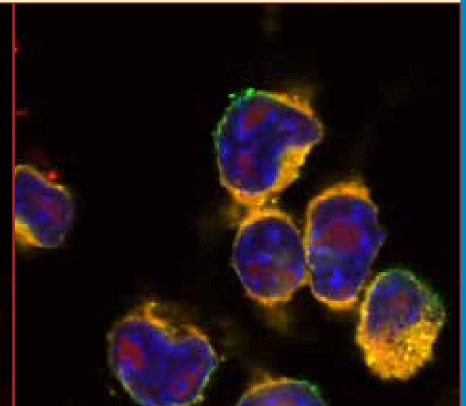
In this context, it is fortunate that THSTI is a part of the Faridabad cluster and surrounded by reputed universities, engineering and medical schools. The success of THSTI in the long run will also depend on how connecting the faculty and scholars are and the extent to which all these neighbouring institutions develop capacity for opportunity landscaping, the pillar of medical biotechnology arena. For now, THSTI, well begun. Pay as much attention to the unmet challenges as to your successes. Your journey has to be one of its own kinds. A decade from now, may you have answered for us as to how translatable indeed is the concept of the very idea of translational institutes.

Vaccine and Infectious Disease Research Centre









An Overview

Sudhanshu Vrati

Infectious diseases remain a major challenge globally and Indian population is particularly inflicted with several such infections. Vaccine and Infectious Disease Research Centre (VIDRC) has the mission to study infectious diseases and pathogens relevant to India to produce knowledge that could be translated to novel prophylactics and therapeutics. A large number of viral infections persist in India that are reported sporadically or have become endemic. In addition, several of the viral infections break out as frequent epidemics in various parts of the country. HIV/AIDS continues to be a major challenge in India and an effective vaccine is most desirable. In this context, we have a joint research program between THSTI and the International AIDS Vaccine Initiative (IAVI) aimed at developing HIV vaccine candidates. The other viral diseases that VIDRC focuses on are associated with the poor hygienic conditions prevalent in many parts of the country. Thus, studies are directed against rota- and hepatitis A viruses that are transmitted feco-orally though contaminated drinking water. VIDRC has been partnering with the Society for Applied Studies (SAS) through the Population Science Partnership Centre (PSPC) of THSTI and SAS for the clinical development of rotavirus vaccine candidates. The other viruses that are studied at VIDRC include Dengue and Japanese Encephalitis viruses, which are highly prevalent in India and are transmitted through mosquito bite. In terms of the bacterial infections, tuberculosis remains a major medical challenge for India and VIDRC is making considerable efforts to study *Mycobacterium tuberculosis*, the pathogen responsible for the disease, with a view to identify novel genes/proteins/pathways that could be potential drug targets or vaccine candidates.

Vaccine research and development at VIDRC

Our strategy for the development of the HIV vaccine candidates involves the use of broadly neutralizing antibodies (bNAbs) to engineer the antigen that could induce them. In this context our scientists are screening plasma from Indian HIV-1 (Clade-C) patients for the presence of bNAbs for isolation of human monoclonal antibodies from the elite neutralizer. A total of 200 plasma samples obtained from slow progressing HIV-1 positive donors were screened against a comprehensive Env-pseudotyped virus panel. Eleven plasma samples showed IgG-mediated neutralization breadth across different clades. Our scientists comprehensively mapped one very broad and potent neutralizing plasma sample that recognized epitopes in the viral envelope that have been reported for other broad and potent neutralizing antibodies.

Efficient cleavage of HIV envelop on the cell surface is required for preferential binding to bNAbs. This, therefore, is a desirable property for developing a vaccine immunogen. Our scientists have identified a naturally occurring Indian Clade-C envelop (4-2.J41) that is efficiently cleaved on the cell surface and binds specifically to bNAbs and not to non-neutralizing antibodies. The codon-optimized 4-2.J41 showed high level of expression of envelop on the cell surface and maintained its native conformation making it suitable for genetic vaccination. Our scientists are currently developing soluble versions of this envelop to mimic the native protein on the viral membrane to be used as an immunogen for animal studies.

Rotavirus vaccine

Over the years, scientists at VIDRC have been collaborating with the Society for Applied Studies and others on the clinical development of rotavirus vaccine 116E. The phase III clinical trial of the vaccine in a large number of infants in India found it to be safe and efficacious to prevent severe rotavirus gastroenteritis. Based on these findings the oral 116E vaccine Rotavac® was licensed, and released for use in children by the Prime Minister of India earlier this year.

PM launches **Rotavac** vaccine
Livemint - 09-Mar-2015
New Delhi: The oral rotavirus vaccine **Rotavac**, which has been developed by Hyderabad-based Bharat Biotech and the department of ...
Prime Minister launched India's first indigenously developed ...
Jagran Josh - 10-Mar-2015
PM Narendra Modi Launches Rotavirus Vaccine Developed in India
NDTV - 09-Mar-2015

Research on medically important viruses and viral infections

VIDRC scientists have been attempting to define the early biomarkers to predict the dengue disease severity and are studying the correlates of disease severity in pediatric patients. These studies are being conducted on dengue patients in collaboration with clinicians from the All India Institute of Medical Sciences, New Delhi; School of Tropical Medicine, Kolkata; and the GTB Hospital, Delhi. Our studies showed that the dengue viremia did not correlate with disease severity. However, patients with secondary infections had prolonged viremia as compared to primary infections. Severe patients had lower levels of type-I interferons and higher levels of IL-10. Our scientists are currently investigating the link between the various secreted factors and disease severity.

Safe and effective dengue antivirals are highly desirable and repurposing/ repositioning drugs found safe for human use in other conditions could be an attractive approach for this purpose. Our scientists have screened a library of pharmacologically active compounds and have identified inhibitors completely inhibiting dengue virus production in cell culture. Currently, the targets of these inhibitors in the pathways that are involved in viral replication are being identified. Targeting host factors required for the virus life cycle presents an alternate approach to dengue antiviral development. Tyrosine kinases regulate a diverse range of cellular processes and many viruses have been shown to exploit them at various stages of viral life-cycle. Using a siRNA library screen, VIDRC scientists have identified c-terminal Src kinase (Csk) as one of the kinases involved in dengue virus replication. Future efforts will

focus on understanding the mechanism of action of Csk in virus replication and how this knowledge could be exploited for dengue antiviral development.

Understanding the receptor system and the cellular entry mechanism will greatly help in designing novel antivirals. Studies at VIDRC have shown that Japanese encephalitis virus entry in neuronal cells occurs via a clathrin independent endocytic mechanism whereas in fibroblasts it is clathrin-dependent. We are using a siRNA screen to identify host membrane trafficking genes involved in virus life cycle- entry, replication and egress in human neuronal and epithelial cells. Our studies have identified GRP78 as a potential receptor for JEV on mammalian cells. We would now test if pharmacological inhibition of GRP78 can inhibit JEV infection in the mouse model.

Lack of an efficient cell culture system remains the major challenge to studying the biology of Hepatitis E virus (HEV). VIDRC scientists have developed an EGFP based replicon model of HEV in human hepatoma cells. A new virus-encoded factor, which plays an essential role in genotype-1 HEV replication by modulating the viral RNA-dependent RNA polymerase (RdRp), has been identified. This will help in establishing a better cell culture model to study HEV replication. Additionally, HEV RdRp has been purified from bacterial and mammalian cells for the development of an assay to characterize viral RNA replication. This should help in development of novel direct-acting antivirals.

Tuberculosis research at VIDRC

Availability of novel tools for rapid manipulation of mycobacterial genome would greatly facilitate the development of novel vaccine and drug candidates for countering *Mycobacterium tuberculosis* (Mtb). The CRISPRi system recently developed for targeted gene regulation in *Escherichia coli* can repress gene expression by as many as thousand-fold. VIDRC scientists have implemented the CRISPRi system in both fast-growing *Mycobacterium smegmatis* (Msm) and slow-growing Mtb-complex bacteria. By using this approach our scientists were able to efficiently repress diverse sets of genes in both Msm and Mtb-complex to negligible levels. Additionally, CRISPRi was found to be effective in knocking down specific domains of a protein in mycobacteria. These tools will allow us to rapidly test the gene essentiality in mycobacteria and thus accelerate our understanding of Mtb biology.

Very little is known about the nutritional requirements of Mtb while replicating inside the host cell. Various studies have demonstrated that cholesterol is required for the maintenance of the persistent stage of Mtb infection. We hypothesize that this carbon switch is very critical for Mtb to slow down its replication and metabolic rate thereby activating a more latent form of infection. VIDRC scientists are attempting to generate an interactome map of the regulatory pathways of cholesterol utilization in Mtb. Based on our transcriptome data and a previous study that identified genes essential for cholesterol utilization, a set of 40 genes have been identified that could possibly be critical for carbon specific regulation of Mtb physiology and metabolism. Our scientists have generated deletion knockout strains specific to 15 of these genes. Molecular and functional characterization of each of these genes is in progress. The goal here is to identify critical cholesterol catabolic pathway genes as novel target for developing a live-attenuated vaccine candidate against tuberculosis.

An important player in adaptation of bacteria to various stress conditions is polyphosphate (polyP). In bacteria, enzymes involved in polyP metabolism are polyphosphate kinase-1 and -2. VIDRC scientists have demonstrated that *M. tuberculosis* responds to various stress conditions by accumulating higher levels of polyP. A mutant of *M. tuberculosis* devoid of activity associated with these enzymes was significantly impaired for growth in guinea pigs. Similarly, *M. tuberculosis* mutant lacking the MazF toxins was highly attenuated in guinea pigs. These mutants thus are attractive vaccine candidates and we hope to test their potential in an animal challenge model.

M. tuberculosis secrets various molecules into its surroundings for its pathogenesis, besides for important physiological functions that allow it to survive in the hostile host environment. Its stockpile consists of a combination of lipids, proteins, sugars and small molecules. So far very few Mtb effectors have been characterized. In order to understand the Mtb virulence mechanisms and design novel vaccine and drug candidates it is important to identify its entire virulence artillery, delineate their host-specific functions, and define their host molecular targets. VIDRC scientists are developing tools to identify Mtb protein effectors that access macrophages. Besides the effector molecules, mycobacteria are known to shed in their surroundings membrane vesicles (MVs) packed with proteins that may be pathogenic or have important physiological functions. VIDRC scientists are engineering these MVs to incorporate the desired proteins with antigenic properties and use these as vehicle for delivering the vaccine antigens. To this end, our scientists are producing recombinant MVs from *Mycobacterium smegmatis*, a non-pathogenic mycobacterium species.

HIV Vaccine Translational Research (HVTR) Program

Bimal Chakrabarti

The HIV Env protein is responsible for cell entry and it is also the target of neutralizing antibodies. In its active form, it is composed of gp120 and transmembrane gp41 polypeptides, derived from the cleavage of gp160 precursor protein. The cleaved envelope proteins, which form a trimer by non-covalent association on the viral membrane, bind to the primary receptor CD4, followed by co-receptor, to mediate entry into host cells. A key strategy of HIV vaccine design is to identify immunogens that elicit antibodies that recognize the native Env and thus block viral entry into target cells. The recent isolation of several broadly neutralizing MAbs demonstrates that the human B cell repertoire can generate broadly neutralizing antibodies targeting Env. However, the target of these inhibitory antibodies, the HIV Env, displays a high degree of genetic and structural variability, requiring the elicitation of broadly reactive antibody responses to functionally conserved elements. Recent progress in isolating potent, broadly neutralizing monoclonal antibodies (MAbs) from HIV-infected individuals and characterization of their cognate epitopes has increased the number of potential Env antibody targets. Several of these new targets recognize trimeric Env, suggesting that in some cases it is the functional trimer that drives the elicitation of broad neutralization during natural infection.

The mission of the HVTR program is to identify candidate immunogen that elicits broadly neutralizing antibody responses against HIV-1 by establishing an innovative discovery program employing high throughput technology. This joint program between THSTI and the International AIDS Vaccine Initiative (IAVI) also envisages developing a unique set up for accelerating the effort for vaccine development for IAVI and other R&D partners, and integration of this unique facility in other labs of IAVI and appropriate R&D partners globally.

The program is currently organized to pursue the following projects through the various projects detailed below.

- Screening for cleaved functional Envs
- Development of rapid and high-throughput screening of Env immunogens
- Isolation and characterization of Broadly Neutralizing Antibodies from Indian Patients

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Screening of HIV-1+ plasma for presence of broadly neutralizing antibodies and isolation of human monoclonal antibodies from elite neutralizer (Protocol-G)

Broadly neutralizing antibodies isolated from a minority of elite neutralizers have provided clues of vulnerable targets on HIV-1 envelope (Env) glycoprotein associated with immune evasion; however, it is not known whether pathogenesis established by majority of the circulating clade C strains in Indian patients mount neutralizing antibody response against any of those known targets. A total of 200 plasma samples obtained from slow progressing HIV-1 positive donors were screened against a comprehensive Env-pseudotyped virus panel. Eleven plasma samples were identified those showed neutralization breadth across different clades. We confirmed that the virus neutralization by plasma samples was IgG mediated. We also comprehensively mapped one very broad and potent neutralizing plasma samples (G37080) that we identified through screening and found that it does recognize epitopes reported for other broad and potent neutralizing antibodies such as epitopes that form CD4 binding site (CD4bs) and membrane proximal external region (MPER) in the viral envelope. Furthermore by depletion studies using monomeric and trimeric soluble envelope as well as chimeric autologous viruses, we found that G37080 neutralizing plasma antibodies targets novel conformational epitopes in V1 loop on viral envelope. Our data provides information that will guide us to prepare suitable antigen bait in carrying out antigen-specific memory singe B cell sorting towards isolation of broadly neutralizing monoclonal antibodies.

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Identification and characterization of an efficiently cleaved Indian clade C and clade A HIV-1 Env

The envelope protein on the HIV-1 viral membrane is the primary target for a prophylactic vaccine. Env is a polypeptide of 160 KDa molecular mass that undergoes cleavage into the gp120 and gp41 subunits which form a trimer of a heterodimer to form the functional Env. It has been demonstrated that efficient cleavage of naturally occurring Env on the cell surface is a pre-requisite for preferential binding to broadly neutralizing antibodies (bNAbs) – a desirable property for developing a vaccine immunogen. We have identified a naturally occurring Indian Clade-C Env (4-2.J41) that is efficiently cleaved on the cell surface and binds specifically to bNAbs and not to non-Nabs (non-neutralizing antibodies). Furthermore, codon-optimized 4-2.J41 shows elevated levels of expression of Env on the cell surface and is able to maintain its native conformation. This suggests that 4-2.J41 is suitable for genetic vaccination wherein only a single plasmid is required. We are currently developing

soluble versions of this Env that mimics the native Env on the viral membrane using different methods - to be used as immunogen in animal studies. The clade A Env, BG505 shows a cleaved near-native trimeric conformation in its SOSIP.664 form. However full-length BG505 Env is not efficiently cleaved. Our aim is to screen and identify a clade A Env that is efficiently cleaved on the cell-surface. We have screened and identified a clade A Env (HVTR-A5) that is efficiently cleaved on the cell surface and now are in the process of extensive characterization of this Env.

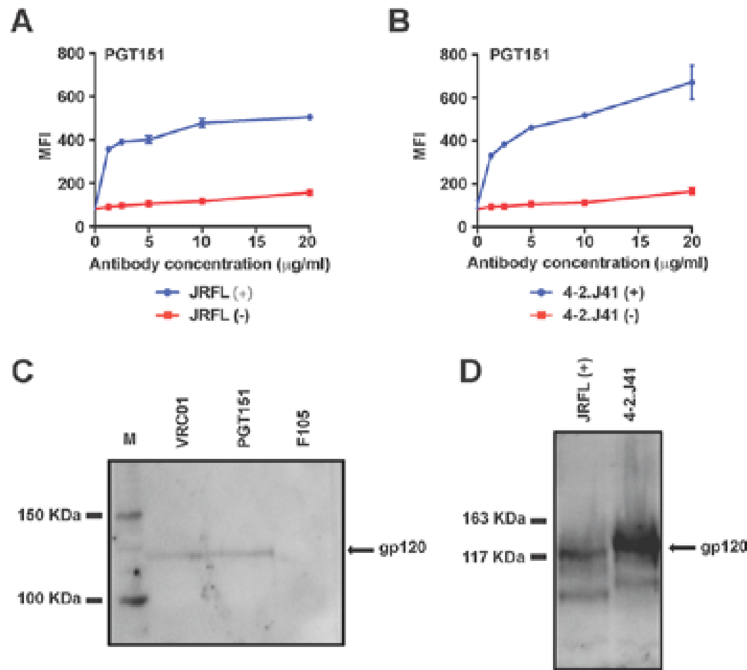

Figure 1. Efficient cleavage of 4-2.J41 Env on the cell surface. (A-B) FACS-based cell surface binding curves of wild type and cleavage-defective JRFL (A) and 4-2.J41 (B) Env to cleavage specific antibody PGT151. The wild type and cleavage-defective Envs are designated by (+) and (-), respectively. Mean fluorescence intensities (MFI) of antibody binding are shown. The graphs shown here are derived from the same representative experiments. Bars at each antibody concentration indicate the SEM values for duplicate samples. (C) Western blot analysis of immunoprecipitated 4-2.J41 Env protein from plasma membrane fraction. Proteins from the plasma membrane fraction of Env transfected 293T cells were immunoprecipitated with VRC01, PGT151 and F105 antibodies and analyzed by Western blot using HIVIG as probe. M = molecular weight marker. (D) Western blot analysis of Env glycoproteins from cell surface biotinylation. Cell surface expressed JRFL(+) and 4-2.J41 Envs were biotinylated, lysed and immunoprecipitated with neutravidin-agarose before analysis by Western blot and probed with HIVIG.

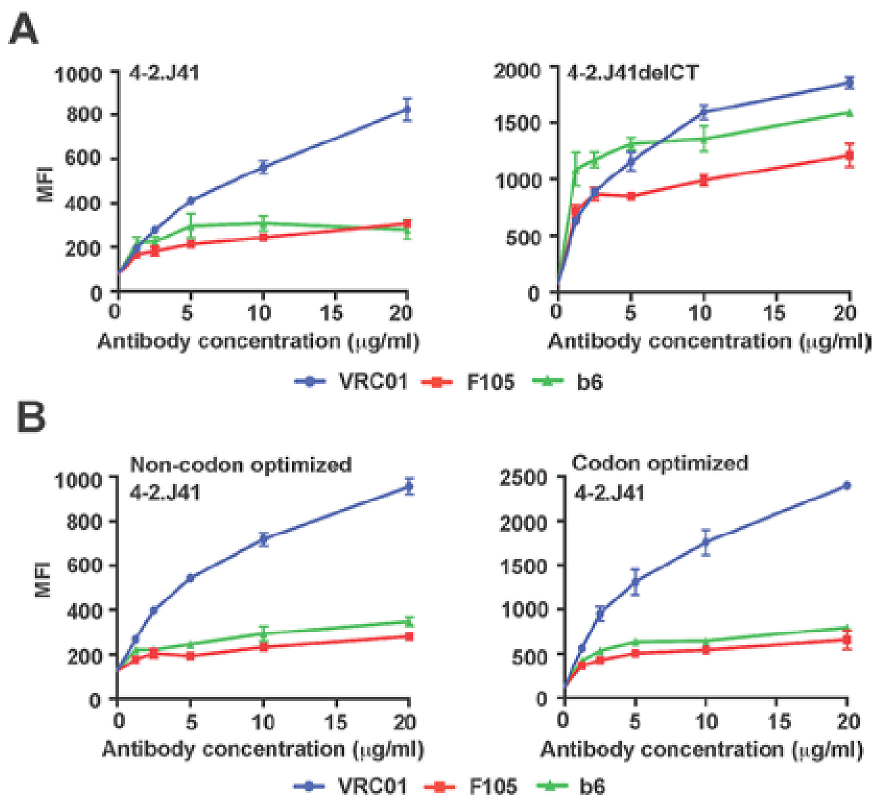

Figure 2. Effects of increased cell surface expression of 4-2. J41 Env to antibody binding. (A) Binding curves of VRC01, F105 and b6 antibodies to wild type and cytoplasmic tail deleted 4-2.J41delCT Env. (B) Binding curves of VRC01, F105 and b6 antibodies to wild-type and codon-optimized 4-2.J41 Env. The graphs shown here are derived from the same representative experiments. Bars at each antibody concentration indicate the SEM values for duplicate samples.

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A comparative immunogenicity study in rabbits of cleavage competent Indian clade-C Env (4-2.J41) or with clade-B JR-FL Env or in combination of these two by using DNA priming followed by soluble trimeric protein boost immunization format

In this project, we would like to evaluate recently identified cleavage competent Indian clade-C envelope (4-2.J41) alone and in combination with JR-FL Env for priming in the form of plasmid DNA followed by relevant protein boost. The sera from animals primed with three times DNA have been examined for the induction of nonnative trimeric Env binding antibodies. High antibody titers against JRFL Env in sera were detected by ELISA in plate coated with JRFL gp140-Foldon (FT) trimeric proteins. On the contrary, except the sera from control group and the animals immunized with plasmid DNA expressing JRFL Env alone sera from animals immunized with plasmid DNA expressing either 4.2.J41 Env or in combination with JRFL Envs showed high binding antibody titer to trimeric 4-2.J41 Env (4-2.J41-FT Env) protein as measure by ELISA in plate coated with 4-2. J41 gp140-Foldon (FT) trimeric proteins. However, the animals immunized with plasmid DNA expressing JRFL-FT DNA elicited equivalent antibody binding titer to both JRFL-FT and 4-2.J41-FT trimeric proteins. The neutralizing antibody response is currently being tested.

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To stabilize the cleaved native-like conformation of cytoplasmic tail deleted Indian clade-C 4-2.J41 Env at cell surface level

One of the immune evasion strategies of HIV-1 Env is low number of Env spikes on virion. The cytoplasmic tail of gp-41 carries trafficking signals and conserved motifs that allows internalization of Env from the plasma membrane for degradation or recycling and also alters its conformation. This project aims for rational modifications of trafficking signals and conserved motifs in the cytoplasmic tails of HIV-1 Indian clade C (4-2.J41) Env that can positively regulate Env cleaved native-like conformation, which will further help us we to derive either DNA or virion-based immunogens with greatly enhanced levels of Env trimers at cell surface. Preliminary studies in our lab have shown that when whole cytoplasmic tail (CT) of 4-2.J41 Env is deleted, unlike JRFL del-CT which binds to neutralizing antibodies and less moderately to non-neutralizing antibodies on cell surface level, CT truncated 4-2.J41Env binds to both neutralizing and non-neutralizing antibodies (higher extent).

Furthermore, we found that the cleavage is not affected in cytoplasmic tail truncated 4-2.J41 Env. To map the CT, we made several Env constructs with deletions of CT of various length and motifs. We found that the presence of a conserved hydrophilic epitope restores the Env conformation as that of wild type Env conformation on cell surface. We are currently characterizing and evaluating the mechanism, which might give us insight to design novel soluble immunogens for HIV-1 Indian clade C Env.

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Validation of characterization of role of C-terminal domain in restoration of wild type conformation for 4.2.J41 Env with deleted cytoplasmic tail (CT) at protein level

Preliminary studies and current research work done in our lab it has shown that unlike JRFL del-CT Env, which binds to neutralizing antibodies well but no or poorly to non-neutralizing antibodies; the cytoplasmic tail (CT) deleted of 4-2.J41 Env binds to both neutralizing and non-neutralizing antibodies. This project aims for the validation of rationale and to check for the effect of CT residues of 4-2.J41 in maintaining or restoring the conformation of envelops lacking CT regions similar to wild type Env at the protein level and to develop a soluble immunogen with extended broadly neutralizing epitopic region, membrane proximal external region (MPER). Different soluble constructs with varying length (of C-terminal) has been designed to validate the importance of c –terminal residues. Structural guided deletion and various mutations were introduced to stabilize the native conformation of Env. Preliminary experimental results indicated that designed constructs could express secretary protein and it could bind to most of the neutralizing antibodies tested. Furthermore, characterization of the designed immunogen is under way. Purification of the soluble protein to homogeneity is being performed using negative (non-neutralizing antibody; F105)/Positive selection (Neutralizing antibodies; PGT151) selections. Validation of role and importance of C- terminal residues in conformation stabilization, as well as for the immunogenic potential has been targeted for future directions.

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Designing of native trimeric HIV Env as candidate vaccine (with approach of fusion protein)

Soluble, trimeric Env are potential target for vaccine development. An approach of biological trimeric protein or trimeric domain as a fusion protein has been utilized to design and express native trimeric HIV Env as a candidate for successful vaccine development. In order to express HIV Env protein in its native trimeric form as successful vaccine candidate an approach of trimeric fusion tag has been targeted. Several biological trimeric proteins with known structure (www.rcsb.org) have been analyzed for this purpose with a selection criteria based on distance of closest approach between the terminals of each subunit. Four different biological trimers were selected and constructs were prepared using codon optimize YU2 sequences. Designed constructs also included the immune-dominant MPER regions along with the others with deleted MPER. Preliminary pull down experiments with different neutralizing antibodies shows that the construct binds with several neutralizing antibody, however show weak binding with cleavage dependent PGT151. Further characterization and validation of fusion protein tag HIV Env as successful vaccine candidates is going on by checking its antigenicity (binding experiments using reutilizing and non-neutralizing antibodies). Further modifications that aim to enhance immunogenic response for broadly neutralizing specificity will be applied to envelope-Fusion protein construct, and will be tested in rabbits, to determine antibody titres and neutralizing responses.

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Design, characterization and validation of native-like trimeric HIV Env as immunogen

The elicitation of broadly neutralizing antibodies (NAb) remains the primary and most challenging goal in human immunodeficiency virus (HIV)-1 vaccine development. DNA priming and protein boost with JR-FL SOSIP.R6 gp140 has been shown to elicit Ab that neutralize HIV-1JR-FL and certain other representative primary isolates in pseudovirus and/or whole-virus assays. Along with JR-FL SOSIP; soluble, cleaved BG505 SOSIP.664 Env trimer, which is stabilized and antigenically near-native in conformation provides a solid platform for the structure based immunogen design. Here we are using our recently identified clade C Env, 4-2.J41, as a target for soluble immunogen designing. 4-2.J41 reported as efficiently cleaved on the cell surface and binds to cleavage-dependent antibody, PGT151 and several Env-specific conformation dependent antibodies. Envelops with Cys-Cys (SOS) and Ile to Pro (IP) mutations has been shown previously to maintain a native trimeric conformation, however full length SOSIP conformation of 4-2.J41 shows minimal or negligible binding with cleavage-dependent antibody, PGT151 at cell surface. At soluble protein level, initial data obtain with same point mutations altered the conformation of the Env, which is evident by the binding inability of the modified Env to cleavage specific antibody, PGT151. This data prompts us to assume that upon above mentioned medication 4-2. J41 Env, the conformation of this Env is changed to non-native conformation. So, different structure guided modifications are needed to stabilize the the

core of 4.2-J41 (HIV-1, Clade C) Env so that 4-2.J41 Env forms a native like, cleaved, trimeric closed conformation. Thus gp41-gp41 trimer-stabilizing mutation screening and Cysteine tethering of the gp120 subunits to reduce the metastability of the Env core is under way. In another approach we are grafting regions from a stable Env (chimeras) to 4.2-J41 Env to stabilize the native like, trimeric closed conformation.

Stabilization of trimeric native HIV Envelope using Cys tethering approach

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After cleavage, the protomers of trimeric envelope are loosely attached with each other and also with gp41. This gives room for metastable open conformation and shedding of gp120 from the gp41 base. The objective in this proposal is to put cystein residues on each protomers of gp120 and also between gp120/gp41 regions where they are in very close proximity. Such cystein will form disulphide bond in correct folded conformation and will help in stabilizing a closed conformation like a native trimers and have the potential as immunogen candidate.

Alteration of gp41 domain of 4-2.J41 Env for stabilizing native-like trimeric structure

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The BG505.SOSIP is so far the best HIV envelope that is amenable to detailed structural studies at atomic resolution. It maintains a very stable near native trimeric conformation and binds very efficiently to native like cleavage specific antibody PGT151. But same is not true for 4-2.J41.SOSIP.664. The objective in this approach is to stabilize the gp120 core of 4-2.J41 by swapping some identified regions from BG505.SOSIP to 4-2.J41. Important trimeric core stabilizing interactions occur between residues of gp120 and gp41. Due to lack of electron density for some parts of gp41 from the structure of BG505. SOSIP.664, it is difficult to identify the key residues determinant for stable structure of BG505.SOSIP. As part of this project, we are swapping regions of gp41 from BG505 to 4-2.J41. Such a chimera will retain the core gp120 from a clade C virus (4-2.J41) that may have potential for an immunogen.

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Identification and characterization of HIV envelop surface exposed areas to be used as immunogen for successful vaccine development

Targeting regions around CD4 binding site will give us alternative approach to probably masking, inhibiting or blocking CD4 binding to gp120, which has been so far not successful by targeting CD4 binding site. We identified 3 regions around CD4 binding sites and targeted them for scaffold designing. For scaffold designing we collaborated with Dr M. S. Madhusudhan (Associate Professor, IISER Pune). Using several in-house analysis programs, *in-silico* grafting experiments as well as on the basis different biochemical parameters 3 scaffolds for the “region 1” were selected for further studies. The region 1 envelop sequences has been grafted into the scaffold protein and the probable epitope harboring scaffold proteins has been clone, overexpress and purified through bacterial expression system up to 95% of homogeneity. In order to characterize the immunogenicity and proper surface exposure of the cloned epitope-scaffold protein before immunization studies we feel the immense need of polyclonal antibodies sera against envelop-peptide. Immunize sera against peptide epitope is showing binding antibodies, further experiments in order to characterize the epitope region and its significance towards vaccine development is going on.

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Determinants of direct access of CD4i antibodies to Env CoRbs

The current model of HIV-1 entry suggests that following Env attachment to CD4 receptor, the co-receptor binding site (CoRbs) is formed by rearrangement of the bridging sheet. In this project, we are investigating whether the CoRbs can be pre-formed or accessible to CD4i antibodies (17b, X5) before primary receptor engagement and what are the determinant in the HIV-1 Env that regulate such access. We have shown that direct access to CoRbs prior to CD4 attachment varies among different Env isolates and the length of V1 loop plays a significant role in determining such access.

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Indo-South Africa Program-Identification of neutralizing antibody epitopes on Indian and South African HIV-1 subtype C viruses for HIV-1 vaccine design

Plasma samples from a total of 181 antiretroviral naïve donors chronically infected with HIV-1 from three different geographical regions in India were screened for presence of broadly neutralizing antibodies capable of neutralizing a panel of HIV-1 clade C viruses from Indian and South Africa. Clade C is the predominant strain circulating in both India and South Africa. 36/181 plasmas were found to have the broadly neutralizing antibodies out of which seven showed maximal breadth and potency. Overall, our data indicated that the Indian plasmas neutralized Indian viruses better than South African viruses and indicated presence of common neutralizing epitopes in both Indian and South African envelopes which would be focus for further research. We have obtained a number of functional HIV-1 clade C *env* genes from plasma of one of the broad neutralizer (DST-YRG2007) and currently exploiting these *env genes* in deciphering targets that vulnerable for immune evasion by the broadly neutralizing antibodies. Such information would inform rational engineering of immungen capable of eliciting antibodies capable to cross neutralize HIV-1 clade C viruses circulating both in India and South Africa.

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Screening and selection of HIV Env immunogen based on their potential to induce B cell activation

To develop a B cell activation assay**,** we have been sorting naïve B cells (CD19+IgD+) from the frozen PBMCs of the healthy donors using negative selection strategy. Purified naive B cells at the concentration of 1million/ml are stimulated with different B cell mitogens; anti human IgM with or without dextran, CpG motifs; B and P Class, and combination of IgM and Cp G motifs. After stimulation B cells are harvested at 12hrs, 24hrs, 48 hours, 64 hours and 96 hours for the expression of B cell activation (CD69, CD80, CD86 and HLADr) and proliferation (Ki67) markers. Our data shows that naïve B cells express activation and proliferation markers upon IgM stimulation at the concentration of 10ug/ml. So far we have seen 2 fold increase in the expression of B cell activation markers (CD69, CD80, CD86 and HLADr) and 5 fold increase in proliferation (Ki67) marker as compared to the expression in unstimulated cells using intracellular flowcytometery. In addition we are also trying to detect the expression of various cytokines (IL-4 , IFNg and IL-2) at the intracellular level in the B cells stimulated with IgM or and CpG motifs at different concentrations. Stimulated B cells express the above mentioned cytokines after 18 hrs, we have detected an increase of upto 30 folds in the expression of IL-4 upon stimulation with B class CpG motifs at 1uM concentration. We are in the process of optimizing the expression of activation/proliferation markers and cytokines in naïve B cells upon IgM/ CpG motifs or other B cell mitogen induced stimulation. Once optimized, we will use this system for in vitro screening of HIV envelope immunogens inducing the expression of cytokines, activation or proliferation markers alone or in combination

Rotavirus vaccine clinical development

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Rotavirus infections are estimated to cause ~527,000 deaths annually, predominantly in developing countries. In India, by the age of 5, nearly every child will have an episode of rotavirus gastroenteritis. We have been involved in the clinical development of an Indian rotavirus vaccine based on a neonatal rotavirus strain 116E. This vaccine recently completed a multicentre phase III clinical trial in India. Based on the safety and efficacy data the vaccine has been licensed and was launched for public use by the Prime Minster of India early this year. We are now conducting a phase III, randomized, double blind, placebo-controlled trial to assess non-interference of the oral rotavirus vaccine (ORV) 116E to the childhood vaccines and clinical consistency in the immune responses to the three production lots of ORV 116E.

Although commercial rotavirus vaccines are currently available and have been demonstrated to be safe and effective in low-income, high-burden populations, they are not affordable in developing countries. Serum Institute of India is developing a live attenuated bovine-human (UK) reassortant pentavalent rotavirus vaccine for oral vaccination against human rotavirus gastroenteritis in healthy infants and plans to establish the proof of vaccine efficacy.

These studies are being conducted in collaboration with the Population Science Partnership Centre (PSPC) of THSTI and the technical details of the studies are presented in the PSPC report.

Development of vaccine/gene delivery vectors

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Adenoviruses have been extensively used as model systems to study various cellular processes and as gene/vaccine delivery vectors. Although human adenoviruses are extensively tested as delivery vectors, some of the recent clinical studies reported some serious safety concerns. As animal adenoviruses are not known to cause disease in humans and that humans are not known to possess alarming levels of neutralizing immunity to animal adenoviruses, we initiated a project on the isolation of novel animal adenoviruses from domestic animals and birds, and characterize them for their suitability as delivery vectors. In this direction, we have isolated several adenovirus isolates from different animal species. Until 2013-14, we had sequenced three bovine adenovirus serotypes procured from ATCC and one novel fowl adenovirus isolate, FAV/B1-7, isolated from apparently healthy domestic fowls. We had also established the complete genome sequence of FAV/B1-7 and identified it as a member of fowl adenovirus species C through BLAST analysis.

Fowl adenovirus species C includes two serotypes, type 4 and 10. During 2014-15, an extensive bioinformatic analyses of the genome sequence was carried out to establish the true identity of FAV/B1-7 isolate. Our analyses clearly demonstrated that the FAV/B1-7 genome has undergone multiple recombination events with other isolates of FAV4 as well as with the FAV10. Precisely, intra-serotype recombination events occurred majorly in the left end of the genome, while inter-serotype events occurred at the right end. As part of its characterization, anti-FAV/B1-7 neutralizing immunity in

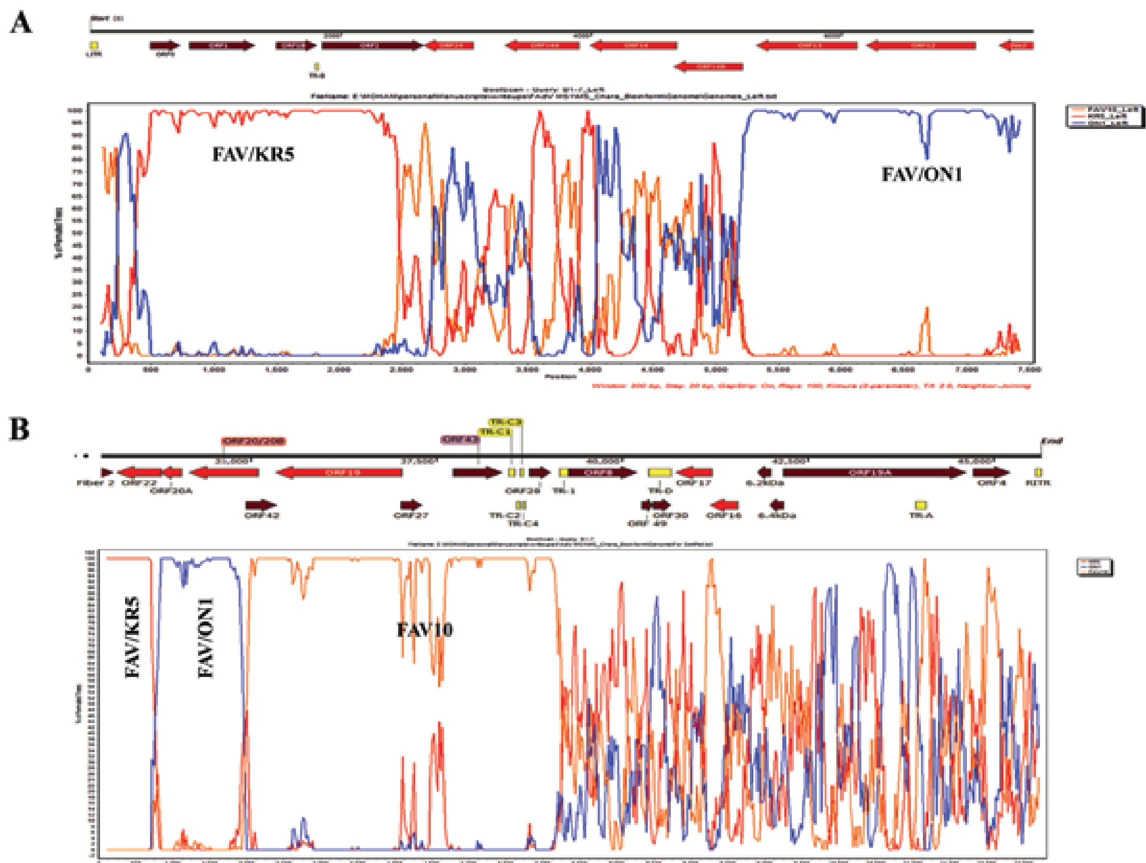

Figure 3. Recombination events in the FAV/B1-7 genome. (A) Bootscan analysis of the first 7.5 kbp region at the left end in the FAV/B1-7 genome with the corresponding sequences reported for FAV10, FAV/ON1 and FAV/KR5. (B) Bootscan analysis of the last 12.5 kbp region at the right end in the FAV/B1-7 with the corresponding regions reported for FAV10, FAV/ON1 and FAV/KR5. Organization of different genomic elements in the genome fragments analyzed in the study is represented above the graphs for easy mapping of recombination events.

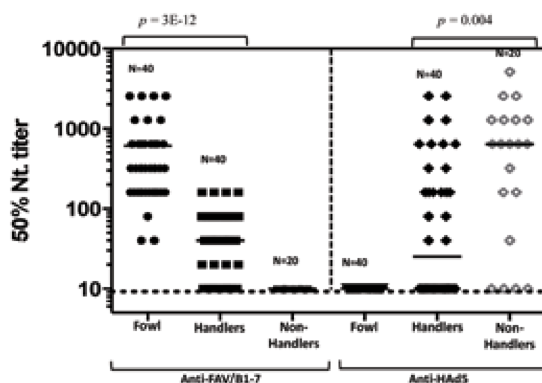

Figure 4. Seroprevalence of anti-FAV/B1-7 and anti-HAd5 neutralizing immunity. Serum samples prepared from the blood samples obtained from poultry birds, human beings working in the poultry farms (poultry handlers) and human beings from cities without known contact with the infected poultry birds (Non-handlers) were assayed for the presence of anti-FAV/B1-7 and anti-HAd5 neutralizing immunity by end-point titration assays on QT35 cells. Represented are the neutralizing antibody titers that provided ≥50% protection of the QT35 monolayers from FAV/B1-7 infection. Statistical significance is represented by p values and a $p < 0.01$ is considered highly significant.

human and fowl sera was estimated. Our data suggested that poultry workers had moderate levels of this immunity, but the levels were significantly lower compared to that present in poultry birds. Interestingly, urban population were completely naïve to the anti-FAV/B1-7 neutralizing immunity. Also, ability of FAV/B1-7 to infect and replicate in different human and animal cell types was tested, which suggested that this isolate probably has a higher affinity for cells of hematopoietic origin. During this period, a detailed transcriptome analyses of FAV/B1-7 isolate was also initiated to identify the regions for insertion of heterologous sequences. Sequencing followed by bioinformatic analyses of the RNA-seq data is completed and we are currently working on validating the bioinformatics data. With respect to the development of FAV/B1-7 based vector, our early attempts to generate an infectious clone through homologous recombination was not successful and currently we

are employing alternate approaches to achieve the same. Similarly, we are also planning to investigate the immunogenicity of FAV/B1-7 in fowl species to develop an attenuated poultry vaccine as prophylactic to prevent hydropericardium syndrome. Besides, complete genome sequencing of EAV isolate H9/NS has also been completed and currently we are in the process of resolving the ambiguities in the genome assembly. Also, the pathogenicity of the EAV/H9/NS has been tested in a mouse model and our data suggested that this isolate is only mildly pathogenic in the murine model. Similar to FAV/B1-7, the EAV isolate will also be characterized and attempts will be dedicated towards generating an infectious clone of this virus.

Biology of medically important viruses and the viral infections

A large number of viral infections persist in India that appear sporadically or have become endemic. Many of these viral infections present themselves as frequent epidemics in various parts of the country. We are interested in studying the viral infections that are associated with poor hygienic conditions such as those spreading though mosquito bites or feco-orally though contaminated drinking water. Thus, we are studying mosquito-borne viruses such as Dengue and Japanese encephalitis, and feco-orally transmitted Hepatitis E virus. Our studies are focused on the biology of the pathogen as well as the infection.

Identification of correlates of disease severity in pediatric dengue patients

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India contributes to about 30% of global dengue infections and several areas of the sub-continent are hyper-endemic with multiple serotypes co-circulating throughout the year. A cohort study to comprehensively investigate the correlation between clinical parameters, plasma factors, viral load and the innate immune response from India has been lacking. We established a pediatric dengue cohort in New Delhi and are characterizing the correlation between the viral load, thrombocytopenia, plasma cytokine profile and severe disease in pediatric patients. The Objective of the study is to evaluate the viral and immunological factors that correlate with severe dengue disease.

We enrolled a total of 97 patients in the last three years and have extensively characterized the clinical features and immune response in these patients. Table 1 shows the clinical features of the patients enrolled in the cohort. About 40% of our study patients had primary infections and 30% of these patients had severe disease as against 65% of those with secondary infection. Although dengue viremia did not correlate wit h disease severity (Figure 5A), patients with secondary infections had prolonged viremia as compared to primary infections (data not shown). Severe patients had lower levels of type-I interferons and higher levels of IL-10 (Figure 5B and 5C). Reduced IFN-γ and elevated IL-10 levels in severe cases suggest a bias towards Th2 responses in severe dengue disease. We are currently investigating the link between various secreted factors and disease severity.

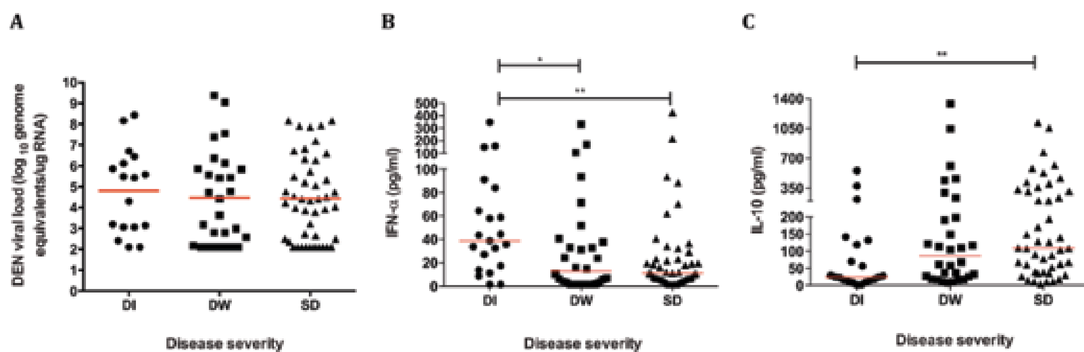

Fig. 5 Correlates of dengue disease severity. (A) Relationship between dengue genome copy numbers and disease severity at the time of admission, DI – Dengue infection, DW- Dengue with Warning signs and SD – Severe Dengue. Geometric mean value is shown. (B) Interferon-a and IL-10 levels in the plasma of dengue patients with the indicated severity were measured by multiplex magnetic bead assays. Median value of cytokines are indicated. Statistical significance was determined by Mann-Whitney test. * P< 0.05, ** P< 0.01.

Investigating the role of tyrosine kinases in flavivirus infection

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Currently there are no vaccines or antivirals available for dengue (DEN) disease. Approaches that target host factors required for the virus life cycle, instead of virus-encoded proteins, open up a new perspective to counteract viral infections and are being applied to different families of viruses. Tyrosine kinases (TKs), comprising of receptor tyrosine kinases and cytosolic tyrosine kinases, regulate a diverse range of cellular processes from cell division to apoptosis and many viruses have been shown to exploit TK signaling at various stages of viral life-cycle. Drugs targeting host TKs are in commercial use for conditions such as acute myeloid leukemia and other cancers, which suggest that TKs can be an attractive drug target in virus infections. Objective of this study is to identify human TKs involved in DEN virus infection in cell culture models.

We screened a siRNA library targeting human tyrosine kinases and identified c-terminal Src kinase (Csk) as one of the kinases involved in dengue virus replication. Cells where Csk expression was suppressed by siRNAs showed decreased amounts of dengue virus titers in the culture supernatant and reduced levels of viral RNA in infected cells (Figure 6A and 6B). The effect was not due to decreased viral entry (data not shown). This effect was also observed with Japanese encephalitis virus, a related virus from the same family as dengue virus (data not shown). Csk colocalized with viral replication compartments and the antiviral effect of Csk was independent of its regulatory activity of Src kinases. These results identified Csk as a host tyrosine kinase involved in dengue virus replication and future efforts will focus on understanding the mechanism of action of Csk in flavivirus replication.

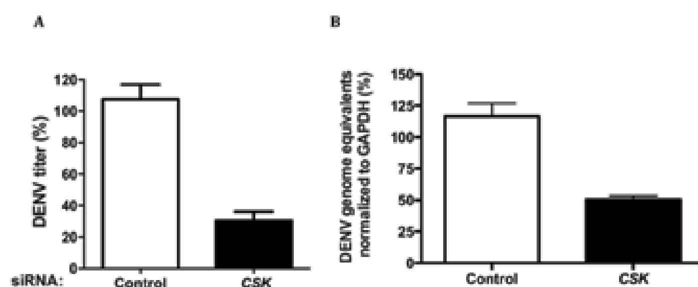

Fig. 6. Csk knock-down inhibits DENV replication. (A) Huh-7 cells were transfected with 10 nM of siRNAs targeting CSK or non-targeting control and 48 h post-transfection, cells were infected with 1 MOI of DENV2. Viral titers in the infected culture supernatants were measured at 24 h post-infection (pi) by plaque assay. (B) RT-PCR analysis to measure DENV2 RNA levels in total RNA isolated at 24 h pi from Huh-7 cells transfected with siRNAs and infected with DENV2 as described above. The data are representative of at least three experiments performed with two or more replicates and indicate mean with SEM.

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Identification of DENV inhibitors from a pharmacologically active compound library

A number of reports have identified inhibitors of dengue virus protease and polymerase by using *in vitro* assays. However, there are no candidate drugs that have progressed to clinical trials. Repurposing/repositioning drugs that have been shown to be safe for human use in other conditions for treating dengue could be an alternative approach to bring anti-dengue drugs to market. The Objective here is to identify inhibitors of dengue virus by high-throughput screening of a library of pharmacologiclly active compounds

We have screened a library of pharmacologically active compounds by using a immunofluorescence-based high-throughput screening approach for DENV2 infection in Huh-7 cells. We have identified 6 inhibitors that completely inhibit production of virus in cell culture out of which three compounds had IC_{50} values in the low micromolar range (data not shown). Two compounds inhibited dengue RNA replication as observed by reduced viral RNA levels in inhibitor-treated cells as compared to DMSO-treated controls (Figure 7A and 7B). We are in the process of identifying the targets of these inhibitors and to characterize the pathways that are involved in viral replication.

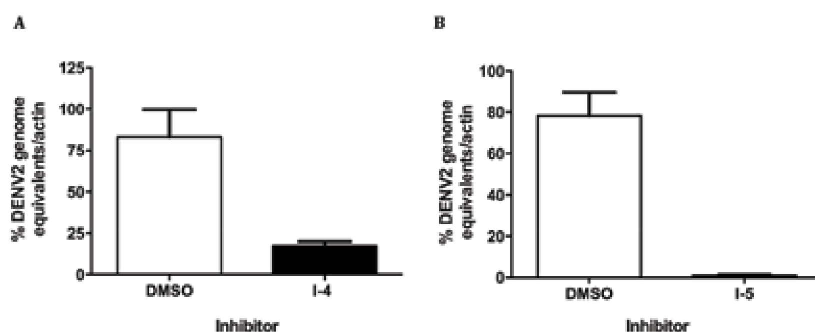

Fig. 7. Identification of dengue replication of inhibitors. (A) Huh-7 cells were infected with 1 MOI of DENV2 and grown in medium containing inhibitor I-4 or I-5. DMSO is used as a vehicle control. DENV RNA levels in total RNA isolated at 24 h pi from infected cells were measured by RT-PCR. The data are representative of two experiments performed with three replicates and indicate mean with SEM.

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Effect of viral infections on zinc homeostasis and permeability barrier functions

The inhibitory effect of zinc ions on virus infections has been reported earlier and zinc supplementation studies in humans also indicate a beneficial effect of zinc in reducing severity of certain viral infections. However, a clear understanding of the effect of virus infections on intracellular zinc metabolism, on the efflux and influx of zinc ions and intracellular storage and accumulation of zinc during viral infections has not been investigated. In addition, as zinc has been shown to play a crucial role in maintaining the permeability barrier functions, it is not clear if alteration in zinc homeostasis affects endothelial and epithelial barriers. The Objective of this study is to characterize the effect of viral infections on zinc homeostasis and study its relationship with permeability barrier functions. We have begun to investigate the modulation of zinc homeostasis in three different models of virus infections: (i) dengue virus as a model for studying endothelial barrier functions and (ii) respiratory syncytial virus and rotavirus as a model to study epithelial barrier functions. We hope that our study will lead

to an understanding of how virus infections affect zinc homeostasis and with this information we will be able to assess if changes in zinc homeostasis has any bearing on the pathogenesis and disease outcome.

Transcriptome analysis for identification of novel biomarker for disease progression in Dengue patients

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Dengue virus infection is now recognized as one of the most important mosquito-borne human infections of the 21st century. The virus is known to promote vascular permeability, cerebral edema leading to Dengue hemorrhagic fever [DHF] or Dengue shock syndrome (DSS). The global epidemiology of dengue fever/DHF is changing fast. Dengue infection is known to be endemic in India for over two centuries as a benign and self-limiting disease. In recent years, the disease has changed its course, manifesting in the severe form as DHF and with increasing frequency of outbreaks. Delhi has experienced 11 outbreaks of dengue virus (DENV) infection since 1997 with the last one reported in 2010. There are no vaccines available so far against Dengue and no biomarkers available which can help us to predict the disease outcome. The ability to predict which patient may develop DHF and DSS may improve the triage and treatment.

The focus of this project is to study the early transcriptional signature in the peripheral blood mononuclear cells (PBMCs) in a large number of clinically and virologically well characterized patients with mild and severs dengue infection and to establish their correlation with disease progression. Besides, this should help us understand host transcriptome disregulation in patients infected with different DENV serotypes. The proposal would also study differentially expressed microRNAs in PBMCs of these patients to identify those that may be specific to infection with a particular DENV serotype or related to disease severity. To our knowledge, no information is available on the microRNA profile in dengue patients and how it could control some of the transcripts that are disregulated during the course of infection. These pieces of information on a large cohort of dengue patients would prove to be valuable in identifying early biomarkers for disease severity.

Patients' whole blood samples were collected from suspected Dengue fever cases as per the WHO guidelines. PBMCs were separated from whole blood. Platelets were counted and samples were categorized into two groups according to platelet count (>50000 and <50000). So far, we have collected 262 blood samples. Among the 262 samples, 42.58% patients were positive for dengue infection. Majority of dengue positive patients were from the age group 18-35 years with a male preponderance. The most prevalent dengue strains circulating during the year 2014 (Sep-Nov) were DENV-2 (74%) with co-circulating DENV-1 and DENV-3. Among Dengue positive and negative samples 19 PBMC samples were selected for Next Generation Sequencing. Total RNA sequencing of all the 19 samples (11 Dengue positive and 8 Dengue negative) has been completed and the bioinformatics analysis of the whole transcriptome is currently under progress. We have followed up 12 Dengue positive patients and collected blood samples at 5 days interval. We are now planning to do the whole transcriptome sequences from these follow up samples.

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Understanding the role of extracellular microRNAs in mediating Neuroinflammation during viral infection

Microglia, the resident immune cells in the central nervous system (CNS) are considered the key cellular mediators of neuroinflammatory processes. Several viruses including Japanese encephalitis virus (JEV) can activate microglia and play important role in amplifying virus induced neuropathology by increasing pro-inflammatory mediators. JEV is one of the most important causes of viral encephalitis worldwide. There is no specific treatment for Japanese encephalitis (JE) and no effective antiviral drugs have been discovered. It is evident that microglia can have both neuroprotective and neurotoxic effects. Over-activation and dysregulation of microglia contribute to neuronal damage in neurodegenerative diseases. Therefore several drugs including Minocycline have been tested to control microglia over activation. Minocycline is a semisynthetic second-generation tetracycline that exerts anti-inflammatory and anti-apoptotic effects. It is reported previously that Minocycline can act as neuro-protector, can reduce microglial activation, and viral replication following JEV infection. However, the exact mechanisms remain unclear. Recently, extracellular microRNA containing exosomes have gained importance as they play vital role in cell–cell communication and act as mediators of neuroinflammation. Here, our focus is to understand the role of extracellular microRNAs released in exosome from activated microglia / neuronal cells during JEV infection. The hypothesis is that virus may use the exosomal machinery of the host for viral dissemination and induce pathogenesis. We further hypothesize that exosome released from minocycline treated microglia contains specific microRNAs and are able to modulate neuronal cell fate upon JEV infection. Using small RNA deep sequencing, we will try to identify microRNAs signature in the exosomes released from JEV-infected as well as minocycline-treated cells and assess their role in neuron protection. So far, we have developed human microglia and neuronal cell line that can release GFP labelled exosome in their culture supernatant. We have standardized and characterized the exosome released from uninfected and JEV-infected cells. Now, we are doing the experiments to check for the presence of viral components in exosomes and studying the content of the exosome (mainly microRNAs) released from uninfected and JEV-infected cells. We have collected the human CSF samples from acute encephalitis cases (both JEV and non-JEV etiology) and currently doing microRNA arrays on them.

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Identification of the Japanese Encephalitis Virus Attachment and Receptor System

Binding of a virus to its specific receptor is a key event that initiates infection. Identification of the virus receptor can aid in the development of anti-virals that can block virus infection at the first step. To identify the JEV receptor we have employed the JEV-Envelope Protein Domain III (ED3) as an exploratory system. The envelope (E) protein of JEV folds into three structural domains, and the third domain (ED3) mediates viral attachment to the host cells, and carries epitopes that elicit a neutralization response. JEV-ED3 was expressed and purified using a bacterial expression system. The recombinant JEV-ED3

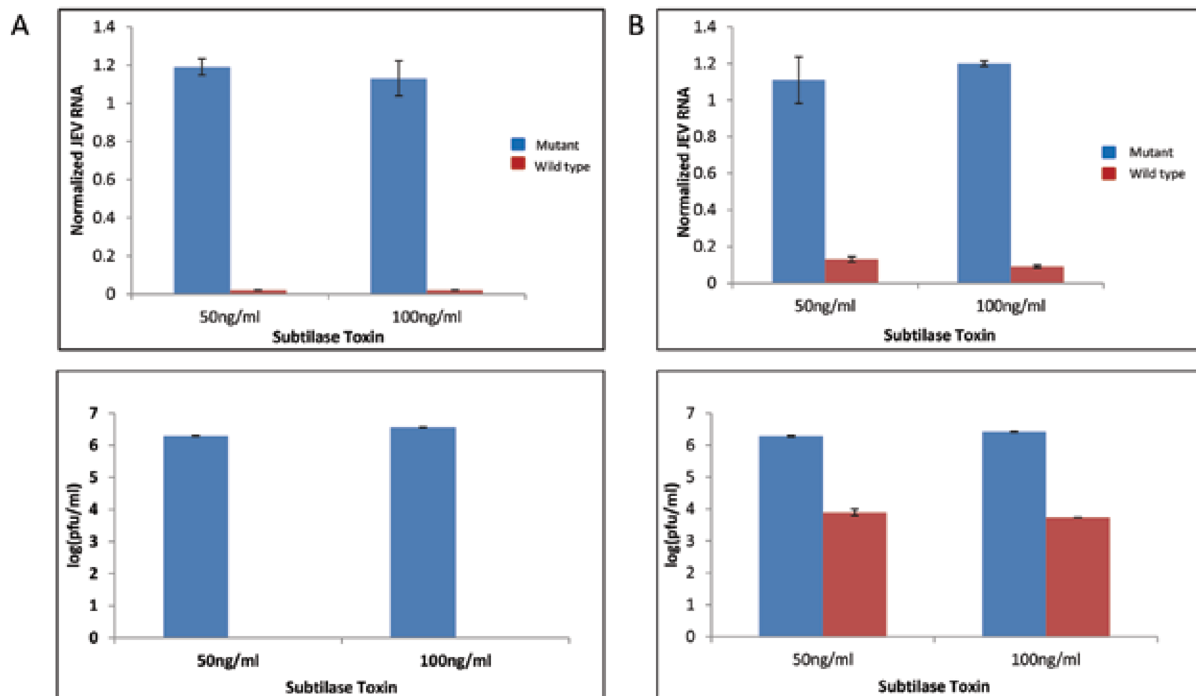

Figure 8. Clevage of GRP78 by Subtilase toxin inhibits JEV replication and production of infectious virions. JEV infected Neuro2a cells were treated with wild type (brown bars) and mutant Subtilase toxin (blue bars) at (A) 1h.p.i and (B) 6h.p.i. JEV-RNA levels were quantified at 24h.p.i by RT-PCR (upper panels) and virus titres were monitored by plaque assays (lower panels). The wild type Subtilase toxin cleaves GRP78 and results in decreased virus replication while the mutant toxin has no effect. The inhibition by the toxin is most pronounced when it is added early after infection.

binds to the surface of host cells and can compete for infection by JEV, thus establishing it as a valid tool to initiate receptor studies. Biochemical Studies to find interacting partners of JEV-ED3 were done and membrane proteins that specifically interact with JEV-ED3 were resolved on 2D gels and specific proteins/spots were analysed by Mass Spectroscopy. These experiments identified GRP78 as a JEV-ED3 binding protein. GRP78 (glucose-regulated protein of 78 kDa) is traditionally regarded as a major ER chaperone facilitating protein folding and assembly, protein quality control and regulating ER stress. GRP78 is also expressed on the surface of cells where it regulates cell signalling and cell viability. GRP78 also serves as a co-receptor for Dengue virus serotype 2 and Coxsackie virus. Our studies indicate that GRP78 is expressed on the surface of several cell lines. Antibodies directed against GRP78 block JEV infection highlighting the possible role of GRP78 as a virus receptor. The interaction between JEV-Envelope and GRP78 was further validated by Mammalian 2- hybrid studies. Docking studies between GRP78 and JEV-E were done. By these studies we have been able to identify putative interacting residues between these proteins. We have established the role of GRP78 in both virus entry and as a chaperone for virus replication and egress. Depletion of GRP78 by RNA interference inhibited virus entry significantly validating the role of GRP78 as the virus receptor. Virus infection leads to transcriptional upregulation of GRP78. We have also employed the Subtilase AB toxin derived from Shigatoxigenic E.coli that can cleave GRP78 into 44-kDa and 28-kDa fragments, corresponding to the N-and C terminal regions of the protein. As a control the Subtilase AB toxin mutant that lacks this catalytic activity was used. Following treatment with subtilase toxin

JEV replication and production of infectious virus particles was substantially suppressed indicating that GRP78 also plays an important role in virus life-cycle at the post-entry step. [Figure 8] We plan to further extend our studies and test if pharmacological inhibition of GRP78 can inhibit JEV infection in mouse model.

Role of microRNAs in establishment of Japanese Encephalitis Virus infection and disease progression

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Microglia are the CNS-resident macrophages that play an important role in both the innate and adaptive immune responses in the CNS. Microglia function in normal and diseased CNS can be regulated through small (~22 nucleotide) non-coding RNAs, called microRNAs. Japanese Encephalitis Virus (JEV), a leading cause of viral encephalitis in South East Asian countries, can use microglia as long term reservoir and may cause changes in microRNA (miRNA) and mRNA profiles. These changes in the global microRNAome may play important role in determining the pathology of encephalitis caused by JEV infection. In order to comprehend the global changes in host microRNAome, we profiled the cellular miRNA and mRNA expression using Affymetrix microarray platform, at multiple time points during JEV replication in human microglial cells. *In silico* analysis revealed a phased pattern of miRNA expression, associated with JEV replication and provided unique signatures of the infection. Target prediction and pathway enrichment analysis identified biological relevant pathways including TLR, JAK-STAT signaling pathway, complement cascade, apoptosis, NGF pathway, cholesterol biosynthesis and NOTCH signaling pathway in microglia.

Our mRNA array data suggested an up-regulation of NOTCH-1, DLL-1, JAG1 and Hes1 expression upon JEV infection (Fig. 9A). This was further validated by qRT-PCR (Fig. 9B). Notch mRNA expression was also upregulated in mice infected brain tissues (Fig. 1C). We further examined activated NOTCH (NICD) expression in control and JEV infected mice brain. Immunofluorescence images showing NICD expression in activated microglia labeled with Iba; (green). The expression is intensely augmented both in the cytoplasm and nucleus after JEV infection compared with the control (Fig. 9D). Activated NOTCH expression was also elevated in infected mice brain as shown in Fig. 1E. Transcription of IRF8 is directly regulated by NOTCH pathway. We have previously reported that IRF8 is overexpressed during JEV infection in CHME3 cells. Here, we further showed that IRF8 expression was also increased in JEV infected mice brain. Together, these results suggested that NOTCH pathway is activated during JEV infection.

Recent reports suggested that a subset of miRNAs, termed as NeurimmiRs, co-exist in the brain and peripheral organs. These miRNAs can affect both neuronal and immune functions and thus constitute important therapeutic targets for those diseases that affect both the immune system and brain functions. Among them, miR-155 and miR-146a are multifunctional and widely reported to modulate different stages of innate immune response during inflammation and infection. Since JEV is a neurotropic virus it is likely that NeurimmiRs play an important role in virus replication and immunopathology. Using a global miRNA array we have identified differentially expressed NeurimmiRs in human

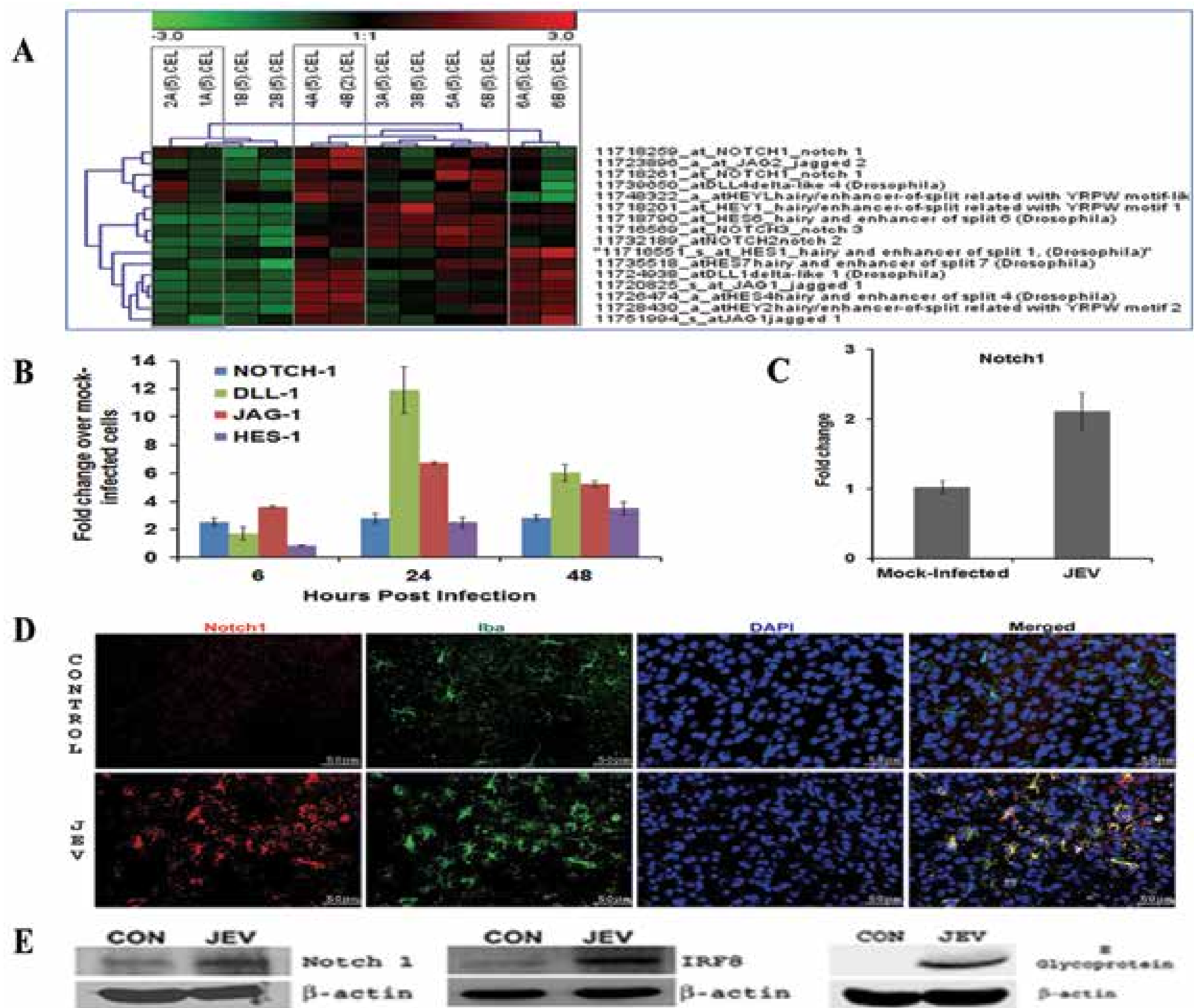

Figure 9. NOTCH pathways is activated during JEV infection. **(A)** Heat map image of NOTCH pathways genes as observed in microarray. ***(B)*** Validation of NOTCH 1 receptor, ligand DLL1, JAG1 and their target HES1 expression in JEV infected CHME3 cells at three different time points by qRT-PCR. All qRT-PCR data are represented as means ± SD. Each graph represents mean absolute fold change of triplicate experiments for each mRNAs at three individual time point as compared to mock infected controls. ***(C)*** Elevated NOTCH1 mRNA expression is shown in JEV infected mice brain as compared to mock infected mice by qRT-PCR. ***(D)*** Activated NOTCH expression is enhanced in infected mice brain tissue as compared to control mice. Immunohistochemistry was done on infected mice or control brain section. Activated microglia is stained with iba (green), activated NOTCH (NICD) is stained with red and DAPI is used to stain nucleus. ***(E)*** Western blot analysis of activated NOTCH, IRF8 and JEV are shown in infected mice brain lysate. Mouse Actin is used as internal control.

microglial cells during the course of JEV infection. Of these, we have focused our study on miR-155 and miR-146a and have investigated their effect on JEV replication and their role in the modulation of microglia-mediated innate immune response during JEV infection. For this purpose, in vitro studies were performed in JEV-infected human microglial CHME3 cells. Our results indicate that miR-155 induction might have a beneficial role for the host by limiting JEV replication through modulation of microglia-mediated innate immune responses.

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Cellular entry mechanisms of Japanese encephalitis virus

A complex network of endocytic pathways is operational at the eukaryotic plasma membrane, which can be exploited by pathogens to gain entry into a permissive cell and establish infection. The route of virus entry can differ between cell types. In addition to utilizing the already-operational endocytic pathways, in several cases viruses can induce pathways conducive to entry by receptor binding and signaling events. For flaviviruses, the receptor mediated endocytic pathway has been shown to be preferred internalization route, as low pH of the sorting endosome facilitates viral uncoating and fusion. However, endocytic pathways display extensive cross-talk with respect to molecular players and cargo sorting and recent studies have demonstrated that a high degree of plasticity exists in eukaryotic cells. We are interested in defining the endocytic pathways utilized by JEV for entry into cells in terms of key molecular players. Studies in our lab have shown that JEV entry in neuronal cells occurs via a clathrin independent endocytic mechanism. By using fluorescently labeled virus particles, a combination of pharmacological inhibitors, RNA interference (RNAi), and dominant-negative (DN) mutants of regulatory proteins involved in endocytosis we established that JEV infects fibroblasts in a clathrin-dependent manner, but it deploys a clathrin-independent mechanism to infect neuronal cells. The clathrin-independent pathway was shown to require the scission molecule- dynamin and plasma membrane cholesterol. Virus binding to neuronal cells leads to rapid actin rearrangements and an intact and dynamic actin cytoskeleton, and the small GTPase RhoA plays an important role in viral entry. We are further extending

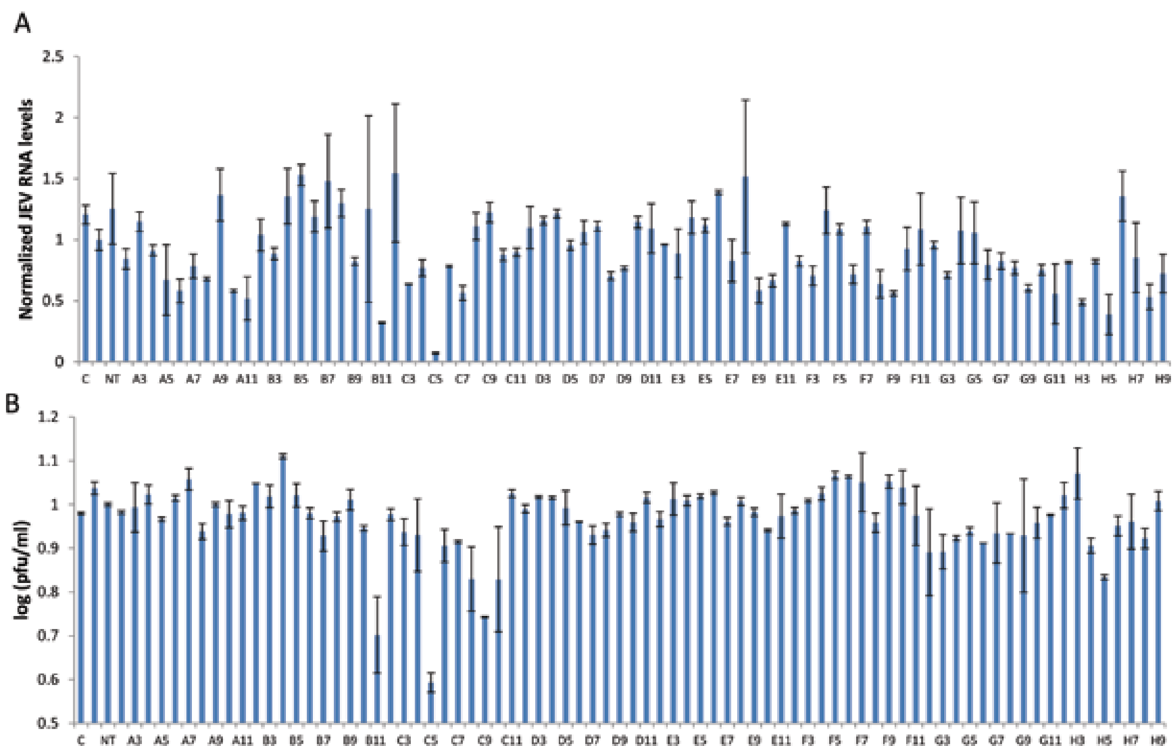

Figure 10. siRNA based screen of membrane trafficking genes inhibiting the JEV life-cycle. HeLa cells were transfected with non-targeting (NT) or specific siRNA (72 genes) for 36 h followed by infection with JEV for 24 h. JEV-RNA levels were calculated by qPCR (A) and production of virus particles was calculated by plaque assays (B)

these studies to identify host membrane trafficking genes involved in Japanese Encephalitis Virus life cycle- entry, replication and infectious virus particle production, by RNA interference screen (of 144 membrane trafficking genes) in human neuronal and epithelial cells.

The RNAi screen was standardized in HeLa (epithelial) cells and IMR-32 (neuronal) cells. A total of 72 genes have been tested for their role in JEV life-cycle in HeLa cells. Several hits have been obtained implicating a crucial role of these genes in the virus life-cycle. [Figure 10] We now plan to test all the 144 genes in epithelial and neuronal cells for their role in the virus life-cycle.

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Role of the host autophagy pathway in the Japanese Encephalitis Virus infection process

Autophagy is an important cellular process that maintains cellular homeostasis. Autophagic cargo such as long-lived cytoplasmic proteins and dysfunctional organelles are sequestered by double-membrane vesicles (autophagosome) and are degraded after autophagosome-lysosome fusion. The autophagic mechanism is constitutive and generally operates at a basal level in all cells, but is up-regulated in response to extracellular or intracellular stress, and pathogen infection. It is also an important component of the innate and adaptive immune response against a variety of viral and bacterial pathogens. Viruses can abrogate and/or exploit the autophagic process to enhance their replication or transmission.

We have examined the role of autophagy in the life-cycle of JEV. We observe that JEV infection leads to induction of autophagy in several cell types. Quantitative real-time PCR shows that JEV infection leads to transcriptional upregulation of key autophagy genes and accumulation of autophagic vesicles in the cell. To elucidate the role of autophagy in the JEV life-cycle we employed cells where autophagy was disrupted by depletion of key autophagy genes. We observed that JEV replication was significantly enhanced in neuronal cells where autophagy was rendered dysfunctional by ATG7 depletion, and in Atg5 deficient Mouse Embryonic Fibroblasts (MEFs), resulting in higher viral titers. Autophagy was functional during early stages of infection however it becomes dysfunctional as infection progressed resulting in accumulation

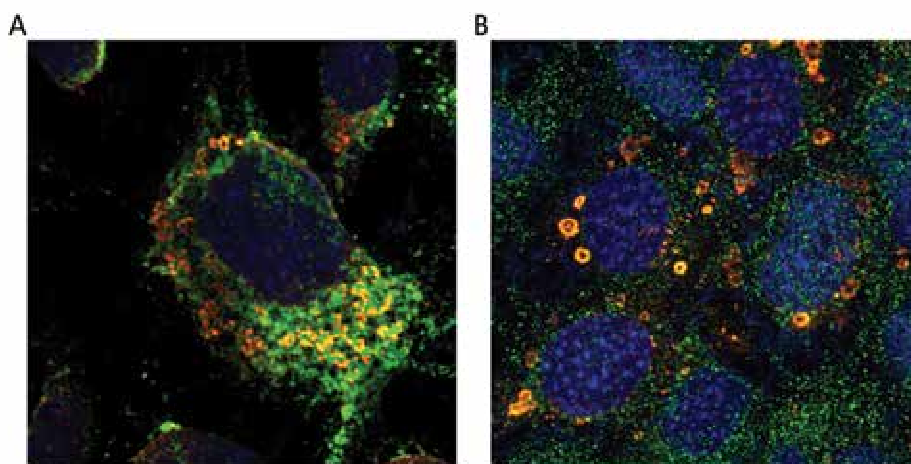

Figure 11. Autophagy is anti-viral for JEV and autophagy deficient cells show enhanced JEV replication. (A) *atg*5-/- mouse embryonic fibroblasts (MEFs)infected with JEV. Replication complexes are stained with JEV- NS1 (red) and NS3 (green) antibodies. (B) Autophagy proteins play autophagy independent roles in JEV life-cycle. *atg*5-/- fibroblasts showing JEV replication complexes (red) overlapping with autophagy protein LC3 (green). Nuclear stain DAPI (blue).

of misfolded proteins. Autophagy deficient cells were highly susceptible to virus induced cell death. We also observed that JEV replication complexes that are marked by Non-structural protein 1 (NS1) and dsRNA colocalized with endogenous LC3 but not with GFP-LC3. Colocalization of NS1 and LC3 was also observed in Atg5 deficient MEFs, which contain only the nonlipidated form of LC3. Viral replication complexes furthermore show association with marker of the ER Associated Degradation (ERAD) pathway- ER Degradation Enhancing α-Mannosidase-like 1 (EDEM1). Our data suggests that virus replication occurs on ERAD derived EDEM1 and LC3-I positive structures referred to as EDEMosomes. While silencing of ERAD regulators EDEM1 and SEL1L supressed JEV replication, LC3 depletion exerted a profound inhibition with significantly reduced RNA levels and virus titers. While autophagy is primarily anti-viral for JEV and might have implications for disease progression and pathogenesis of JEV, nonlipidated LC3 plays an important autophagy independent function in the virus life-cycle. [Figure 11]

Our current studies are focussed on determining the autophagy trigger in response to JEV infection and the mechanism of the anti-viral role of autophagy. We observe that two major cellular responses of JEV infection- oxidative stress and ER stress contribute to autophagy induction. Expression of virus non-structural proteins can also independently induce autophagy. JEV induced autophagy is mTOR independent as pharmacological inhibition of mTOR does not inhibit but rather enhances virus production, a phenomenon that is recapitulated in autophagy deficient cells. We also observe key differences in transcriptional activation of innate immune markers in wild-type and autophagy deficient cells in response to JEV infection. We plan to extend our studies to establish the links between autophagy and innate immune responses to JEV infection, and to check if pharmacological induction of autophagy can exert a protective effect in JEV mouse model.

Role of cellular endoplasmic reticulum stress pathways in flavivirus replication

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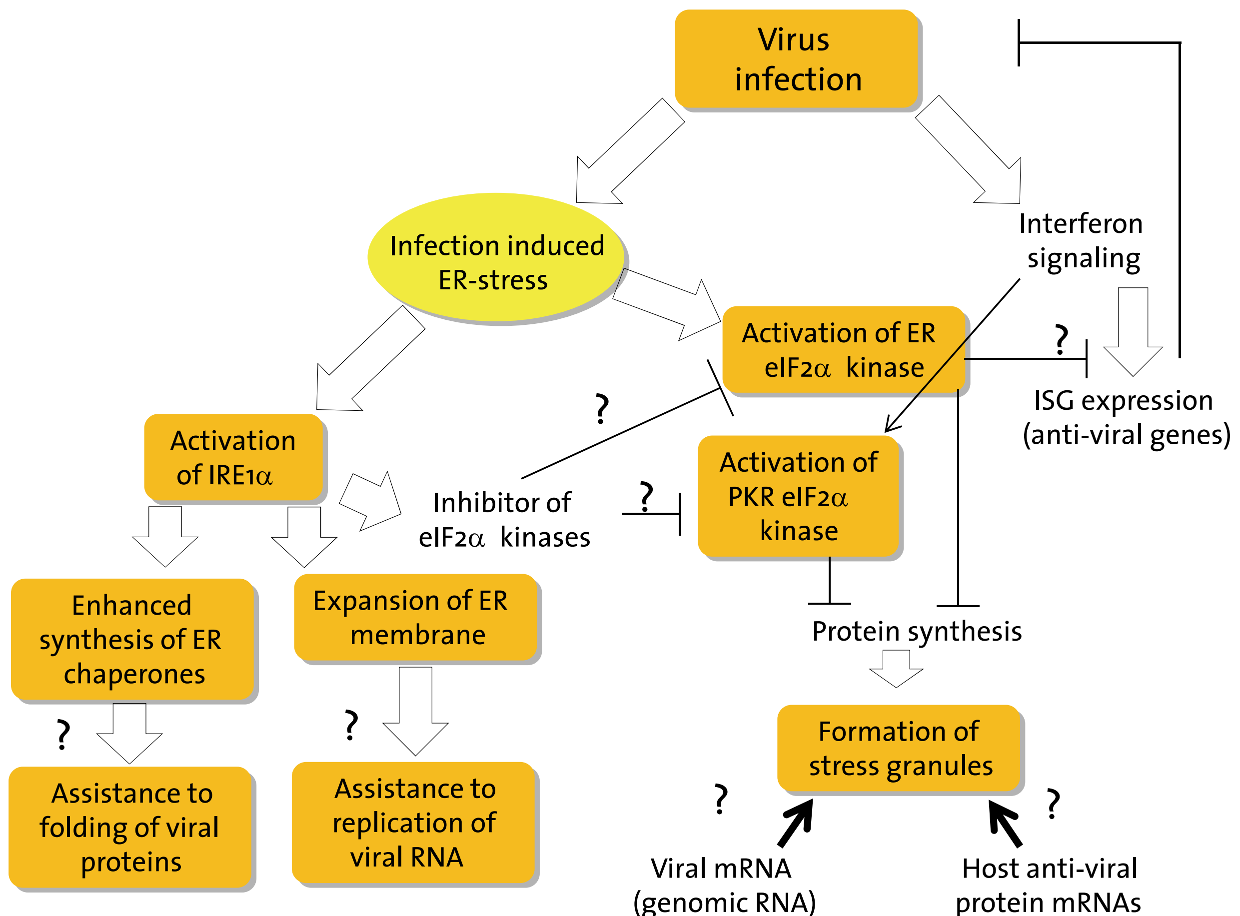
Infection by flaviviruses (JEV and DENV) induces stress pathways in host cells, which have characteristics of the host unfolded protein response (UPR). The primary purpose of UPR is to restore cellular homeostasis failing which the cell is committed to an apoptotic death. The cellular and molecular changes that ensue as part of UPR can have either pro-viral or anti-viral function. The potential pro-viral functions include transcriptional and/or translational inhibition of the synthesis of specific anti-viral proteins, expansion of the ER-membrane which provide the platform for assembly of flaviviral replication complexes etc. The possible anti-viral functions include inhibition of synthesis of the viral proteins, degradation of ER-resident viral proteins etc. UPR is initiated through detection of unfolded proteins in the ER-lumen by three ER-membrane resident sensors, followed by their activation. The downstream signals that emanate from these activated sensors execute the cellular and molecular changes mentioned above. An inhibition of one of these sensors (IRE1alpha) showed its activation to have a positive effect on the synthesis of viral proteins through mechanism which is not yet clear. Activated IRE1alpha performs cytoplasmic splicing of the *Xbp1u* mRNA thus generating a new

transcript which is translated to produce a transcription factor (TF), XBP1. One of the targets of the XBP1 TF is p58[IPK], which is known to inhibit kinases that phosphorylate eIF2alpha. The merits of this hypothesis to explain the above observation is currently being tested.

The UPR pathway involves synthesis and/or activation of multiple TFs, some of which generate TF cascades. The genes targeted for regulation by most of these TFs and their precise role in either homeostatic or apoptotic efforts, are not known yet. Our results show regulation of multiple innate anti-viral genes by activation of the UPR sensors. However, it is not yet clear as to what role the proteins coded by these anti-viral genes play in replication of flaviviruses. In addition to protein coding genes we observed differential regulation of certain non-coding RNAs which include a few long non-coding RNA (LncRNA) genes in addition to microRNAs, following activation of these UPR pathways. The role that these might play in viral replication is not known yet.

Currently efforts are on to characterize the effect of activation of the UPR pathways in different infection model systems. Our results have indicated that the pro- and anti-viral functions, of some of the molecular changes during UPR, probably balance each other. Further, it seems that different flaviviruses have evolved to utilize this balance in different manner. Future studies would involve a detailed characterization of these pathways and their precise effect on the synthesis of viral proteins and/or replication of viral RNA.



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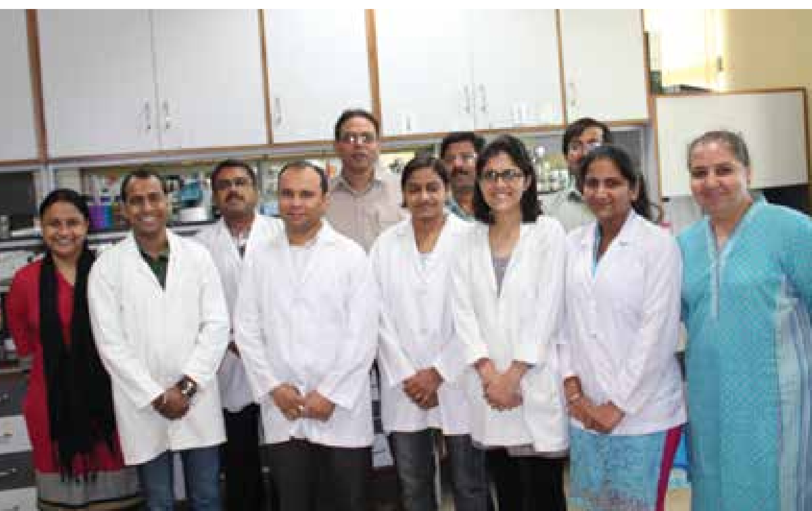
Role of host proteins interacting with flavivirus RNA in viral replication

Virus infection causes a multitude of stresses in the host cells, which respond by mounting highly conserved molecular and cellular changes. In mammalian cells, different types of physiological stresses lead to the formation of stress granules (SGs) in the cytoplasm. SGs are RNA-protein complexes without any limiting membrane and serve as a temporary repository of the mRNAs, the initiation of translation on which has been inhibited by stress. The composition of SGs includes mRNAs in association with ribosomes, translation initiation factors and other unique proteins like G3BP etc. Following the subsidence of the stress stimulus that lead to formation of SG, these mRNAs are relocated to polysomes, where they again serve as template for protein synthesis. Formation of SGs has a negative influence on either synthesis of viral protein or replication of viral nucleic acid, which is why many viruses have evolved mechanisms to inhibit formation of SGs in the infected host cell. Further, viruses from different genera have evolved to utilize proteins that form crucial components of SGs, to aid replication of their genome.

The genomic RNA of flavivirus is of the plus-sense and is single-stranded. Therefore, it can directly serve as a template for synthesis of the viral proteins, three serving structural and seven serving non-structural functions. Following synthesis of these proteins, initiation of translation on the plus-sense genomic RNA is terminated, and instead it serves as the template for transcription of the negative-strand viral RNA, catalyzed by the virus encoded RNA-dependent RNA polymerase (non-structural protein 5 or NS5). The negative strand RNA subsequently serves as the template for transcription of multiple copies of the plus-sense genomic RNA, which are packaged with the structural proteins (envelop or E, membrane or M and core or C) before secretion of mature virus particles through the host secretory pathway. The genomic RNA contains a single open reading frame (ORF) flanked by untranslated regions (UTRs) on either side. Among these, the 3'UTR has been shown to play a major role in influencing efficiency of viral RNA replication through specific interactions with multiple host proteins. Interestingly, many of the host proteins that interact with viral genomic RNA have been shown to be components of SGs. This has given rise to the hypothesis that through an unknown mechanism the interaction of the viral RNA with these proteins prevents their natural function *i.e.* to form SGs in infected cells. Therefore, interaction with the SG proteins seems to serve dual purposes in the viral life cycle, by inhibiting formation of SGs and playing a crucial in the replication of viral RNA. Pour research here is directed to find new interacting partners of the viral negative strand RNA and in deciphering the role of the known interacting proteins in greater detail. An understanding of the dependence on host proteins for efficient replication of viral RNA could be utilized to develop unique therapeutic strategies based on the principle of disrupting such interactions.



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Construction of the protein interaction map of Hepatitis E virus to understand the virus life cycle and identify crucial targets for therapeutic intervention

Protein-protein interactions (PPI) are essential for relaying information and maintaining physical integrity of an organism. Since viruses depend on host cells for survival, interaction between virus & host proteins are crucial for survival of the virus. No report exists regarding the network of PPI among Hepatitis E virus (HEV) and host proteins except for one on ORF3 protein. We propose to establish a map of PPI network among HEV and host factors, which will advance our understanding of the molecular mechanism of HEV life cycle & unravel new targets for anti-HEV intervention strategy.

We intend to identify direct and indirect interactions among HEV and host proteins by following two different approaches: Yeast two hybrid library screening and Affinity Purification-Mass spectrometry, using HEV proteins as bait. These data will be assembled to construct the map of protein-protein interaction network between HEV and host factors. Relevance of these interactions during the life cycle of HEV will be initially evaluated using the HEV replicon model developed in my laboratory. Depending on the outcome, further studies will be planned to identify crucial targets for therapeutic intervention.

Following the human fetal brain cDNA library screening, 52 host proteins have been identified as direct interaction partner of HEV proteins. Pathway and function wise enrichment of HEV interacting partners were analyzed by the web-based software “PANTHER”. Pathway analysis of HEV Human brain library interacting proteins unraveled that HEV manipulates various host cellular processes like apoptosis, immune system, metabolic and cellular machinery (Figure 12) with more than 50 % of them involved in host cellular and metabolic pathways.

Functional classification of HEV interacting proteins revealed that significant number of proteins are categorized under membrane trafficking, nucleic acid binding, enzyme modulator, cell adhesion and many other functions with

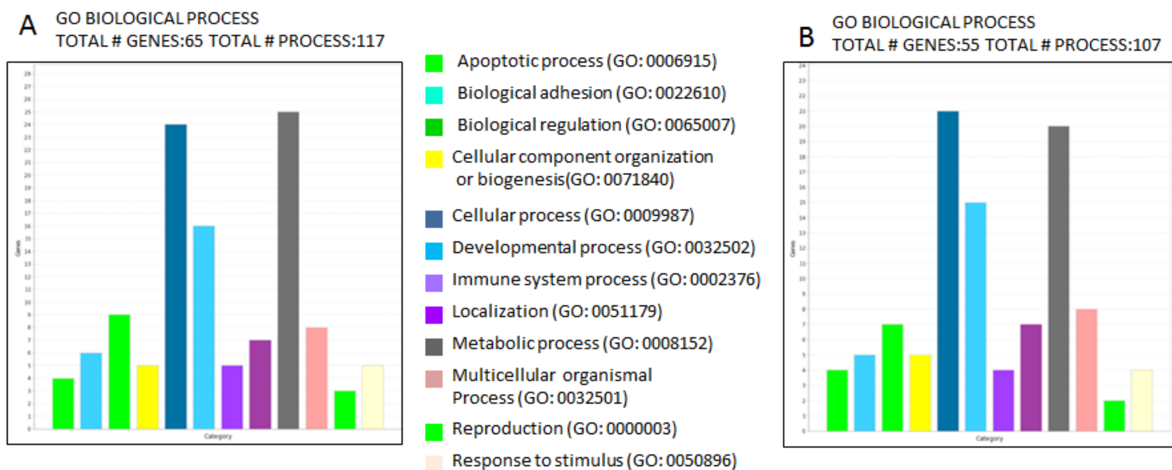

Figure 12. Pathway analysis of HEV targeted Human proteins. Pathways are displayed in the X-axis and number of interacting proteins is represented in Y-axis A: Total population, B: curated.

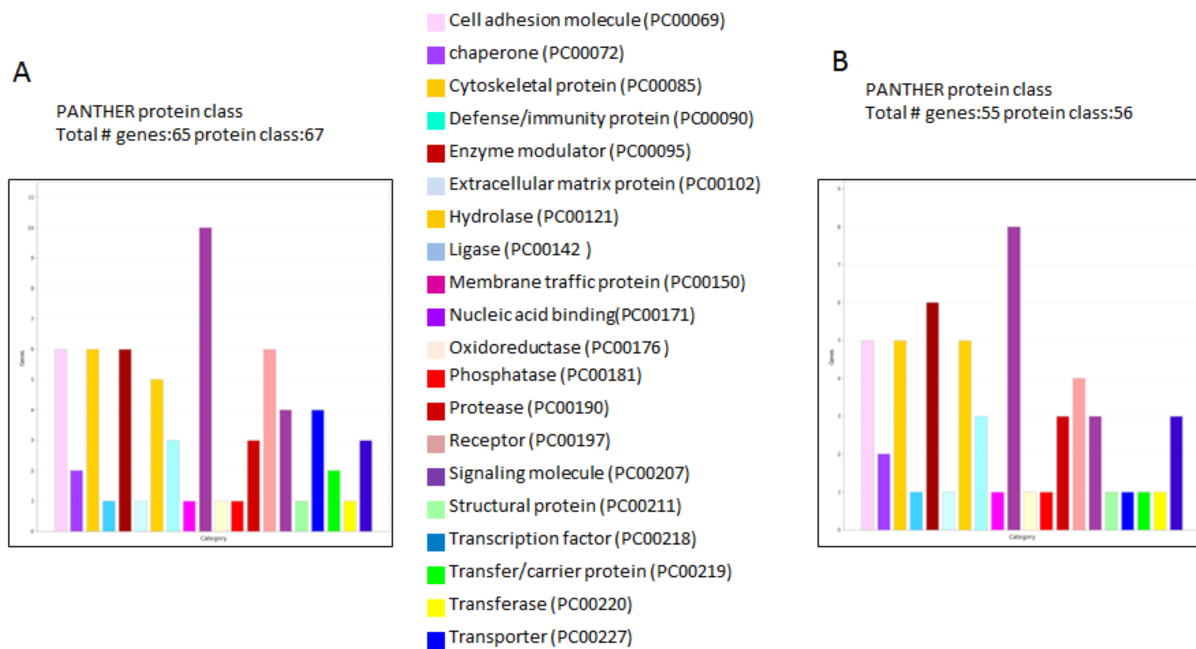

Figure 13. Functional classification of HEV interacting Human proteins. Functional category are represented in the X-axis and number of interacting proteins is represented in Y-axis. A: Total population, B: curated

the major population of nucleic acid binding proteins (Figure 13). Pathway and functional analysis of both curated (only coding sequence “in frame” with Gal4-AD) and total population (only coding sequence “in frame” with Gal4-AD activation and 5’UTR+ coding sequence “in frame” with Gal4-AD) gene population did not show significant difference in terms of pathway enrichment. This suggests that even genes with 5’UTR sequence may be bonafide interacting partners, which remains to be experimentally verified.

Towards developing an efficient model system for studying HEV life cycle: exploring the mechanisms dictating poor replication efficiency of genotype-1 HEV.

Investigators
Vidya Padmanabhan Nair
Saumya Anang
Subramani Chandru
Milan Surjit

Despite sharing similarity in genome organization and encoding similar proteins, genotype-3 HEV replicates more efficiently in cell culture system than genotype-1 HEV. We have been exploring the possible mechanisms underlying this discrepancy using a variety of biochemical and molecular biology techniques. Our study has revealed a new virus encoded factor, which plays an essential role in genotype-1 HEV replication by virtue of its ability to modulate the activity of viral RNA dependent RNA polymerase. Ongoing work aims at further characterizing the function of this protein and utilize the information thus obtained in establishing a better laboratory model of genotype-1 HEV.

Investigators
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Sheetal Kaul
Milan Surjit.

Establishment of a mammalian cell culture based HEV expression system to study the viral life cycle

One of the major hurdles in studying genotype-1 HEV life cycle (which is more prevalent in India) in the laboratory is attributed to lack of an efficient cell culture based or small animal model system. Two different host systems are being explored for establishing a cDNA based model of genotype-1 HEV infection, which include (A) mammalian cells (such as human hepatoma cells), (B) Yeast (Saccharomyces Cerevisiae). Up to now, we have been successful in establishing a EGFP based replicon model of HEV in human hepatoma cells. These cells (named ORF2-Huh7 HEV-EGFP) stably express EGFP fused HEV genome (central part of ORF2 coding sequence is replaced with that of EGFP) and flag-tagged ORF2 from two different expression cassettes. Upon replication of the viral genome, EGFP is expressed, as indicated by green fluorescence. Trans-expressed ORF2 allows encapsidation and release of the EGFP encoding genome to the culture medium, which can be quantified by ELISA using antibodies against ORF2 and Flag. These secreted virions are able to infect normal Huh7 cells, although at a lower efficiency. As it allows performing two independent assays to monitor viral replication and release, this replicon model would be instrumental in studying the mechanism of HEV life cycle as well as for screening anti-virals against HEV. Ongoing work aims at further characterizing the model.

Investigators
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Milan Surjit

Expression in yeast (Pichia pastoris) and evaluation of antigenic potential of recombinant virus like particles (VLP) of Hepatitis E virus

No vaccine against HEV is available in India and most of the world except for China, where a bacterially expressed recombinant HEV VLP based vaccine is commercially available. Though suggested to be efficient, protective potential of Chinese vaccine remains to be thoroughly established. We have generated Pichia clones, which secrete bulk of the major capsid protein (ORF2) of HEV in a methanol inducible manner. Immobilized metal affinity chromatography mediated purification followed by biochemical characterization revealed this protein to be N-linked glycosylated and assemble into VLPs. Ongoing studies aim at further purifying this protein and evaluate its antigenic potential. If successful, Pichia expressed HEV VLPs would not only be useful for studying the biology of HEV but also find application in HEV diagnostics and vaccination studies.

Interference of innate immunity response by hepatitis E virus

The outcome of any virus infection depends upon the nexus between viral processes and the cellular innate immune responses. Viral RNAs harbor molecular signals known as pathogen associated molecular patterns (PAMPs) that are detected by innate immunity receptors to activate a suite of cellular

Investigators

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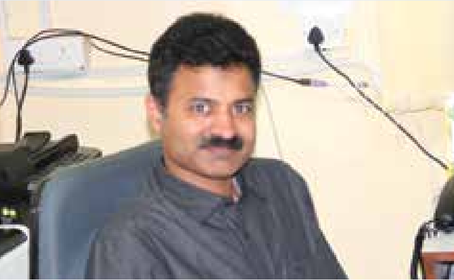

Ranjith Kumar

defense responses. Viruses are known to have evolved strategies to interfere with the host responses either by blocking recognition of molecular patterns or by cleaving adaptor proteins.

We are interested in characterizing biological role of the HEV proteins during viral infection with special focus on their involvement in interfering with innate immunity response. We observed that innate immune receptor RIG-I was activated by HEV RNA. The role of HEV proteins in interfering with RIG-I signaling was investigated and we observed that the papain-like cysteine protease (PCP) blocked RIG-I mediated interferon production.

Even though PCP was suggested to be a protease, its protease activity was not involved in the inhibition of RIG-I signaling. Instead, PCP physically interacted with one of the adaptor proteins required for activation of RIG-I response. In addition to PCP, HEV capsid protein also inhibited innate immune response. Further characterization of how these proteins interfere with host innate immunity is underway.

Investigators

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Collaborator

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Characterization of hepatitis E virus RNA-dependent RNA polymerase and its associated proteins in the replicase complex

HEV is a difficult virus to cultivate in cells. Absence of efficient cell culture system has long hindered HEV research and replicon mediated approaches has been the primary method used to study HEV replication. However, these approaches have its limitations especially to study mechanism of action and regulation of RdRp. Unlike RdRps from hepatitis C virus and poliovirus, there is very little information available on the mechanism of action of HEV RdRp.

The RdRp is an essential enzyme found in all RNA viruses and hence is a potential target for drug design and development. The viral replicase complex, which is responsible for the replication of the viral RNA, is formed by the association of RdRp with other viral non-structural proteins and some of the host proteins. In spite of the critical role of RdRp in the viral life cycle, the replication of HEV is poorly understood. Understanding viral replication will be crucial for the development of more effective antivirals.

We have purified HEV RdRp from bacterial and mammalian cells and are in the process of developing a non-radioactive assay to characterize viral RNA replication. Furthermore, role of HEV encoded structural and non-structural proteins along with host proteins involved in regulation of RNA synthesis are being identified and characterized. Understanding the viral replicase complex will provide important information on the replication of HEV and help in development of better direct acting antivirals.

Investigators
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Screening of small molecule compounds to identify inhibitor(s) of hepatitis C virus (genotype 3a) RNA-dependent RNA polymerase

Hepatitis C virus (HCV) is a global pathogen and according to World Health Organization, it affects more than 180 million individuals worldwide. In India, nearly 12.5 million individuals are suffering from HCV infection, with more than hundred thousand people getting infected every year. Chronic infection of HCV is one of the leading causes of liver cirrhosis and hepatocellular carcinoma. The high rate of chronicity along with the lack of successful vaccine makes HCV a serious health issue. Effective antiviral drug for the treatment of HCV remains a serious need for the entire world in general and for India in particular. Since HCV genotype 3a is the most prevalent form of HCV in India we focused on its RNA dependent RNA polymerase (RdRp) for developing antivirals. We established a cell-based assay to characterize HCV genotype 3a RdRp in mammalian cells and screened small molecule compound library with the goal of identifying HCV 3a RdRp specific inhibitor. Upon screening around 3500 compounds, about 10 compounds were found to inhibit the RdRp at a concentration lower than 10 μM.

The efficacy of these compounds on HCV replication was tested on a replicon-based assay system and we identified one compound that showed concentration dependent inhibition of HCV replication without any cytotoxic affects. Biochemical analyses with recombinant HCV RdRp suggested that the compound did not directly interact with the viral polymerase and probably interacted with a host protein required for viral replication. Furthermore, this compound inhibited hepatitis E virus (HEV) replication suggesting that the host protein may be important for multiple hepatotropic viruses. We are in the process of further characterization of this inhibitor.

Biology of Mycobacterium tuberculosis

India has the highest burden of tuberculosis (TB). The World Health Organization (WHO) statistics for 2013 gives an estimated TB incidence of 2.1 million cases for India out of a global incidence of 9 million. It is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent rather than active TB. We are studying the biology of Mycobacterium tuberculosis, the TB causative agent with a view to identify novel genes/proteins/pathways that could be potential drug targets or vaccine candidates.

Investigators
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Understanding the role of polyphosphate kinases and polyphosphatases in physiology of Mycobacterium tuberculosis.

Stringent response is one of the regulatory mechanism by which bacteria adapts to poor nutrient conditions through the production of various alarmones such as guanosine pentaphosphate ((p)ppGpp) which regulates various metabolic processes and bacterial virulence. Another important player in adaptation of bacteria to various stress conditions is polyphosphate (polyP). In bacteria, enzymes involved in polyP metabolism are polyphosphate kinase - 1 (PPK-1) which catalyzes the reversible transfer of the terminal ( ) phosphate of ATP to form polyP and polyphosphatase (PPX) that processively hydrolyzes the terminal residues of polyP to liberate Pi. Polyphosphate kinase -2 (PPK-2) is another enzyme involved in polyP metabolism that drives synthesis of GTP and ATP using polyP as phosphate donor. In this project proposal we have demonstrated that *M. tuberculosis* responds to various stress conditions by accumulating higher levels of polyP. *M. tuberculosis* possesses a single homolog of *ppk-1* and we have generated Δ*ppk1* mutant strain of *M. tuberculosis*. The *ppk-1* mutant strain exhibited negligible levels of intracellular polyP, decreased expression of *sigF* and reduced growth in stationary phase. The polyP deficient strain showed survival defect in nitrosative stress and in THP-1 macrophages as compared to the wild type strain. In addition, polyP accumulation was also observed upon exposure of mycobacteria to various drugs. Deletion of *ppk-1* in *M. tuberculosis* genome significantly reduced the number of persisters in the presence of isoniazid or levofloxacin. The mutant strain survived as well as the wild type strain in oxidative stress and acidic conditions. Our results suggest that polyP accumulation is required for persistence of *M. tuberculosis invitro* and it plays an important role in physiology of bacteria residing within human macrophages and guinea pigs. The mutant strain was significantly impaired for growth in both spleens and lungs of infected animals at both 4 weeks and 10 weeks post-infection.

We have also biochemically characterized polyphosphate kinase – 2 and polyphosphatase proteins of *M. tuberculosis*. We have shown that PPK-2 enzyme utilizes polyP as substrate for the synthesis of GTP. We have also observed that accumulation of PolyP is associated with improved survival of *M. tuberculosis* under low oxygen growth conditions. In guinea pig experiments, we observed that *ppk-2* mutant strain of *M. tuberculosis* was impaired for growth in both lungs and spleen, with thie growth defect being more prominent in spleens. The genome of *M. tuberculosis* possesses two homologs of polyphosphatase enzymes, Rv0496 and Rv1026. Using DAPI and malachite green based assays we show that both these enzymes are exo-polyphosphatases and show substrate specificity towards long-chain polyphosphates. We have also constructed both single and double mutant strains of *M. tuberculosis* devoid of activity associated with these polyphosphatases and have shown that the double mutant strain was significantly impaired for growth in guinea pigs. We also demonstrated that polyP accumulation in *ppx* double mutant strain impairs its ability to survive upon exposure to drugs as well as to form biofilms. Future experiments includes using microarray, mass-spec and biochemical pull-down studies to identify pathways regulated by these polyP levels. We are also planning to evaluate the efficacy of these attenuated strains as an

alternative vaccine against *M. tuberculosis*. We have also standardised 96-well assay systems in the lab and screening experiments are in progress to identify small molecule inhibitors for polyphosphate kinases and polyphosphatases enzymes from *M. tuberculosis*.

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Investigating the role of TA modules in Pathogenesis and Persistence of M. tuberculosis

Bacterial drug-tolerance is reported to result from lower metabolic requirements for processes that characterize actively growing cells such as transcription, translation, replication and cell wall synthesis. An attractive hypothesis for the origin of these persisters is that they arise from stochastic over expression of endogenous regulators of macromolecular synthesis in a subset of cells. The best studied of these systems are the “toxin-antitoxin” (TA) modules found within many prokaryotic genomes. These modules are generally expressed from a bicistronic operon wherein the upstream gene encodes an unstable antitoxin and the downstream gene encodes a stable toxin. The antitoxins neutralize their cognate toxins by forming tight protein-protein complexes that abrogate toxicity of toxins if both modules are present in equimolar concentration. TA systems can be broadly subclassified into 5 different classes depending on the nature of antitoxin component (RNA or protein) and their mode of regulation. Bioinformatic analysis revealed that genome of *M.tuberculosis* $H_{37}Rv$ encodes >80 TA modules belong to either MazEF, RelBE, ParDE, CcdAB, HigBA and VapBC. In contrast, the related obligate intracellular parasite *M. leprae* appears to have lost all functional toxin genes due to the unchanging nature of the niche *M. leprae* occupies in the human host. Several of these TA modules have been bio-chemically characterized but very little is known about the role of these TA modules in physiology of *M. tuberculosis*. In our lab we are focusing on functional characterization of MazEF and VapBC family of TA systems.

Using anhydrotetracycline based expression systems we have shown that overexpression of 3 MazF homologs induces bacteriostatic effect in *M. tuberculosis*. No growth inhibition was observed when we overexpress the remaining 6 MazF homologs. We also report that these MazF toxins are differentially regulated under various disease relevant stress conditions and upon exposure to anti-tubercular drugs. In order to understand the role of these MazF toxins in survival of *M. tuberculosis* under various disease relevant stress conditions and drug induced persistence, we have constructed single, double and triple mutant strains of *M. tuberculosis* devoid of activity associated with MazF toxins. We observed that triple mutant strain was impaired in its ability to survive in oxidative and nutritional stress. We also observed that growth kinetics of parental strain and MazF triple mutant strain was comparable in macrophages at different time points. For animal experiments, guinea pigs were infected with various strains of *M. tuberculosis* via aerosol route and intracellular bacteria were enumerated at 4 weeks and 8 weeks post-infection in lungs and spleens. Aerosol infection was carried out such that it resulted in implantation of 100 – 200 bacilli at Day 1 post-infection. We observed that deletion of these MazF toxins significantly impaired the growth of *M. tuberculosis* by 10-fold in both lungs and spleens of guinea pigs

at 4 weeks post-infection. This growth defect in the survival of MazF triple mutant increased to 50-fold at 10 weeks post-infection in both tissues.

Using Atc inducible vector systems, we have functionally characterized VapC homologs from *M. tuberculosis*. We observed that despite the presence of PIN domain these homologs differ from each other in their ability to inhibit growth of *M. tuberculosis*. These VapC homologs are also differentially regulated upon exposure to various stress conditions or drugs. Using temperature sensitive mycobacteriophages, we have generated few mutant strains of *M. tuberculosis* devoid of activity associated with these individual VapC toxins. Our preliminary results suggest that these mutant strains displayed similar growth kinetics in liquid cultures and were attenuated for growth in lungs and spleens of guinea pigs. Future experiments include to construct other VapC deficient strains from *M. tuberculosis* and to compare the survival of parental, mutant and complemented strain in guinea pigs and upon exposure to various stress conditons. We are also performing RNA-seq and Mass-Spec analysis to identify targets for these ribonucleases. The identified mRNA targets for these ribonucleases would be validated in our *in vitro* transcription assays.

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Screening of synthetic compounds for anti-mycobacterial activity and identification of Novel mycobacterial drug target pathways

The situation of TB has worsened due to BCG failure to combat adult TB and emergence of various drug resistant strains of *M. tuberculosis*. In the lab we are also screening of librarues to identify novel scaffolds and validating newer drug target pathways for *M. tuberculosis*. The scaffolds tested for anti-tubercular activities are;

***Diamine derivatives*:** Out of twenty-seven compounds tested, four compounds having substitution at *p*-position on the aromatic ring exhibited activity with MIC_{99} value ranging from 12.5 - 25 µM. Diamine derivative having *i*-propyl group substitution at *p*-position was found to be the most potent among all the tested compounds with MIC_{99} value of 12.5 µM against *M.tb* $H_{37}Rv$ strain.

Isoniazid derivatives: Most of the isoniazid-amidoether derivatives exhibited potent activity against *Mycobacterium tuberculosis* H37Rv strain with MIC_{99} values ranging from 0.39 to 3.125 µM *in vitro*. Five compounds were equally potent to the reference compound isoniazid. The most active compound was also evaluated for *in vivo* activity and significantly reduced the bacillary load in both lungs and spleen. However the effect was more pronounced in case of spleen. We have also evaluated anti-tubercular activity 1,2,3-triazole derivatives of isoniazid. Most of the compounds (synthesized in the present study) exhibited potent activity against *Mycobacterium tuberculosis* H37Rv strain with MIC_{99} values ranging from 0.195 to 1.56 µM *in vitro*. One compound showed better *in vitro* activity than the reference, whereas five compounds were equally potent to the reference compound isoniazid. The cytotoxicity of these compounds was studied against THP-1 cell line and no toxicity was observed even at 50 µM concentration. The compound with most potent *in*

vitro activity was evaluated for *in vivo* activity in murine model of tuberculosis and we observed that daily dosage of 25mg/kg significantly reduced the bacillary load in both lungs and spleen at 10 weeks post-treatment. However this clearance effect was more pronounced in the case of spleen. Based on these studies, future studies would involve further optimization of these molecules which may lead to the potent anti-tubercular scaffold.

Bile acid amphiphiles: In a collaborative study with Dr. Bajaj, anti-tubercular activity of 32 bile acid amphiphiles featuring different hydrophilic and hydrophobic cationic charged head groups using four different bile acids, Lithocholic acid, Chenodeoxycholic acid, Deoxycholic acid and Cholic acid was evaluated. We present a new concept that antimycobacterial activity of amphiphiles is driven by their hydrophobic interactions with mycobacterial lipids. We report that hard-charged hydrophobic amphiphiles interact with mycobacterial trehalose dimycolates and penetrate through rigid mycobacterial membranes via barrell-stave mechanism. Contrastingly, primary amine amphiphiles specifically inhibited growth of *E. coli/S. aureus via* electrostatic interactions. We report that fine tuning of the charge head group of amphiphiles modulates their specificity against bacterial membrane and might be a useful strategy for designing of more specific amphiphiles to combat drug resistant bacteria.

Screening of libraries: In another project we have screened nearly 5,000 compounds in our whole cell based assays. Based on these screening efforts, we have identified scaffolds that are more potent against slow growing mycobacteria in comparison to fast growing mycobacteria and *E. coli*. These observations suggest that these scaffolds target a metabolic pathway that is specific for slow growing mycobacteria. The exact MIC_{99} values for some of these compounds is <1 μM, with the most active compound has a MIC_{99} value of 300 nM. Most of the compounds (~30) were observed to be non-cytotoxic in THP-1 cells even at 100 μM concentration (highest concentration tested in the study). Based on these readings, therapeutic index (MIC_{99}/TC_{50}) was calculated for all these 40 compounds and compounds with Ti value >25 were evaluated for their ability to inhibit intracellular mycobacterial growth using BCG infected macrophages. In our macrophage experiments, these shortlisted compounds demonstrated dose dependent killing of bacteria in THP-1 macrophages. These compounds were able to inhibit bacterial growth by 50-fold at 4 days post-treatment. Similar levels of killing were observed in the case of isoniazid, positive control used in the study. We are currently collaborating with various medicinal chemists to make derivatives of these scaffolds in an attempt to improve upon their activity.

Validation of Phosphoserine phosphatase as a drug target: The emergence of drug resistant strains of *Mycobacterium tuberculosis (M. tuberculosis)* makes identification and validation of newer drug targets a global priority. Phosphoserine phosphatase (PSP), a key essential metabolic enzyme involved in conversion of O-phospho-L-serine to L-serine was characterized in this study. The *M. tuberculosis* genome harbors all enzymes involved in L-serine biosynthesis including 2 PSP homologs, Rv0505c (SerB1) and Rv3042c (SerB2). In the present study we have biochemically characterized SerB2 enzyme and developed malachite green based high throughput assay system (HTS) to identify SerB2 inhibitors. We have identified 10 compounds that were structurally different from known PSP inhibitors and few of these scaffolds

were highly specific in their ability to inhibit SerB2 enzyme, non-cytotoxic against mammalian cell lines and inhibited *M. tuberculosis* growth *in vitro*. Surface plasma resonance experiments demonstrated the relative binding for these inhibitors. The two best hits identified in our screen, clorobiocin and rosaniline were bactericidal in activity and killed intracellular bacteria in a dose dependent manner. We have also identified amino acid residues critical for these SerB2-small molecule interactions. This is the first study where we validate that *M. tuberculosis* SerB2 is a druggable and suitable target to pursue for further HTS screening.

The future experiments in the lab include screening of more libraries for activity against M. tuberculosis *in vitro*. We are also developing an assay system to identify inhibitors that are active against intracellular bacteria. Using reverse genetics approach, we are also trying to identify targets for the shortlisted active compounds. We are also developing high-through put enzyme based assay systems to screen inhibitors against enzymes involved in other essential amino acid metabolic pathways.

Carbon metabolism in Mycobacterium tuberculosis and its implications on mycobacterial pathogenesis

Investigators
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Indu Bisht
Amit Kumar Pandey

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Very little is known about the nutritional requirements of *Mycobacterium tuberculosis* (Mtb) while replicating inside the host cell. After infecting host macrophages, Mtb replicates logarithmically for the first three weeks. On induction of host mediated adaptive immune response, the rate of Mtb growth declines, but maintains a constant level throughout the course of the infection. Although, Mtb is thought to survive on lipids inside the host macrophages, the exact intra-cellular diet of Mtb is not very clear. Various research findings suggests sugars along with lipids, derived from the host could be a major source of carbon for Mtb during the intracellular logarithmic growth phase. After the onset of the host mediated adaptive immunity, Mtb infection moves into the persistent stage. Various studies have demonstrated that cholesterol is required for the maintenance of the persistent stage of Mtb infection. We hypothesize that this carbon switch is very critical for Mtb to slow down its replication and metabolic rate thereby activating a more latent form of infection. A better understanding of the type of intracellular carbon source available and the carbon source specific genetic signature would widen knowledge of the disease process. This would extend the range of potential genes that can be targeted for better therapeutics. The proposal involves generation and characterization of Mtb strains lacking genes critical for cholesterol utilization Information from the project on regulatory genes and the motifs of the related regulatory proteins would be very helpful in unraveling the complex regulatory network. The ultimate goal will be to generate an interactome map of the regulatory pathways of cholesterol utilization in Mtb. We did a preliminary study on genes that are differentially regulated under cholesterol specific media. In this microarray-based study Mtb was grown in media containing cholesterol and glycerol as the sole carbon source. A preliminary analysis of the transcription signature thus obtained indicated a re-wiring that explains the reduced growth rate specific to cholesterol sensing and metabolism. Interestingly, a majority of the differentially regulated Mtb

genes were associated with cell growth and metabolism. These observations very strongly associate cholesterol metabolism to Mtb replication and growth.

Based on our transcriptome data and a previous study that identified genes essential for cholesterol utilization, we identified a set of 40 genes that could possibly be critical for carbon specific regulation of Mtb physiology and metabolism. Currently, we have generated clean deletion knockout strains specific to almost 15 of the above genes. Molecular and functional characterization of each of these genes is in progress. Majority of the genes included in this study belong to genes that either directly or indirectly regulate the Mtb transcriptome under specific growth and stress condition. The list includes genes from the toxin-antitoxin system, transcription factors, two-component systems, adenylate cyclases and gene of unknown function. We intend to extend this study and incorporate other physiologically relevant carbon source available to Mtb inside the host viz., fatty acids, amino acids, low and high-density lipoproteins.

Integrative genomics of host-pathogen interaction to identify new drug targets against persistent Mycobacterium tuberculosis

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Tuberculosis (TB) remains one of the world's most important infectious diseases, with an estimated 10 million cases and 2 million deaths per year. Due to the emergence of multi drug resistance (MDR) & extremely drug resistance (XDR) strains of *Mycobacterium tuberculosis* (Mtb), the etiological agent of TB, and a sudden increase in the incidence of human immune deficiency virus (HIV) and diabetes in the population, TB, a seemingly treatable disease is turning out to be a major public health hazard. One of the most challenging aspects of TB treatment is the presence of a slow growing, non-replicating, metabolically inactive "persister" population of bacilli inside host cells that requires extremely long treatment regimen. Clinical and experimental evidences shows that the capacity of Mtb to enter a dormant state leading to latent infection is the key to (i) the survival of Mtb in its host and (ii) induce chronic infection, thus delaying the efficacy of current therapy. Inhibiting dormancy or altering the metabolic state of dormant Mtb could increase the effectiveness of antibiotics and shorten treatment duration.

We hypothesize that the differentially regulated critical metabolic pathways triggered by the intracellular nutrient availability and requirements contribute significantly towards the generation of Mtb persisters. We have earlier demonstrated that Mtb could metabolize and survive on media containing cholesterol as a sole carbon source and that cholesterol metabolism is very critical for Mtb persistence. This indicates that Mtb actively modulates the host biosynthetic machinery for the generation of nutrients required for its own survival. Utilizing genetic and high dimensional informatic approach we will identify differentially regulated metabolic pathways both in Mtb and its host, which could lead to (i) better understanding of host-pathogen symbiosis and thus Mtb pathogenesis, and (ii) designing of novel intervention strategies targeting persisters.

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Cholesterol utilization pathway genes as therapeutics target

Current tuberculosis treatment regimen involves multiple drugs for a prolong period. The duration could be from three months to two years depending on the type of infection. Prolong treatment leads to non-compliance and emergence of newer drug resistance strains. Shortening the therapy would go a long way in alleviating this problem. It is widely perceived that the major culprits are the so-called non-replicating and metabolically inactive "persister" population. The importance of cholesterol metabolism during the persistence stage of Mtb infection and its potential role in generation of persisters is very intriguing. In light of the above facts and hypothesis the focus of the current proposal is to screen for chemical inhibitors that specifically target these pathways. The long-range goal would be identify novel anti-tubercular drugs that specifically targets "persisters". These novel compounds in combination with the standard frontline anti-tubercular drugs would significantly enhance the success rate in tuberculosis therapy. We are in the process of screening a library of almost 2000 compounds for scaffolds that specifically inhibit growth of Mtb in media containing cholesterol as a sole carbon source. We have sucessfully standardized an alamar blue dye based whole cell growth inhibition protocol in a 96-well plate format. We have identified several compounds that demonstrated carbon source specific growth inhibitory activity. The compounds demonstrating a very low MIC and cellular toxicity would further be tested for antimycobacterial activity in an in-vitro cell culture based model.

Our lab is also exploring the potential of targeting cholesterol catabolic pathway genes as novel therapeutic target against Mtb. We have generated several Mtb strains deficient in genes critical for cholesterol catabolism and are in the process of characterizing these strains. The long term goal is to identify critical cholesterol catabolic pathway genes as novel target for developing a live-attenuated vaccine against tuberculosis.

Investigators
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Genetic essentiality study of Mycobacterium tuberculosis under various growth and stress conditions

Advancement in new cost-effective high throughput sequencing techniques has led to the identification of complete genome of various pathogens. The volume of the data generated, failed in its objective of further understanding of microbial pathogenicity. Genetic essentiality study of a pathogen is one such technique where in a gene is functionally characterized and associated with a phenotype. In this laboratory the process of standardizing protocol to study genetic essentiality of Mtb under various growth and stress condition is in progress. To achieve the goal, the use of mariner based mycobacteriophage system for generating high-density transposon mutant library is planned. The library will passage through different growth and stress conditions and the genetic essentiality would be determined by comparing input and output libraries. Since it is demonstrated that cholesterol is required only during

the late stages of Mtb infection, the hypothesis, that a genetic essentiality screen for the gene required for bacterial growth in cholesterol would be more relevant physiologically, if done under hypoxic conditions, is proposed. A better understanding on cholesterol metabolism at the molecular level under physiologically relevant conditions would definitely help in designing of effective therapeutic solutions for TB. We are currently in the process of constructing a mariner phage based Mtb transposon library. The future work involves the construction high-density Mtb and *M bovis* transposon libraries. These transposon libraries would then be used for performing conditional essentiality studies in mycobacteria.

Investigators
Gunjan Arora
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Characterization of M. tuberculosis acetylome and its implications on carbon source utilization

Regulation of metabolic pathways at the transcriptional level is well documented but recent reports highlight the importance of post-translational modification of enzymes modulating critical biochemical pathways. One such modification is Lysine-acetylation of proteins. Information on reversible lysine acetylation of metabolic enzymes in various pathogens also exists. It is therefore hypothesized that the lysine acetylation of proteins in Mtb might contribute significantly towards regulating metabolic pathways specific to various growth and stress conditions. In this project, lysine acetylation profiles of proteins from Mtb grown in cholesterol and glycerol media will be catalogued. The differential acetylation pattern will be further analyzed for its effects on cholesterol metabolism in Mtb. Initial lysine acetylation profiling of total Mtb proteins isolated from different growth conditions are intriguing. Standardization of the protocols for further analysis is underway. Initial observation suggests that a majority of Mtb proteins get acetylated and a detailed analysis of the lysine acetylome of Mtb under various growth and stress conditions would certainly reveal its implications on mycobacterial pathogenesis.

Investigators
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Development of a novel tool based on the CRISPR interference approach for knocking down the expression of genes in mycobacteria.

An urgent demand to produce effective vaccines and drugs for eliminating *Mycobacterium tuberculosis* (Mtb) has exhilarated the development of advanced tools for rapid manipulation of mycobacterial genome. The most common approach for the functional analysis of genes is to introduce genomic deletions by homologous recombination. However, recombination-based techniques are not efficient in creating specific chromosomal mutations in mycobacterial genome because these strategies are largely dependent on transformation efficiencies and often yield low numbers of mutants despite consuming significant quantities of DNA. To overcome these limitations, a conditionally replicating (temperature-sensitive) shuttle phasmid was developed which produce a specialized transducing mycobacteriophage, carrying allelic exchange substrate. However, due to involvement of multiple steps of transformation and selection, or construction of transducing phages, this strategy is complicated, time-consuming and expensive. Recently a recombineering approach was developed by overexpressing mycobacteriophage-encoded recombination proteins in mycobacteria that enhances recombination efficiency of DNA substrates. However, a major drawback of recombineering technique is the requirement of toxic proteins Gp60 and Gp61 for stimulating recombination. It remains to analyze the off-site effects of these proteins in mycobacteria. Importantly, none of these methods are suitable for targeting essential genes.

Recently, various other strategies were reported for targeted gene regulation in other microbial systems such as RNA interference (RNAi), engineered transcription-activator-like effector (TALE) proteins and CRISPR (clustered regularly interspaced short palindromic repeats)-interference (CRISPRi) that need to be tested in mycobacterial species. Out of these, CRISPRi offers a simple and cost-effective tool principally applicable to all microorganisms for targeted gene regulation. The CRISPRi system was recently developed

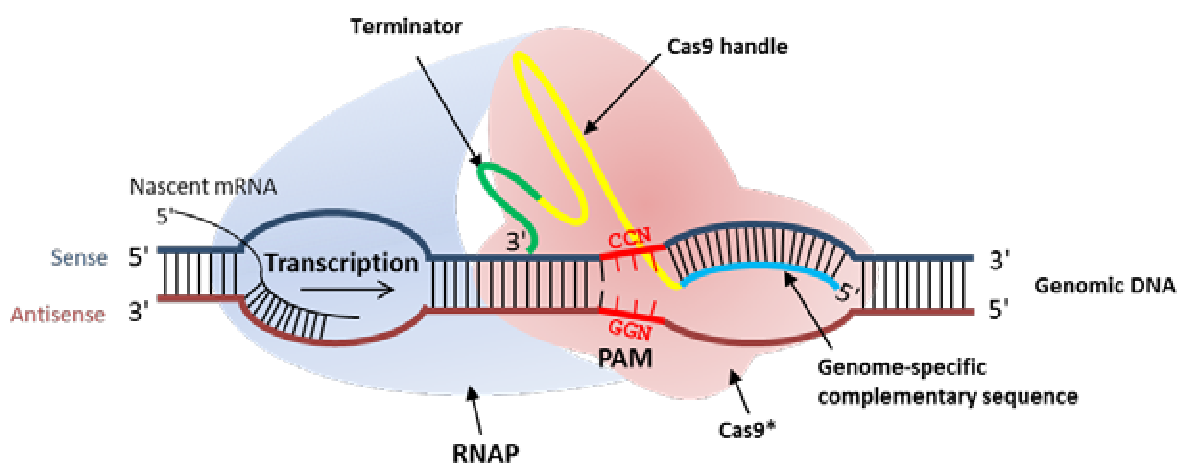

Fig. 14: Schematic of the CRISPRi approach. Cartoon depicting regulation of gene expression by CRISPRi due to interference of RNAP transcription by nuclease-deficient dCas9. The nuclease deficient dCas9 containing two substitutions in the nuclease domains (D10A and H840A) makes complex with sgRNA, which is targeted to specific DNA sequence. If the target DNA sequence belongs to the protein-coding region, the dCas9–sgRNA–DNA complex blocks the movement of RNAP and subsequent transcription elongation.

for targeted gene regulation in *Escherichia coli* by using endonuclease-deficient Cas9 containing two mutations in the nuclease domains (D10A and H840A) (designated as dCas9. The modified CRISPR system was termed as CRISPR interference (CRISPRi) due to the ability of dCas9 to interfere with DNA transcription (Fig.14) which can repress gene expression by as many as thousand-fold.

Here, we implemented the CRISPRi system in both fast-growing *Mycobacterium smegmatis* (Msm) and slow-growing Mtb-complex bacteria. We show that codon-optimized dCas9 can be stably expressed in mycobacteria for up to fifteen days without any toxic or off-target effects. Further, we constructed an *E. coli*-mycobacteria shuttle plasmid which contains unique restriction endonuclease sites for cloning of gene-specific complementary sequences next to dCas9-binding region under the control of a tetracycline-inducible promoter, Pmyc1tetO. By using this approach we were able to efficiently repress diverse sets of genes in both Msm and Mtb-complex bacteria to negligible levels. We also demonstrate that CRISPRi is effective in knocking down specific domains of a protein in mycobacteria. Finally, by using CRISPRi system we were able to rapidly test the gene essentiality in mycobacteria. Currently, we have established collaboration with several International and National institutes and are working on constructing knockdown strains targeting various essential and non-essential genes.

Toward the characterization of multiple P-loop GTPases in mycobacteria

Investigators
Eira Choudhary
Nisheeth Agarwal

Collaborators
Ramandeep Singh

GTPase superfamily of proteins is universally present in all forms of life, regulating essential cellular pathways such as protein synthesis, cell cycling & differentiation and hormone signaling. A survey of genome sequences of different mycobacterial species reveals the presence of conserved P-loop GTPases namely Era, Obg, EngA, HflX and YchF that have not been characterized and their role has remained obscure in these organisms. Based on the conserved occurrence of P-loop GTPases in different mycobacterial species, we hypothesize their involvement in essential metabolic pathways. We aimed at investigating the role of multiple P-loop GTPases in the biology of mycobacteria to explore this class of proteins as novel drug targets.

We began with cloning, expression and purification of these GTPases using *E. coli* expression system and observed that HflX GTPase is lethal for *E. coli*, whereas other GTPases are expressed well in *E. coli*. Also, our results showed that YchF is not a GTPase, rather it is an ATPase. Further we demonstrated that EngA is associated with ribosome, and GTP is critical for interaction of $EngA^{MS}$ with 50S subunit of ribosome. Subsequently, we analyzed localization of these GTPases by expressing GFP-fusion proteins in Msm; our results indicated that EngA, ObgE and YchF are localized to both cell envelope and cytoplasm, whereas the Era GTPase is restricted to only envelope. Similar observations were made in Mtb, which also revealed that EngA is not only present in the cytoplasm, but is also exposed to the extracellular environment which is susceptible to degradation by proteinase K. By constructing the EngA depletion strain, we observed that it is essential for *in vitro* growth of Mtb.

We screened a library of small molecule inhibitors against purified EngA GTPase. Our preliminary results reveal that 5 out of 2300 compounds exhibit >40% inhibition in the GTPase activity of EngA. Subsequently, we also tested the MIC of each of these compounds against Mtb, however, we did not observe any substantial effect on *in vitro* growth of Mtb. Our future goal is to better understand the role of EngA in Mtb and to test the effect of anti-EngA small molecules on its function.

To characterize the role of putative preprotein translocase in Mycobacterium tuberculosis

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Collaborators
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RCB, Faridabad

Membrane organization of a pathogen plays significant role in determining its virulence. The host-pathogen interaction is driven by the unique arrangement of several membrane proteins on the envelope of both the partners. The fate of these membrane proteins is in-turn governed by specialized transporters known as protein translocases. There are several proteins embedded in both the cell membrane and outer wall of *M. tuberculosis* which regulate important biological processes crucial to mycobacterial virulence such as transport of materials across the membrane, interaction with host and subsequent elicitation of host immune response upon infection etc. These membrane proteins are specifically localized to envelop by a group of proteins known as pre-protein translocases.

In this project, we are characterizing the role of a putative pre-protein translocase annotated as YidC in Mtb, which is encoded by the gene *Rv3921c*. A thorough analysis of genome sequence indicates that *Rv3921c* is placed in the genome adjacent to essential genes namely *rnpA* and *rpmH*, and this genomic arrangement is conserved across different mycobacterial species. Although Rv3921c lacks N-terminal periplasmic domain, which is present in the well-characterized YidC protein from *E. coli* and its close homologs, analysis of Rv3921 sequence by THMM program and fluorescent microscopy of mycobacterial cells overexpressing Rv3921c-GFP fusion demonstrated that Rv3921c is exclusively present on the envelope. A reverse transcription-PCR study showed that *Rv3921c* homologue in *M. bovis* BCG, *BCG_3979c* is co-transcribed with upstream genes *BCG_3978c*, *rnpA* and *rpmH*. By quantitative RT-PCR analysis it was observed that *BCG_3979c* is constitutively expressed in mycobacteria. By overexpressing Rv3921c in both E. coli and mycobacteria, we concluded that expression of Rv3921c is tightly regulated in these organisms, and any perturbation in its level leads to cessation of bacterial growth. Proteome analysis by 2D-PAGE revealed that overexpression of Rv3921c in Msm results in depletion of two proteins of ~80kDa and 40kDa molecular masses that were identified as MSMEG_2299 and MSMEG_3476 encoding ribonucleotide reductase and a peptidase of M48 superfamily, respectively. We argue that this apparent reduction in levels of MSMEG_2299 and MSMEG_3476 on the cell membrane owing to increased expression of Rv3921c is probably due to disruption of functional preprotein translocon.

Subsequently, we constructed the Rv3921c and MSMEG_6942 (an orthologue of Rv3921c) conditional depletion strains of *M. tuberculosis* and *M. smegmatis* respectively, by using the CRISPRi approach. The *in vitro* growth analysis of

depletion strains provided a surprising observation which indicated that Rv3921c is essential whereas MSMEG_6942 is dispensable for bacterial growth (Fig. 15).

We are studying the proteome profile of Rv3921c-depletion strain of Mtb by LC-MS. We have also overexpressed Rv3921c-FLAG fusion in Mtb and a Co-IP experiment is underway to identify its interacting partners. Modulation of cell envelope composition upon overexpression of Rv3921c is an indicative of altered host immune response to infection. Thus we will also study the effect of Rv3921c depletion on intracellular growth of Mtb and Th1/Th2 response in THP-1 macrophage line.

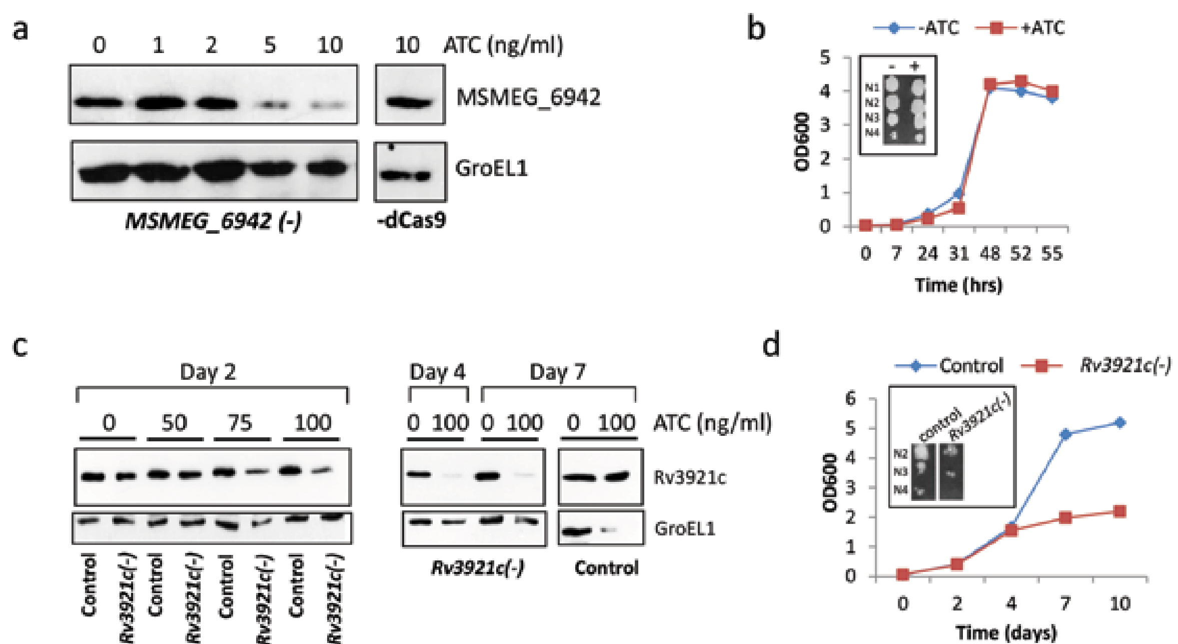

Fig. 15: Effect of depletion of MSMEG_6942 and its orthologue in Mtb (Rv3921c) on in vitro growth of bacteria. a) Effect of CRISPRi on protein synthesis in Msm. Whole-cell extracts of the respective Msm strains were prepared after 24 h of treatment with different ATC concentrations and subjected to immunoblot analyses using specific antibodies, which show that expression of MSMEG_6942 and not of an unrelated protein GroEL1 disappears following ATC treatment in a dose-dependent manner. (b) Influence of CRISPRi-mediated silencing of gene on in vitro growth of Msm. In vitro growth was determined by measuring OD600 of MSMEG_6942 knockdown strain, in the presence (+) or the absence (-) of 50ng/ml ATC. Also, an aliquot of each culture, after 24 h of growth, was subjected to 10-fold serial dilution and spotted on 7H11 agar plates with (+) or without (-) 10ng/ml ATC; dilutions are as follows: N1: 10-1, N2: 10-2, N3: 10-3 and N4: 10-4, respectively (see insets). (c) Effect of ATC on the expression of Rv3921c protein in Mtb. Expression was analysed by immunoblotting using Rv3921c-specific antibodies and cell lysates of control and Rv3921c(-) depletion strain, after different ATC concentrations for 4 days (left panel) or 100ng/ml ATC for 4 and 7 days (right panel), respectively. The results clearly indicate a complete suppression of Rv3921c expression after 4 or 7 days of treatment with 100ng/ml ATC. (d) Effect of CRISPRi-mediated silencing of Rv3921c on in vitro growth of Mtb. In vitro growth was determined by measuring OD600 of the control and the Rv3921c(-) knockdown strains, treated with 100 ng/ml ATC. Also, an aliquot of each of these cultures, after 48 h of treatment with 100 ng/ml ATC, was subjected to 10-fold serial dilution and spotted on 7H11-OADC agar plates to monitor growth after 4 weeks; dilutions are as follows: N2: 10-2, N3: 10-3 and N4: 10-4, respectively (see insets). These results demonstrate that Rv3921c is essential in Mtb, whereas its orthologue in Msm, MSMEG_6942 is dispensable for growth.

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A systems approach to analyze changes in global phosphorylation status of proteins in macrophages infected with Mycobacterium tuberculosis complex bacteria, and their repercussions on mycobacterial virulence

With several thousand years of its stay in human body, Mtb has well adapted itself to successfully face the innate and adaptive immune responses of the host. This is primarily evident by the ability of Mtb to restrict fusion of mycobacterial phagosomes with lysosomes within infected macrophages, and to undergo persistence under the influence of a combination of drugs. Hence, to survive these onslaughts Mtb must have devised mechanisms to manipulate the host signaling which will eventually allow its replication in phagosome. Upon infection of macrophages both pathogenic as well as non-pathogenic mycobacteria including vaccine strain *M. bovis* BCG exert multiple physiological changes primarily by causing alterations in expression and posttranslational modifications (PTMs) of certain host proteins that result in perturbation of host signaling. Phosphorylation is one such PTM event, which universally happens in host cells under various extracellular stimuli including infections. Like other pathogens, Mtb infection mounts a cascade of phosphorylation and dephosphorylation events in the host that affect intracellular replication of mycobacteria. Although there are multiple studies conducted by different groups that have yielded a valuable piece of information, these have failed to provide a clear view of phosphorylation networks of infected host cells, and majority of areas within cellular signaling networks are yet to be understood. For example, basic information on the global phosphoproteome profile of host cells upon Mtb infection has not been looked thoroughly. Similarly, studies on time course and dose-dependent effects of Mtb infection on global phosphoproteome of host macrophages are urgently required to gain a better insight into the role of these pathways in Mtb infection. In addition, the downstream effects of differential phosphorylation of host proteins on Mtb virulence are also not entirely understood.

This project aims to conduct a comprehensive study of the effect of mycobacterial infection on global phosphorylation status of the host proteome and their consequences on survival of bacteria in the host macrophages. Our objective is to identify previously uncharacterized phosphoproteins that respond specifically to Mtb infection and could serve as important determinants of infection. The outcome of this project will not only shed a light on the host responses to mycobacterial infection, but will also help building a phosphoproteome network specific to infection by Mtb bacilli. The information obtained from the proposed study can be further utilized in designing inhibitors that will target the pathways required for intracellular survival of Mtb, as well as identifying signatures of protection against Mtb infection in humans.

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Deciphering Mycobacterium tuberculosis artillery

Mtb's stockpile consists of a combination of lipids, proteins, sugars and small molecules. Surprisingly, thus far, very few Mtb effectors have been identified and/or characterized. Lack of precise inventory of its stockpile impairs our understanding of how Mtb wields its artillery. This in turn dilutes our strategies to initiate a co-ordinated assault on the pathogen. Thus, to better understand its virulence mechanisms and design superior vaccines and therapeutics against Mtb, it is critical to (i) identify its entire virulence artillery, (ii) delineate their host-specific functions, (iii) define their host molecular targets.

To identify Mtb's protein effectors that access macrophages, we designed a genetic approach that exploits Cre-recombinase as a reporter. Using Gateway technology we first tagged each ORF with NLS-Cre. Then we moved them into virulent Mtb to infect recombinant macrophages carrying *loxP-nptII-loxP-gfp* genetic element under a ubiquitous promoter. When such a Cre-fused Mtb protein accesses host environment, the NLS promotes its entry into the nucleus. The hitch-hiking Cre then promotes recombination of *loxP* sites to bring promoterless *gfp* (the reporter for this screen) in close proximity to the ubiquitous promoter for continued expression. Macrophages that receive an Mtb-fused protein thus turn green.

Previously, we procured the Mtb ORFeome entry clone library from BEI Resources, USA. We had earlier designed and constructed two complex Gateway destination vectors that help fuse Mtb ORFs in frame at their C- and N-termini with NLS-Cre, and had moved ~500 Mtb genes into the N-NLS-Cre vector and PCR amplified at least 50 missing (from ORFeome library) genes. Currently we have moved around 1000 Mtb genes into the N-NLS-Cre vector and transformed 382 constructs into Mtb. Once the recombinant animals are available, we will generate bone marrow, differentiate monocytes to macrophages and then set them for infection with modified Mtb library. Simultaneously we will continue to move more Mtb genes into the N-NLS-Cre vector and transform Mtb. We have also cloned 48 missing genes that have been also moved into the N-NLS-Cre vector. Additionally, 342 genes have been moved into C-NLS-Cre vector too.

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Mycobacterial membrane-derived vesicles: Role in pathogenesis and exploration as novel subunit vaccine vehicles against Tuberculosis

Despite worldwide use of BCG, TB continues to prevail. Though effective in children, it fails to protect adolescents and adults. Neither boosting BCG nor using BCG as booster works. Subunit vaccines that can supplement BCG as boosters are currently being explored. Most boosters involving 1-4 purified antigenic-Mtb candidates put together with an adjuvant have failed or are in clinical trials. Experts predict that an ideal subunit vaccine should contain multiple antigens targeting different stages of Mtb pathogenesis. As an alternate to liposomal-derived boosters, here we explore if membrane vesicles of Mtb could serve similar purpose.

Most bacteria generate membrane/outer-membrane vesicles (MVs/OMVs). Pathogenic bacteria exploit them towards pathogenesis. MVs are nanoscale (approx. 10-300 nm) proteoliposomes produced naturally and thus constitute a unique system in which the antigens and the delivery vehicle *per se* are naturally derived from the pathogen. Additionally, MVs circumvent safety limitations of attenuated/killed organisms administered as vaccines. Finally, MVs can be engineered to include several naturally un-incorporated antigens. The prediction is, since the pathogen *per se* delivers the vaccine antigens of interest into recombinant OMVs, they retain native conformations to immune-stimulate better.

We intend to generate recombinant MVs (rMVs) from *Mycobacterium smegmatis* (Msmeg), a nonpathogenic mycobacterium species. Towards this, we had standardized conditions to enrich MVs from large volumes of culture filtrates of Msmeg grown *invitro* in minimal media. Using Mass Spectrometry, we had identified ~110 Msmeg proteins in MVs.

Currently, we are cloning these genes to (a) 3X FLAG-tag them for their detection in MVs; and (b) mCherry fuse them to evaluate if any or all can deliver a heterologous reporter into Msmeg MVs. These analyses will help us decipher ways to (i) understand the mechanism by which proteins get loaded into MVs and (ii) generate Msmeg rMVs with Mtb proteins. We are also currently modifying conditions using Optiprep/sucrose gradients to eliminate protein complexes that might pellet down together with the MVs. Further, using the electron microscopy we are evaluating different fractions obtained after density gradients for their contents. We are also currently evaluating nucleic acid contents of MVs.

Peer-reviewed publications

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Patents

1. Cleaved functional clade C envelope glycoprotein: U.S. provisional patent application Serial No. 62/068,202
2. Native trimeric ENV immunogen design: U.S. Patent Application No. 62,155,673
3. HIV-1 CLADE C ENVELOPE GLYCOPROTEINS: US provisional application. 62/189,418.
4. 7-substituted 2-nitro 6,7- dihydroimidazo [2,1-b][1,3] oxazine derivatives of their optical isomers, pharmaceutical composition containing the same as an active ingredient. Korean application no. DP-201-40911-01.

Seminars and conferences

Arup Banerjee

Participated in 15th Indo-US Flow Cytometry workshop on stem cells held at Institute of Liver and Biliary Sciences, New Delhi on October 27-28; 2014.

Invited speaker in 4th Molecular Virology Meeting April 16-17, 2015, held at RGCB, Thiruvananthapuram.

Bimal Chakrabarti

Co-chaired session on : Adjuvants and Immunogens
Name of the meeting: HIV Research for Prevention - 2014
Place and date: Cape Town, October, 2014

Invited talk: *Designing of native trimeric Env immunogen based on naturally and efficiently cleaved clade C Env from India*
Name of the meeting: Neutralizing Antibody Consortium in
Place and date: La Jolla, California, USA, April, 2015

Jayant Bhattacharya

Participated in the meeting
Name of the meeting: Neutralizing Antibody Consortium in
Place and date: La Jolla, California, USA, April, 2015

Saikat Boliar

Delivered talk on: *An Efficiently Cleaved HIV-1 Subtype C Env that is Selectively Recognized by Neutralizing Antibodies: a Platform for Immunogen Design*
Name of the meeting: HIV Research for Prevention 2014
Place and date: Cape Town, October, 2014

Guruprasad Medigeshi

Title of the talk: *Challenges in R&D for Dengue in India*
Name of the meeting: Symposium on 'Dengue Prevention and Control'
Place and date: India Habitat Center, New Delhi, 29th September 2014.

Title of the talk: *Role of C-terminal Src Kinase in dengue virus replication*
Name of the meeting: International Union of Microbiological Societies congresses; XVIth International Congress of virology
Place and date: Montreal, Canada 27th July to 1st Aug, 2014.

Title of the talk: *Viremia and immune response in dengue pathogenesis – cause or effect of severe disease?*
Name of the meeting: National Conference on Recent Trends in Molecular Virology – 2014
Place and date: Jamia Millia Islamia, Nov 17th to 19th, 2014.

Title of the talk: *Identifying factors of disease severity in dengue infection*
Name of the meeting: Molecular Immunology Forum meeting - 2015
Place and date: Bhubhaneshwar, Jan 16th -18th, 2015

Title of the talk: *Riding on viruses to learn about barriers*
Name of the meeting: Department of Immunology, University of Toronto visit to NII-NCR Bioscience Cluster
Place and date: National Institute of Immunology, New Delhi, Jan 15th, 2015

Krishnamohan Atmakuri

Title of the talk: Decoding bacterial-imposed neonatal sepsis
Name of the meeting: Indo-Cambridge (UK) Infectious Diseases Network
Place and date: NCBS, Bengaluru, Sept 10th 2014

Title of the talk: Deciphering Mycobacterium tuberculosis Artillery
Name of the meeting: Invited faculty speaker to NCBS, Bengaluru
Place and date: NCBS, Bengaluru, Sept 9th 2014

Title of the talk: TB Vaccine Design: Do we have the answers yet?

Name of the meeting: Invited speaker to World TB Day Symposium
Place and date: AIIMS, March 24th 2014

Manjula Kalia

Title of the talk: *Interplay between the cellular autophagy machinery and Flaviviruses*
Name of the meeting: National Conference on Recent Trends in Molecular Virology
Place and date: Centre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia, New Delhi, 17-19 November, 2014.

Tile of the talk: *Host-Pathogen Interactions of Flaviviruses- Role of Autophagy*
Name of the meeting: Indo-Dutch Workshop on Virology & Host Microbe Interactions
Place and Date: The Leela Palace, New Delhi, 5 November, 2014.

Title of the talk: *All About Ebola*
Name of the meeting: Biotalk Series
Place and date: Department of Biotechnology, Jamia Millia Islamia, 20 October, 2014.

Milan Surjit

Title of the talk: *Understanding the Biology of Hepatitis E Virus and development of drugs and vaccine against it.*
Name of the meeting: Ramalingaswami Fellows Conclave.
Place and date: January 2015, Bhubaneswar, Odisha.

Ramandeep Singh

Title of the talk: *PolyP metabolism in mycobacteria: Role of PPK-1 and PPK-2 in stationary phase survival and virulence of Mycobacterium tuberculosis.*
Name of the meeting: Ramalingaswamy Conclave 2015
Place and date: Institute of Life Sciences, Bhubneshwar, January 2015.

Ranjith Kumar CT

Title of the talk: *Modulation of innate immune response and characterization of viral polymerases for the development of potent vaccines and antivirals.*
Name of the meeting: 4th Ramalingaswami Fellows' Conclave
Place and date: Bhubaneswar, India. January 30 to February 1, 2015.

Sudhanshu Vrati

Title of the talk: *Role of host cell proteins in Japanese encephalitis virus replication*
Place and date: NCBS, Banglore, 5 September 2014

Title of the talk: *Role of host cell proteins in Japanese encephalitis virus replication*

Name of the meeting: Sixth symposium on Molecular Medicine
Place and date: JNU, New Delhi, 13 February 2015

Title of the talk: *Making of a rotavirus vaccine: An Indian success story from research to development*
Name of the meeting: Infection and Disease: A Bug's Life
Place and date: Sri Venkteshwar College, New Delhi, 26 March 2015

Extra-mural Grants

Amit Pandey

Funding agency: Indo-Singapore grant jointly funded by Dept. of Science and Technology (DST), India and Agency for Science, Technology and Research (ASTAR)
Amount: Rs 50 lakhs
Duration: From 2015 to 2018
Title of the grant: Integrative genomics of host-pathogen interaction to identify new drug targets against persistent Mycobacterium tuberculosis

Arup Banerjee

Funding agency: DBT (BT/PR8597/MED/29/764/2013)
Amount: Rs. 140 Lakhs, (Three years).
Duration: From August, 2014 To July, 2017.
Title of the grant: Transcriptome analysis for identification of novel biomarker for disease progression in Dengue patients

Funding agency: DBT (BT/PR6714/MED/29/617/2012)
Amount: Rs. 55.6 Lakhs
Duration: From January, 2013 To December, 2015 (Three years).
Title of the grant: Role of microRNAs in establishment of Japanese Encephalitis Virus (JEV) infection and disease progression

Guruprasad Medigeshi

Funding agency: Department of Biotechnology
Amount: Rs. 65,96,000/-
Duration: From March, 2011 to December, 2014
Title of the grant: Role of tyrosine kinases in the life-cycle of Japanese encephalitis virus and dengue virus

Funding agency: Department of Biotechnology
Amount: Rs. 1,23,84,972/-
Duration: From May, 2012 to October, 2015
Title of the grant: Identification of correlates of disease severity in pediatric Dengue patients in New Delhi

Funding agency: Wellcome trust-DBT India Alliance
Amount: Rs. 3,63,18,986/-
Duration: From October 2014 to September, 2019
Title of the grant: Investigating the effect of viral infections on zinc

	homeostasis as a cause of permeability barrier disruption in polarized epithelial and endothelial cells

Krishnamohan Atmakuri

Funding agency:	Dept. of Biotechnology, Ministry of Science and Technology, INDIA
Amount:	Rs 85.6 lakhs
Duration:	From 2012 to 2017
Title of the grant:	Mycobacterial membrane-derived vesicles: Role in pathogenesis and exploration as novel subunit vaccine vehicles against Tuberculosis
Funding agency:	Dept. of Biotechnology, Ministry of Science and Technology, INDIA
Amount:	Rs 64.6 lakhs
Duration:	From 2012 to 2015
Title of the grant:	Deciphering Mycobacterium tuberculosis artillery

Manjula Kalia

Funding agency :	Indo-French Centre for the Promotion of Advanced Research
Amount:	Rs. 68,14,752
Duration:	From 2015 To 2018
Title of the grant:	Host-Virus Interactions and Antibody Therapy for Japanese Encephalitis

Milan Surjit

Funding agency:	DST, India.
Amount:	25 lakh rupees
Duration:	2012-2015
Title:	Establishment of a mammalian cell culture based Hepatitis E Virus (HEV) expression system to study the viral life cycle and application of the secreted virion as a candidate vaccine.
Funding agency:	DBT, India.
Amount:	32.05 lakh rupees
Duration:	2013-2016
Title:	To identify novel therapeutic compounds that inhibit the interaction between Hepatitis E Virus ORF3 protein and TSG 101 and to explore the molecular mechanisms controlling the release of Hepatitis E virions from infected cells.

Nisheeth Agarwal

Funding agency:	Department of Biotechnology, India
Amount:	Rs. 5880000
Duration:	From October 2014 to September 2017
Title of the grant:	A systems approach to analyze changes in global

phosphorylation status of proteins in macrophages infected with Mycobacterium tuberculosis complex bacteria, and their repercussions on mycobacterial virulence.

Ramandeep Singh

Funding agency: Department of Biotechnology
Amount: Rs. 49, 48,400
Duration: September 2010 - September 2015.
Title of the grant: Understanding the role of polyphosphate kinases and polyphosphatases in physiology of Mycobacterium tuberculosis.

Ranjith Kumar CT

Funding agency: Department of Biotechnology

Amount: Rs. 7706300
Duration: From 2013 To 2016
Title of the grant: Characterization of hepatitis E virus RNA-dependent RNA polymerase and its associated proteins in the replicase complex

Sudhanshu Vrati

Funding agency: Department of Biotechnology
Title: The animal Facility for Research on Infectious Diseases
Duration: March 2014 – Feb 2019
Amount: Rs. 17,14,30,400

Funding agency: Department of Biotechnology
Title: Laboratory assays for the Phase III trial for the non-interference of ORV116E
Duration: May 2014 – Nov 2015
Amount: Rs. 67,92,098

Honours and Awards

Guruprasad Medigeshi

Wellcome Trust – DBT India Alliance Intermediate Fellowship

Travel grant from the Department of Science and Technology to attend the International Union of Microbiological Societies congresses; XVIth International Congress of virology meeting at Montreal, Canada.

Krishnamohan Atmakuri

Ramalingaswami Fellowship (2012-2017)

Ramandeep Singh

Ramalingaswami Fellowship (2010-2015)

Amit Pandey

Ramalingaswami Fellowship (2012-2017)

Pediatric Biology Centre

An Overview

Shinjini Bhatnagar

The Pediatric Biology Centre (PBC) was established at the Translational Health Science and Technology Institute, with a vision to serve as an interdisciplinary research center where research on the biological basis of childhood health and disease leads to the creation of knowledge-driven interventions and technologies that can be sustainably implemented.

The mandate of PBC is to become a national catalyst in designing solutions for maternal, neonatal and infant care engaging multiple expertise groups in this endeavor. It aims to bridge the gap between classical clinical and population epidemiology and the mechanistic biology in order to develop a fascinating science driven approach to solving maternal and child health problems.

Provided below is a summary of the various programs currently underway at PBC.

Understand development of early life immune systems in response to antenatal, perinatal & infant stressors

In order to understand what underlies the increased susceptibility of neonates and particularly those who are growth restricted, to serious systemic infections, we examined differences in the immune phenotype of small for gestational age and appropriate for age neonates. We concluded that the differences seen in diverse cellular lineages of the immune system are more likely to reflect altered maturation of the immune system due to the intrauterine conditions causing growth retardation rather than simply retarded immune system maturation (published in *PloS One 2015*). These initial studies have opened new avenues for examining both the molecular-cellular genesis and the functional-clinical consequences of immune dysfunction in growth-restricted neonates. We are now establishing a cohort of more severely growth restricted infants in a tertiary hospital at New Delhi to further strengthen our understanding of the development and functional properties of the neonatal immune systems in response to antenatal, perinatal and early childhood stressors and evaluate the clinical consequences in the neonatal period and beyond. Further, we have established systems to compare the immune phenotypes and functions of cord blood from a cohort of full-term babies in the U.S. (Stanford) and India (New Delhi) with a specific hypothesis that there are significant differences in the frequency of B-1 cells and immature B-2 B cells between U.S. and Indian neonate cohorts.

Another main objective within this domain is to understand nutritional influences on the immune system, their interactions and to develop nutrition based interventions for improved immune function, growth and well regulated inflammation. To understand vitamin D biology further a clinical trial to assess the effect of daily vitamin D supplementation from birth to 24 weeks on immune responses to vaccines administered in the first few months of life is on at the district hospital in gurgaon. This trial of 900 infants, also allows us to study the immune maturation of infants aged 6 – 24 weeks in the group that has been randomized to the placebo group.

Study molecular basis of disease that has major clinical importance/public health significance; using this knowledge, generate sound individual and population-specific diagnostics, interventions and policies

Multi-institutional, multidisciplinary program on preterm birth and other pregnancy outcomes: In India's 27 million born annually, 3.6 million are born preterm, and over 300,000 of them die each year, contributing to 25% of the overall global preterm (PTB), related deaths. To address important issues in preterm birth we have established a pregnancy cohort at the district hospital in Gurgaon. We are enrolling women in the cohort before 20 completed weeks of gestation. These women will be followed up at least until 42 days after delivery. We hypothesize that risk stratification of mothers for preterm birth facilitates better understanding of mechanisms within these well-defined sub-categories, for which preventive & therapeutic interventions are feasible to develop. Varied bio specimens across pregnancy, at birth and beyond will be collected in addition to well-characterized information on epidemiological determinants & phenotypes with the immediate aim to identify the following correlates of PTB (i) modifiable clinical & epidemiological determinants, (ii) genomic/epigenomic/proteomic signatures and the (iii) quantitative dynamics of the vaginal microbiome. This program has been initiated as a successful grant application to the Dept. of Biotechnology, GOI, National Grand Challenge Program to address the multiple strategic priorities in preterm discovery.

Study epithelial barrier disruption during inflammatory processes in disease: The ongoing study on mechanisms of CD80 mediated proteinuria at the level of cellular and molecular changes in podocyte has opened avenues to investigate the role of CD80 as a general disruptor of barrier function in various other epithelial cells. Our preliminary results have shown that treatment of colonic epithelial cell line Caco-2, with LPS leads to increase in CD80. Experiments are underway to look at functional consequences of CD80 expression and also effect of CD80 expression in other epithelial cell lines.

Study early life infections; evaluate interventions that inform how & why for clinical practice & policy

Serious infections like pneumonia, sepsis, and meningitis contribute to more than 25% of the annual 1 million neonatal deaths in India and are also a major cause of hospitalization in infants. Based on the promising results of our previous trial we propose to undertake a multi center (7), multi country (2) double-blind randomized placebo controlled trial to measure the efficacy of

zinc administered orally as an adjunct to standard therapy to 4000 infants aged 1 day to 2 months hospitalized with clinical sepsis on reducing case fatality. This study will strengthen the evidence of beneficial effect of zinc in infants with sepsis and help guide policy. Within this large clinical trial, we will also investigate the cause for post-infection poor outcomes by assessing defects in the immune host defense and the role of zinc in re programming immune system to overcome the possible immunoparalysis, that maybe responsible for the poor outcomes.

Use and develop modern basic biology and platform technologies to rapidly devise, test and adapt innovative, sustainable solutions for India's child health problems

Diagnostic tests for Celiac disease: Another major area of focus has been to develop diagnostics for childhood illnesses and low cost devices using innovative technology. We collaborated with International Center of Genetic Engineering and Biology, AIIMS and J. Mitra and Co (industrial partner) to develop easily available indigenous, rapid, sensitive and specific diagnostic tests for Celiac Disease. We developed two tests, ELISA and the rapid point of care that were commercialized by the industry in October 2014. (Patent NO 1133/DEL/2011; dated 18.04.2011)

Social Innovation Immersion Program: The Social Innovation Immersion Program (SIIP) hosted by PBC and Center for Biodesign at THSTI has been initiated to create a platform for social innovation in health, for the base of the pyramid in India by taking innovators into the heart of community health. PBC will leverage existing partnerships with GHG and tertiary hospitals including AIIMS, MAMC, and SJH for mentorship and exposure. A multi-disciplinary team is undergoing clinical immersion beginning with community and home-based healthcare followed by brief exposures to tertiary hospitals to provide immersion across a variety of resource settings. They will pick a few need-idea pairs based on burden of disease targeted and likelihood of success and will crystallize the solutions into executable components to create a prototype solution.

Inter-Institutional Program for Maternal, Neonatal and Infant Sciences-A translational approach to studying Preterm birth

Investigators

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Uma Chandra Mouli Natchu
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Collaborators

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Shinjini Bhatnagar

This inter-institutional program on maternal and infant sciences is a multidisciplinary research effort to predict & diagnose Preterm Birth (PTB). It is envisaged that the clinically relevant research outputs from the study will be appropriate characterization of biological, clinical and epidemiological risk factors to achieve appropriate risk stratification of mothers who may deliver before term. More specifically, the aim would be evaluation of putative biomarkers, identification of simple microbiological tool based vaginal risk factors, modulation of vaginal microbiota for therapeutic purposes and evaluation of environmental modification chosen from SNP analysis. It is further envisioned to elucidate causal biological risks and processes of fetal growth and the clinical consequences of PTB and intra uterine growth retardation, most of which are of major public health consequence.

After obtaining the necessary ethics approvals from all the participating institutes, a hospital based prospective cohort study that is enrolling women early in their pregnancy and serially following them through their pregnancy until childbirth and till 42 days (6 weeks) post-partum has been established at the General Hospital Gurgaon (GHG) in the National Capital Region (NCR); which is the main study site. The other study site is Safdarjung Hospital (SJH), New Delhi where the study participants will get referred for further management if their medical condition so demands. Pregnant women coming to the antenatal clinic of the General Hospital Gurgaon during the study duration will be approached by the study nurses to ascertain their eligibility to participate in the study. Women who have a pregnancy of less than 20 weeks gestation as determined by a ‘dating’ ultrasound and who are willing to come to GHG for follow up antenatal visits will be eligible if they are willing to participate in the study and give their written consent for it. Those women who have an extrauterine, molar or heterotopic pregnancy will be excluded. Information related to socio-demographic details, medical history, history of past and present pregnancy will be obtained and recorded in structured and pretested case recording forms (CRF). This will include information on established socio-demographic, environmental and obstetric risk factors for preterm birth and/ IUGR. Collection of biological samples (Maternal blood, stool, urine, high vaginal fluid, saliva) will be done in pre packed collection kits which will be labelled with a unique identification code (UIC) for each participant to ensure confidentiality of participants. This unique identification code will be scanned at each step in the flow of biological samples from collection to immediate processing to temporary storage to transportation and till final storage. Scanning of UICs at each step will inform us about the actual time taken for flow of samples from one point to other, thus will help us to monitor adherence to the protocol while handling these samples and facilitate the quality assurance process of laboratory parameters. The enrolled participants will be followed up at pre-defined periods during the course of the pregnancy. They will be asked to come at 18-20 weeks, 26-18 weeks, 30-32 weeks, labor and delivery and then at 42 days post-partum. At each of the time points clinical data will be collected along with the anthropometry and a clinical examination in structured and pre-tested follow up forms. Biospecimens (maternal blood, urine, saliva, high vaginal fluid and additionally

cord blood, cord tissue and placental tissue at delivery) will be collected at 18-20 weeks, 26-18 weeks, delivery and 42 days post-partum following the same rigor as described for the enrolment samples. Serial ultrasounds will be done at 18-20 and 30-32 weeks, essentially to assess the cervical length, placental position, size, echogenicity and vascular flow.

This cohort will also serve as a platform for wider and larger research programs. Investigators, who may or may not belong to the initial phases of this program, will be encouraged to develop additional research questions through discussions using a trans-disciplinary approach. The clinical, imaging data and biospecimens longitudinally collected on enrolled women and the child will serve as resource for such studies. Creation of this resource will also reduce time and cost for the conduct of future research in this area.

Zinc as an adjunct for the treatment of very severe disease in infants younger than 2 months

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Shinjini Bhatnagar

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Uma Chandra Mouli Natchu
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Mamta Jajoo

More than 3 of the 7.6 million global deaths in children under 5 years of age occur in the neonatal period and severe infections like pneumonia and sepsis contribute to 25% of these deaths and are also a major cause of hospitalization in infants. Despite appropriate antimicrobial therapy, the outcome of these severe infections in early infancy is poor. It is important to develop inexpensive, effective and accessible interventions that can be added to standard therapy for severe infections to improve clinical outcomes and to reduce case fatality. In a recent randomised controlled trial in India, we found that daily oral zinc treatment had an efficacy of 40% against treatment failure in 7-120 days old infants with probable serious bacterial infection. The trial was not powered to evaluate the effect of the intervention on the risk of case fatality. Based on the promising results of the above-mentioned trial, a large, multicentre study powered to examine the effect of zinc on case fatality from very severe disease would contribute evidence towards revising treatment recommendations for low resource settings in South Asia and elsewhere.

This is an individually randomized double-blind placebo-controlled hospital-based trial involving 4000 young infants aged 1 day up to 2 months hospitalized in 7 hospitals of India and Nepal with very severe disease as defined by WHO IMCI. The intervention is daily oral administration of 10 mg of elemental zinc along with standard antibiotic therapy. Placebo along with standard antibiotic therapy is used as a comparator. If zinc treatment of young infants with very severe disease reduces the risk of death it should be incorporated into global and national guidelines.

The primary outcomes are (i) case fatality (ii) Time to death until 12 weeks post discharge; whereas the secondary outcomes are: (i) primary treatment failure (ii) time from enrolment to cessation of symptoms and signs of very severe disease (iii) time to failure of primary treatment (iv) time to hospital discharge (v) Time to death (vi) Death at any time after discharge from hospital until 12 weeks post discharge (vii) Severe illness or hospitalisation at any time after discharge from hospital until 12 weeks post discharge (viii) an evaluation of the incremental cost effectiveness of zinc supplementation (ix) survival and function of peripheral blood mononuclear cells and innate immunity.

We have identified the 7 hospital sites- 4 in Delhi and 3 in Kathmandu, Nepal and have initiated fortnightly skype meetings with all site investigators to discuss the site set up, ethics committee submissions, regulatory clearances, etc. We are in the process of preparing the documents for DCGI clearance for the regulatory trial.

Cross sectional study of cord blood immune markers in term appropriate for gestational age and small for gestational age neonates

Investigators

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Nitya Wadhwa

While infections are a major cause of neonatal mortality in India even in full-term neonates, this is an especial problem in the large proportion of neonates born underweight (or small-for-gestational-age; SGA). One potential contributory factor for this susceptibility is the possibility that immune system maturation may be affected along with intrauterine growth retardation. In order to examine the possibility that differences in immune status may underlie the susceptibility of SGA neonates to infections, a multicenter cross sectional study was initiated in 2011 to document the immune profile at birth in term appropriate for gestational age (AGA) and small for gestational age (SGA) newborns. We enumerated the frequencies and concentrations of 22 leukocyte subset populations as well as IgM and IgA levels in umbilical cord blood from full-term SGA neonates and compared them with values from normal-weight (or appropriate-for-gestational-age; AGA) full-term neonates.

The study was initiated first at a single site, AIIMS in April 2011. Subsequently, we included 3 more clinical sites: Maulana Azad Medical College (MAMC), and Vardhman Mahavir Medical College & Safdarjung Hospital (VMCC & SJH) in Delhi and General Hospital Gurgaon (GHG) in the National Capital Region. The proposal was sent for review and approved by the Institutional Ethics Committees of each of the hospital sites. Research personnel were recruited and trained in clinical data collection, taking written informed consent from the vulnerable population of pregnant women, collecting umbilical cord blood, immediate processing, temporary storage and transportation. Methods were standardized across the multi clinical sites. Regular standardization exercises were held among the research team members to minimize intra-observer and inter-observer variability within and across the 4 clinical sites.

The recruitment of participants has since been completed, double data entry completed and data analyzed and the manuscript published. An analysis of 502 samples, including 50 from SGA neonates, showed that there are some interesting differences in cellular lineages of the immune system of SGA neonates in comparison with AGA neonates. These differences possibly reflect stress responses in utero associated with growth restriction. Increased susceptibility to infections may thus be linked to complex immune system dysregulation rather than simply retarded immune system maturation.

This first study done at PBC has helped describe and characterize the phenotypes of 22 leukocyte subset frequencies from umbilical cord blood in term neonates and compare the phenotypes in full-term AGA and SGA neonates. It has also helped generate hypothesis for subsequent follow up studies, which we have initiated at PBC and are on-going.

Understanding the distinct development and functional properties of the neonatal immune system and their clinical consequences in the neonatal period

Investigators
Nitya Wadhwa
Shailaja Sopory
Pallavi Kshetarpal
Prasenjit Guchhait

Collaborators
Pratima Mittal
Safdarjung Hospital, New Delhi

India's high neonatal mortality contributes to nearly two-thirds of its high infant mortality, particularly in low birth-weight (LBW) neonates. Infections contribute to more than 25% of neonatal mortality. Immune system limitations are plausible contributors to this problem, particularly in LBW neonates. The neonatal immune system is known to be quantitatively and qualitatively distinct from the adult immune system, but the differences are not well enough understood as yet for explaining neonatal susceptibility to infections and for eventual interventions.

Our preliminary data, from a large-scale cross-sectional study undertaken at the Pediatric Biology Centre, which involved characterisation of 22 leukocyte subsets in umbilical cord blood from term small for gestational age (SGA) or appropriate for gestational age (AGA) neonates, show that SGA neonates have comparatively fewer plasmacytoid dendritic cells (pDCs), a higher myeloid DC (mDC) to pDC ratio, more natural killer (NK) cells, and higher serum IgM levels in cord blood. Further, SGA neonates showed a tendency to having relatively more inflammatory monocytes, fewer immature B cells and lower CD4:CD8 T cell ratios, although these were not robust associations. Moreover, our data show notable differences from Western data in neonate-adult comparisons as well, with B-1 B cell frequencies and higher immature B cell frequencies. Finally, our data also show low patrolling monocyte frequencies in cord blood, along with a widespread deficiency of vitamin D, known to be involved in the development and function of the immune system.

Based on these preliminary data, we propose to explore the neonatal immune system further at multiple levels in the following directions:

A. Immunobiology of newborns and clinical consequences in the neonatal period: We propose to develop a cohort of full-term neonates where we will expand our participant population so as to include more severely growth restricted SGA newborns, characterize their cord blood immune phenotype to test if the associations found in our preliminary data for SGA neonates are robust and follow them through early infancy to examine the correlations between cord blood immune phenotypes and susceptibility to infection-related morbidity.

B. Role of B cells in compromising neonatal immunity: We will purify and characterize the functionality of B-1 B cells, immature B cells and mature B cells from cord blood and adult blood with particular reference to the differences between LBW and NBW neonates, and will examine the mechanistic basis of the differences found.

C. The role of Notch pathway in neonatal T cell development: We will test the hypothesis that the differences in the CD4:CD8 ratio found between adults and neonates, as well as between SGA and

AGA neonates, is related to differences in the Notch signaling pathway.

D. Characterization of neonatal monocyte subsets and the role of vitamin D: We will purify and characterize differences if any in the monocyte developmental potential for hematopoietic progenitor cells from adult and SGA/AGA cord blood and analyse the role of vitamin D this process. We will also purify monocyte subsets from adult and SGA/AGA cord blood to characterise them in functional and molecular terms.

We have just received the sanction for this cohort study to be conducted at Safdarjung Hospital and General Hospital Gurgaon. We will initiate the process of developing the SOPs, recruiting staff and getting the necessary ethics approvals.

Neonatal immune profiles: infections and toxicants

Investigators

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Holden Maecker
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Collaborators

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Satyajit Rath

The immune phenotypes of newborns from different global populations have not been compared, but may hold a key to understanding differences in infection rates and other health outcomes around the world. For example, defects in B cell maturation and/or differentiation might result in a deficient antibody repertoire, which in turn may cause increased infection susceptibility early in life. Preliminary data (now published in PLoS One, 2015) from the Pediatric Biology Centre of Translational Health Science and Technology Institute (THSTI) in the National Capital Region (NCR) has suggested that there may be deficits in B-1 cells and immature classical B cells in Indian newborns compared to published results for Western populations. There is also evidence that environmental levels of toxicants such as arsenic and polycyclic aromatic hydrocarbons (PAH) are particularly high in many places in the developing world including in the New Delhi area. In this project, we propose to directly compare the immune phenotypes and functions of cord blood from a cohort of full-term babies in the U.S. (Stanford) and India (New Delhi). Our primary hypothesis is that there are significant differences in the frequency of B-1 cells and immature B-2 B cells between U.S. and Indian neonate cohorts. We also propose to examine whether these India-specific deficits we expect to find are accompanied by other deficits/differences in cord blood immune phenotypes. Our specific aims are:

- To standardize immunophenotyping and phospho-flow assays between Stanford and THSTI-NII, and to collect data on 50 umbilical cord blood samples from each site;
- To examine frequencies of B-1 cells and immature B-2 B cells between the two neonatal cohorts;
- To determine whether the two cohorts differ in the frequencies of any other sub-population of blood immune cells;
- To examine the functional status of cytokine signaling pathways in cord blood B cells from the two cohorts, in response to a panel of cytokines;
- To determine ranges of arsenic, PAH, and reported neonatal infection-related morbidities in the two cohorts. We will follow up these neonates over the first six months of life to collect data on infection related

morbidity and look for associations between immune profile at birth and frequency of infectious morbidity episodes

We got the necessary ethics approvals from THSTI and the hospital site ethics committees; have recruited the research personnel, developed case reporting forms, standard operating procedures for clinical data collection, cord blood collection and processing and have done the initial training of the staff for this and have standardized processes. Screening and enrolment was initiated in March 2015 and is ongoing.

Vitamin D supplementation to improve immune responses to vaccines administered in early infancy - The NutriVac - D Trial

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Co-investigators
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A large number of Indian infants seem to be deficient or insufficient in Vitamin D in their blood. We have undertaken a clinical trial to study if daily vitamin D supplementation to newborns (one RDA) can improve their responses to vaccines and thereby protect them from diseases like polio, hepatitis B and tuberculosis. Infants are followed up three times a week at home after enrolment in the trial. We are also studying if this supplementation improves growth and reduces infections, hospitalizations and deaths. As part of this study we are also assessing if vitamin D supplementation alters patterns of immune system development in Indian infants.

Uma Chandra Mouli Natchu

Social Innovation Immersion Programme (SIIP)

Investigators
Jonathan Pillai
Uma Chandra Mouli Natchu

Co-investigators
Shinjini Bhatnagar
Nitya Wadhwa

The Social Innovation Immersion Program (SIIP) has been initiated to catalyze innovation in health for the base of the pyramid in India by taking innovators into the heart of communities. PBC leverages existing partnerships with Civil Hospital, Gurgaon and tertiary hospitals including AIIMS, MAMC, and SJH for clinical mentorship and exposure to varying resource settings of healthcare. A team of three fellows is undergoing clinical immersion, beginning with community and home-based healthcare followed by brief exposures to tertiary hospitals. They will pick a few need-idea pairs based on burden of disease targeted and likelihood of success and will crystallize the solutions into executable components to create a prototype solution.

Molecular mechanisms of minimal change disease nephrotic syndrome: Role of CD80

Investigator
Shailaja Sopory

Collaborators
Vineeta Bal
Satyajit Rath

Shailaja Sopory

The most common type of nephrotic syndrome in children is minimal change disease (MCD) and is associated with massive proteinuria. It has been reported that a T- cell co receptor, CD80 (B7-1) is induced in the kidney podocyte and excreted in urine of patients with active MCD. Lipopolysaccharide (LPS) injected mice get proteinuria which is associated with CD80 expression on the podocytes and excretion in the urine. The CD80 null mouse is resistant to LPS mediated proteinuria, suggesting a role of CD80 in mediating disease.

The purpose of this initiative is to understand the mechanism of CD80 mediated proteinuria at the level of cellular and molecular changes in the podocyte. The study is looking at the effect of artificially increasing CD80 levels in podocyte cell lines on expression and localization of various podocyte specific proteins and signaling at the slit diaphragm, at the same time we are using LPS mediated mouse model of proteinuria to understand the upstream signaling processes that lead to increased expression of CD80 on podocytes. We also planned on generating transgenic mice overexpressing CD80 in the podocytes to be used as a model to study CD80 mediated proteinuria.

We had generated podocyte cell lines overexpressing CD80, and detailed characterization of these cells did not show any major changes in the expression of SD proteins as assessed by Western blot and RT-PCR analysis, though there was slight actin rearrangement in these cells. Injecting mice with LPS, poly (I:C) or PamCSK (different TLR ligands) caused CD80 uria. The TLR4 receptor mutant mice were resistant to LPS mediated proteinuria.

Using mouse bone marrow chimera between wildtype (B6) and CD80 null mice and between wildtype (CH3/Ouj) and TLR4 signaling deficient (CH3/Hej) mice we concluded that TLR4 receptor is required on the bone marrow cells and CD80 on the podocye cells to cause proteinuria. We saw that serum from LPS treated mice when added to podocytes in culture could upregulate CD80 expression on podocytes suggesting the presence of a soluble factor which is secreted by the bone marrow cells and acts on the podocytes. By a series of experiments we found TNF-α to be the soluble factor.

In future we would like to know the pathway involved in CD80 induction downstream of TNF-α binding to its receptor on podocytes. We also plan to look for CD80 interacting proteins in the podocyte.

Epithelial Barrier disruption during inflammatory responses

Investigator
Shailaja Sopory

Based on our work being carried out in kidney podocyte cells, where we are interested in looking at the role of CD80 expression in the podocytes during inflammation, we decided to look at the function of CD80 as a universal barrier disrupter based on literature where CD80 has been shown to be upregulated in keratinocytes, bronchiolar, alveolar, gastric and colonic epithelial cells on infectious or allergic stress.

Our preliminary work with Caco-2 cell line has shown induction of CD80 in

these cells on incubation with lipopoly-saccharide (LPS) and Puromycin amino nucleoside (PAN).

Subsequently we generated stable cell lines of CaCo2 expressing CD80 and looked its effect on expression of tight junction proteins. We observed that CD80 expression causes mislocalization of ZO-1 and occludin (Figure 1).

We propose to look at the effect of CD80 induction on other tight junction proteins and also look at functional implication of CD80 expression in terms of Transepithelial resistance and permeability assays.

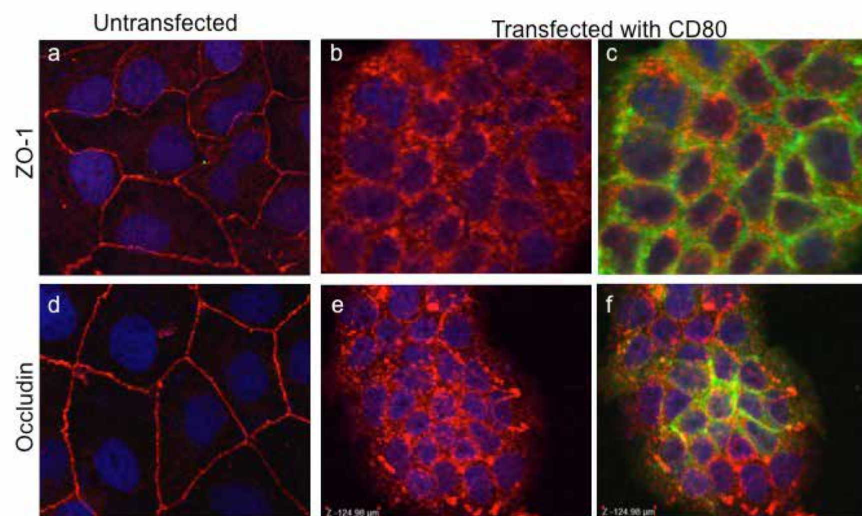

Figure 1. Caco2 cells were transfected with human CD80 construct using lipofectamine LTX. Cells were harvested 48h post transfection and IF was carried out for CD80, ZO-1 and Occludin. The untransfected (UT) cells shown in the figures are untransfected regions from the CD80 transfected cells. a) ZO-1 UT b) ZO-1 in CD80 transfected cells c) merged image ZO-1 red CD80 green d) Occludin UT e) occludin in CD80 Transfected cells f) merge image CD80 green Occludin red. Blue-Dapi

Understanding the distinct developmental and functional properties of the neonatal immune system and their clinical consequences in the neonatal period: Characterization of neonatal monocyte subsets

Investigators
Shailaja Sopory
Nitya Wadhwa
Pallavi Kshetrapal
Prasenjit Gucchait

A large scale immunophenotyping study with ~500 umbilical cord blood samples has been carried out at the Pediatric Biology Centre. Thirty different cellular parameters were analyzed to compare the immune profile from “small for gestational age” (SGA) and “average for gestational age” (AGA) babies. Adult blood was also analyzed at the same time to compare the immune profile between cord blood and adult blood. This study is the first to look at frequencies and number of patrolling and inflammatory monocytes in cord blood and is the largest cord blood data set for all other cellular parameters also. We are interested in characterizing monocyte subsets from cord blood to see if there are any differences at the functional or transcriptional level between adult and cord blood.

HLA-G 5'URR genotyping in small for gestational age neonates compared to appropriate for gestational age neonates

Investigator
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Co- Investigators
Shinjini Bhatnagar
Nitya Wadhwa

Collaborators
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Pallavi Kshetrapal

Small for gestational age (SGA) babies has been a rising public health problem where these babies are at a higher risk for neonatal mortality, morbidity and have a poor neurological outcome. Identification of the materno-fetal problems that lead to birth of SGA babies has been of major concern. There is a growing body of evidence suggesting pregnancy complications such as preeclampsia, recurrent pregnancy loss (RPL), Intra Uterine Growth Restriction (IUGR), and premature birth could be associated with aberrant immunologic interactions at the fetal-maternal interface. These aberrant immunological interactions have been reported to be due to differences in the HLA-G expression in the extravillous trophoblasts lining the maternal-fetal side of the placenta. Human leukocyte Antigen G (HLA-G) is a nonclassical major histocompatibility complex (MHC) class I molecule that exists as membrane bound or soluble isoforms. HLA-G is expressed on the placental trophoblast cells on the feto-maternal interface. Rouas-Freiss et.al, were the first one to show that HLA-G expressing trophoblasts were protected from maternal NK-cell mediated cytolysis. HLA-G binds to the immunoglobulin-like transcript (ILT) receptors, expressed on T lymphocytes and B lymphocytes, NK cells and mononuclear phagocytes, and abates activating signals received by these cells. All these findings indicate that HLA-G molecule plays an important role in fetal-maternal tolerance. Studies have demonstrated an increase in the levels of soluble HLA-G in the maternal serum of pregnant women. High levels of sHLA-G have also been associated to successful implantation in IVF procedures. Circulating HLA-G levels, early in pregnancy, have been reported to be an early predictor for the development of preeclampsia. The expression levels of HLA-G have been linked to genetic variation in the HLA-G gene. Despite evidence for HLA-G playing an important role during pregnancy, the precise relationship between genetic variation in HLA-G and the pregnancy outcome, ie birth of SGA/AGA neonates remains unresolved. The first study to report associations between the HLA-G polymorphisms and birth-weight of a neonate demonstrated the presence of a 14 bp polymorphism in the 3' UTR part of the HLA-G gene increased the birth weight of the neonates in relation to its gestational age. Interestingly no such study that links the HLA-G expression to birth of SGA or AGA babies has ever been done in an Indian population. We plan to study these SNPs at the 5` URR region of the gene. Performing the SNP sequencing for the gene and how these polymorphisms lead to change in expression patterns of this protein will help us investigate the biological phenomenon of HLA-G production by the fetus. We hope that these finding may have an impact on translational medicine.

Statutory clearances were obtained and standardizations of macro-dissections of the human placental tissue was carried out for collecting the desired sections for DNA isolation and histo-pathology. DNA oligomers (nested-primers) were designed, synthesized and used for Sanger sequencing on the PCR amplified promoter region of the HLA-G gene. Reference sequence has been created by sequencing samples from three appropriate for gestational age neonates.

We have initiated the collection of human bio-specimens, namely maternal blood, cord blood and placental tissue. Clinical data is being collected along

with subsequent DNA isolations, such that when we a substantial number of samples is, we would give them for sequencing as the promoter region to identify associations of the single nucleotide polymorphisms.

Role of Notch synergies in acute lymphoblastic leukemia

Investigator
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Collaborator
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Our study focuses on the role of Notch and its synergies in pediatric T and B-ALL. Childhood acute lymphoblastic leukemia (ALL) is an aggressive type of hematologic malignancy that results from malignant transformation of normal developing T or B cells. The most commonly found translocation in pediatric T-ALLs is a rearrangement between chromosomes 7 and 9 that results in the fusion of the coding region of the intracellular region of the NOTCH1 gene to the T-Cell Receptor (TCR) enhancer, driving constitutively an active form of NOTCH1 in T cells. Recent reports about the involvement of Notch 3 and Hes5 in B-ALLs further substantiate our objective to investigate for the Notch synergies in these disease conditions. The Notch signaling pathway plays important roles in the regulation of cell differentiation, proliferation and apoptosis in many developmental systems. Notch receptor activation per se can be oncogenic, as has been shown to be the case in more than 50% of T-cell lymphoblastic leukaemia (T-ALL). Though it is now becoming clear that Notch signals are synergistically involved in many oncogenic events, the complete roster of genes capable of cooperating with Notch receptor activation to influence proliferation events is not yet known. In my earlier work, I carried out a modifier screen utilizing Drosophila genetics and a unique collection of mutations housed at the HMS to search the genome systematically for genes capable of synergizing with a constitutively active Notch receptor to influence proliferation in vivo. Initial analysis of the modifiers obtained from the screen has been encouraging and demonstrates that many of the human homologues of these fly modifiers have be reported and documented to be involved in cancers, like Hepatocellular carcinoma, prostrate cancer, Breast carcinoma, colorectal cancers and many more. Therefore we embarked studies to probe the other candidates to test if these candidates have a role to plan in the human T-ALLs.

We began our investigations by carrying out expression analysis of the candidate genes in Jurkat cell line, a T-ALL specific cell line and standardized Realtime PCR conditions for the gene expression profiling of receptors (Notch), ligands of Notch, candidate synergistic factors. We have now analysed the expression of NOTCH and a few other candidate modifier genes (LMO2, HLF) in pediatric B-ALL samples by qPCR using SYBR green chemistry and normalized the data with GAPDH and compared it with healthy controls.

Literature reveals aberrant induction of LIM domain only 2 (LMO2) in B-precursor ALL with t (17; 19) translocation. Clinically, high LMO2 expression correlated with better overall survival in adult patients. Interestingly we have analysed LMO2 gene expression in ~ 30 samples, among them 88% of the samples reveal down regulation. Thus if high expression of LMO2 is correlated with better outcome, then it would be interesting to follow the LMO2 expression in patients under induction of chemotherapy.

The other candidate gene we are following up in our analysis is Hepatic leukemia factor (HLF). In pro-B cell (ALL), expression of the E2A-HLF gene as a

result of t (17;19) (q22; p13) is associated with poor prognosis. We have analysed HLF expression in ~ 23 samples (pre-B samples) and intriguingly, among them 74% of the sample shows up regulation, 8% does not show any fold change and 4% shows down regulation.

Thus our initial results reveal a trend in the expression pattern of two candidate genes in the patients when compared to the controls, further we would like to understand the mechanism these genes adopt in the progression of the disease using in vitro cell culture approaches. Yet another important link missing is how these genes interact with the Notch pathway and using such approaches may help us unravel this link too.

Population-level heterogeneity of Human Immune cytome and its Biological meaning

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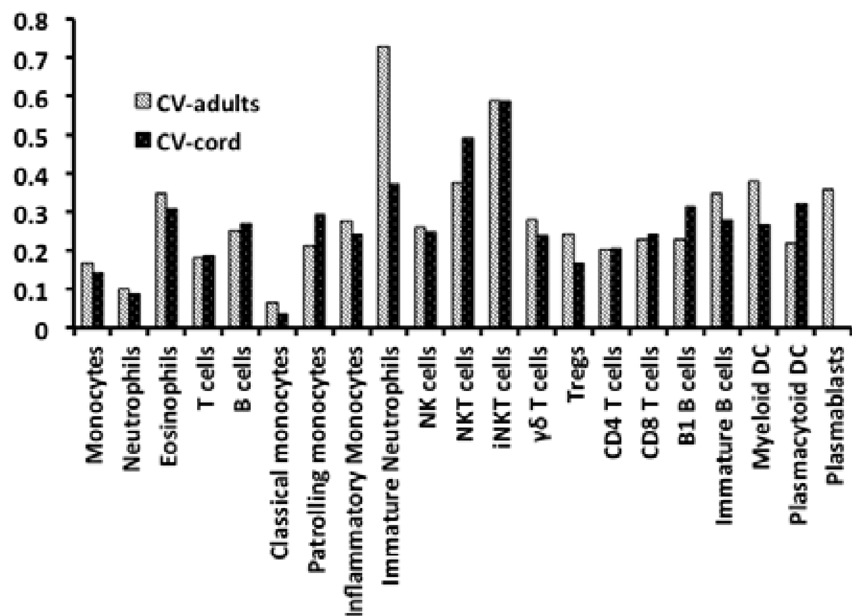
Vineeta Bal

Human populations show considerable inter-individual diversity in immune phenotype and function. Multitudinous factors including genetic, developmental and environmental elements affect various stages of Immune system development and function, and hence, Immune phenotype in individuals. To explore the relative contributions of diversity in environmental and developmental factors in determining immune phenotype diversity at the population level, we compared variances of immune phenotypes in healthy adult volunteers with that of neonatal (umbilical cord) blood using multi-colour flow cytometry. Immune diversity in adult population is likely to be predominantly influenced by environmental factors and that of neonatal population by developmental factors. There was considerable population-level diversity in both adult and neonatal peripheral blood Immune phenotypes. Population-level diversity varied from lineage to lineage, some lineages showing considerably more diversity in comparison to others. On comparing adult and neonatal Immune phenotype diversity for each Immune lineage, some lineages showed higher variance in the adult, suggesting predominant influence of environmental factors. Some lineages showed higher variance in neonates, suggesting predominant influence of developmental factors since the noise due to developmental immaturity in neonates stabilizes and attains mature levels in the adult population. Most of the lineages showed similar variances in adult and neonatal population, suggesting that most Immune lineage phenotypes are predominantly governed by genetic factors. Across lineages, cell types that clearly respond to environmental factors (like plasmablasts), showed much higher variance compared to others. We suggest that such strategies of looking at population-level heterogeneity with respect to phenotype or genotype in natural outbred populations would be an interesting approach to pursue to gain meaningful insights. Population-level-variances represent heterogeneity in complex discrete factors that determine a phenotype, and hence confer important biological information. To further dissect the contributions of heterogeneity in environmental and genetic factors in determining diversity of Immune phenotypes in natural populations, we started a human family-based study. Immune phenotypes that are predominantly genetically determined would show concordance in siblings.



Coefficient of Variation (CV) for various Immune parameters in cord and adult population

After obtaining institutional ethics committee clearance, healthy adult volunteers and healthy neonates born of normal delivery were recruited for the study. A 5-cocktail staining protocol was optimized with the limited amount of blood available. Cord blood was collected from 80 healthy neonates and from 80 adults and immunophenotyping done on these samples. This part of the study has been completed (Figure attached). For the family-based study, we have recruited 75 adult sibling pairs. Protocol for cryopreservation of peripheral blood mononuclear cells has been optimized to ensure cell viability, intensity of surface staining and functional activity. The protocol for complete immunophenotypic characterization using 3 antibody cocktails has been optimized and tested parallely in both fresh and frozen peripheral blood mononuclear cells to ensure accuracy and uniformity. These sibling blood samples are cryopreserved at present and flow cytometry will be done later after collection of all the samples. Peripheral blood from a group of 60 adult volunteers has been collected monthly for a period of one year to look for intra-individual fluctuation in immune phenotypes. Similar to the family-based study, to ensure uniformity in flow-cytometry, the peripheral blood mononuclear cells have been cryopreserved for immunophenotyping later.

Peer-reviewed publications

1. Lodha R, Mukherjee A, Singh V, Singh S, Friis H, Faurholt-Jepsen D, Bhatnagar S, Saini S, Kabra SK, Grewal MH (2014) Delhi Pediatric TB Study Group. Effect of micronutrient supplementation on treatment outcomes in children with intrathoracic tuberculosis: a randomized controlled trial. Am J Clin Nutr 100(5):1287-97.
2. Lodha R, Shah N, Mohari N, Mukherjee A, Vajpayee M, Singh R, Singla M, Saini S, Bhatnagar S, Kabra S (2014) Immunologic effect of zinc supplementation in HIV infected children receiving highly active antiretroviral therapy: A randomized, double blind placebo controlled trial. J Acquir Immune Defic Syndr 66(4):386-92
3. Mukherjee A, Saini S, Kabra SK, Gupta N, Singh V, Singh S, Bhatnagar S, Saini D, Grewal HM, Lodha R; Delhi TB Study group (2014) Effect of micronutrient deficiency on QuantiFERON-TB Gold In-Tube test and tuberculin skin test in diagnosis of childhood intrathoracic tuberculosis. Eur J Clin Nutr 68(1):38-42.
4. Negi R, Dewan P, Shah D, Das S, Bhatnagar S, Gupta P (2014) Oral zinc supplements are ineffective for treating acute dehydrating diarrhoea in 5-12-year-olds. Acta Paediatr 104(8):e367-71.
5. Prasad K, Sharma A, Garg A, Mohanty S, Bhatnagar S, Johri S, Singh KK, Nair V, Sarkar RS, Gorthi SP, Hassan KM, Prabhakar S, Marwaha N, Khandelwal N, Misra UK, Kalita J, Nityanand S; for InveST Study Group (2014) Intravenous autologous bone marrow mononuclear stem cell therapy for ischemic stroke: a multicentric, randomized trial. Stroke 45(12):3618-24.
6. Rathore DK, Nair D, Raza S, Saini S, Singh R, Kumar A, Tripathi R, Ramji S, Batra A, Aggarwal KC, Chellani HK, Arya S, Bhatla N, Paul VK, Aggarwal R, Agarwal N, Mehta U, Sopory S, Natchu UCM, Bhatnagar S, Bal V, Rath S, Wadhwa N (2015) Underweight full-term Indian neonates show differences in umbilical cord blood leukocyte phenotype. PLoS ONE 10(4):e0123589.
7. Singh P, Wadhwa N , Lodha R, Sommerfelt H, Aneja S, Natchu UCM, Kabra SK, Bhatnagar S §, Strand TA (2015) Predictors of time till recovery in infants with probable serious bacterial infections. PLoS ONE 10(4):e0124594.
8. Singh P, Wadhwa N, Chaturvedi MK, Bhatia V, Saini S, Tandon N, Makharia GK, Maki M, Not T, Phillips A, Bhatnagar S (2014) Validation of point-of-care testing for coeliac disease in children in a tertiary hospital in north India. Arch Dis Child 99(11):1004-8.

Seminars and conferences

Nitya Wadhwa

Title of workshop:	Good Laboratory Practice (GLP). An Awareness Program
Place and date:	THSTI, Faridabad, April 22, 2015
Title of workshop:	Causality assessment for Clinical trials in the Indian Scenario
Place and date:	Gurgaon, April 18, 2015

Title of workshop: Current Regulatory Requirements for Members of Institutional Ethics Committee
Place and date: THSTI, Faridabad, April 9, 2015

Title of workshop: CISMAC workshop – From Concept to Study
Place and date: Centre for International Health, Bergen, Norway, September 9-12, 2014

Shinjini Bhatnagar

Title of the talk: Management of persistent diarrhoea in children
Name of the meeting: Expert meeting to strategize the scale up of management of Persistent diarrhoea in children
Place and date: WHO, Geneva (April 7-9, 2014)

Name of the meeting: Discussion to finalize Diarrhoea guidelines- a deliberation with Expert Ministry of Health & Family Welfare
Place and date: Nirman Bhawan, New Delhi (April 28, 2014)

Title of the talk: Celiac disease
Name of the meeting: Indian Society of Pediatric Gastroenterology Hepatology and Nutrition
Place and date: New Delhi (July 18-20, 2014)

Name of the meeting: Meeting on Global Coalition to Advance Preterm birth Research (GCAPR)
Place and date: Washington (July 27-28, 2014)

Name of the meeting: Meeting of Yakult India Microbiota and Probiotic Science Foundation
Place and date: New Delhi (September 27, 2014)

Title of the talk: Multi-Disciplinary Inter-Institutional program to advance scientific knowledge around preterm birth"
Name of the meeting: Grand Challenges Meeting
Place and date: Washington (October 5-8, 2014)

Title of the talk: Clinician-researcher collaboration: a collaborative relationship in the changing Healthcare landscape
Name of the meeting: Workshop on Diabetes and Metabolic syndrome: Networks, Crosstalks & Interventions organised by Joint IUBMB-RCB Advanced School-2014
Place and date: Manesar (Nov 24-28, 2014)

Title of the talk: All Children Thriving
Name of the meeting: Meeting on 'Enigma Convening
Place and date: New Delhi (Dec 8-10, 2014)

Title of the talk: Gut, Its Microbes and Health
Name of the meeting: Yakult India Microbiota and Probiotic Science Foundation
Place and date: New Delhi (March 7-8, 2015)

Title of the talk: Building capacity for Implementation Research
Name of the meeting: 5th Annual Research Symposium of Public Health Foundation of India
Place and date: New Delhi (March 11-13, 2015)

Name of the meeting: Biorepository Synchronization meeting
Place and date: London (April 07-12, 2015)

Extrmural Grants

Nitya Wadhwa

Funding agency: Department of Biotechnology, Govt of India
Amount: Rs. 88, 08576/-
Duration: From March 14, 2014 To March 13, 2016
Title of the grant: Neonatal immune profiles: infections and toxicants

Shinjini Bhatnagar

Funding agency: Research Council of Norway
Amount: 18020 million Norwegian Kroner
Duration: From September 1, 2014 To August 31, 2017
Title of the grant: Zinc as an adjunct for the treatment of very severe disease in infants younger than 2 months

Funding agency: Centre for Intervention Science in Maternal and Child Health, Norway
Amount: 6.5 million Norwegian Kroner
Duration: From June 1, 2015 To February 28, 2021
Title of the grant: Zinc as an adjunct for the treatment of very severe disease in infants younger than 2 months

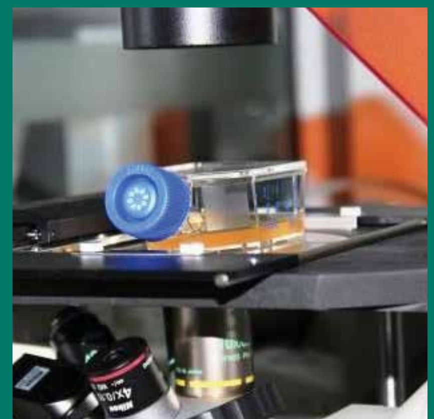
Funding agency: Department of Biotechnology, Govt of India
Amount: Rs. 1,68,57,440/-
Duration: From March 27, 2015 To March 26, 2018
Title of the grant: Understanding the distinct development and functional properties of the neonatal immune system and their clinical consequences in the neonatal period

Patents and Technology Transfer

Technology for ELISA kits for rapid diagnosis of celiac diagnosis were transferred to J. Mitra and Co and were commercialised in October 2014

Bhatnagar S, Khanna N, Natchu UCM, Wadhwa N, Gupta S, Bagga A, Saini S. "A method and device for detection of anti-transglutaminase antibodies" (patent no. 1133/DEL/2011 dated 18-04-2011).

Centre for Biodesign and Diagnostics



An Overview

Shinjini Bhatnagar

The Centre for Biodesign and in vitro Diagnostics (CBD) was established at the Translational Health Science and Technology Institute (THSTI) in 2011 with the mission to create medical technology innovation in India for affordable health care utilizing the biodesign concept and support services that extend from strategic bench work to commercialization. This centre aims to transform the field by creating a novel medical technology enterprise in India for affordable health care through a "bio-design process", which essentially utilizes inputs from clinical-care settings to innovate or improve existing designs. The centre is also promoting an effective translational route for basic findings into routine applications through a multidisciplinary approach, combining new biomarkers, novel technological concepts and clinical insights. CBD also recognizes the need to provide an organizational structure, ecosystem and governance process that ensures long-term sustainability and scope for growth for a new cadre of professionals who work at the interface of biology, engineering and medical science. These readily accessible processes and facilities will support a collaborative model of working, public-private partnership and entrepreneurship development through a multi-disciplinary approach.

The National Biodesign Alliance (NBA) was initiated by DBT to facilitate exchange of ideas, expertise, and resources between the partner institutions in order to collectivity address the whole value chain for development and delivery of affordable diagnostics and medical devices, in an integrated manner. CBD at THSTI operates as the anchoring secretariat of NBA.The CBD-NBA program was initiated in 2010 (functionally initiating operations in 2011) and among a very few of its kind in India with a process of need driven technology innovation with a wide focus of diagnostics, devices, implants and drug and vaccine delivery systems.

The steady addition of faculty, research fellows and staff at CBD in multiple fields and their training both at CBD and at partner institutions have led to the building of an inter-disciplinary research team of antibody engineering experts, genomics experts, proteomics experts, recombinant protein production experts, biomedical engineers and clinicians. In addition, CBD has developed and sustained collaborative networks with many tertiary hospitals in Delhi and NCR in order to work at the interface of clinical need and biomedical innovations. A state-of-the-art laboratory facility have been developed including facilities for high throughput screening of diagnostic

targets, antigen-antibody interaction analysis, high throughput high yield clone selection, bioprocess development and optimization, multiplex array-in-well assay development, rapid diagnostic test prototype development, that are essential for the work initiated by CBD and planned future developments. These facilities are supported by well-trained laboratory teams of technical staff with continuous mentoring by core and program faculty. The resources developed at CBD, both lab facilities and trained staff, are intended to serve as platforms that can be used across multiple innovation partners.

CBD has initiated distinct research programs over the first four years to identify new biomarkers, develop and engineer new antibody reagents for diagnostic use, develop new platform for cost-effective production of diagnostic reagents, design novel assays by need driven product innovation and establishing biodesign process for devices and diagnostics innovations. Based on the initial studies new research programs have been conceptualized to develop novel detection technology for nucleic acid based diagnostics, novel drug delivery platforms and new bioprocess technologies for enhanced protein production and stability. These studies will be platforms to develop futuristic affordable health technology innovation.

As a result of the innovative research and development initiatives, CBD has produced two patent applications for typhoid diagnostic products, one patent on improved mammalian bioprocess for increasing product stability and one patent application on engineered antibodies for improved binding. CBD has also initiated exploring techno-entrepreneurship opportunities by establishing a faculty start-up focused on diagnostics. Apart from these translational outputs, CBD faculties have also published well acclaimed peer reviewed publications in international journals. CBD has worked effectively with intra mural funding and has also been successful in generating significant extra mural funding from DBT, BIRAC and ICMR.

Additional initiatives have been taken on education with the start of a coursework for biodesign, which has set the standard for need driven healthcare product innovation for Ph.D. students in India. CBD has also successfully hosted a bi-lateral post-doctoral fellowship program with the international partner (University of Turku) for training young researchers in the area of new high end diagnostic technology platforms.

Technology platform for simple and efficient production of recombinant antibodies and antigens

Investigator
Gaurav Batra
Collaborator
Navin Khanna
ICGEB, New Delhi

Gaurav Batra

There is an increasing demand of recombinant antibodies and high quality recombinant antigens for the development of ultra-sensitive in-vitro diagnostic immunoassays. Use of recombinant antibody fragments like Fab in immunoassay gives several advantages e.g. elimination of interference caused by heterophilic antibodies, site specific labeling, high performing capture surface through oriented immobilization etc. Use of high quality recombinant antigens removes the risk associated with the cultivation of pathogenic organism for the purification of desired antigens. Also, immunoassays based on highly purified correctly folded recombinant antigens give less background compared to assays based on partially purified native antigens. Despite the advantages of recombinant antibodies and antigens, their use in commercial diagnostic assays is not widespread largely because of the availability, cost and production related issues (expression yields and aggregation). In this project, yeast *Pichia pastoris* based expression toolbox utilizing different media additives and different genetic elements (promoters, signal sequences, folding and secretion aids) is being developed, which can be applied for simple, cost effective, high yield production of recombinant antibodies and antigens.

In this project, we have developed new transformation protocol which allows to achieve >10 times higher transformation efficiency compared to traditional electroporation protocols which result in easy isolation of clones with different copy numbers including very-high copy no. clones (>10 copies). For the high-throughput expression screening of transformants in 96-well plate format, we have optimized the shaking parameter, aeration conditions and architecture of the plate to overcome the problem of cell clumping. In the optimized cultivation conditions in 96-well format, we are able to achieve cell density of 60 (OD_{600}) in minimal media and successfully expressed the proteins with expression levels similar to shake-flask. This is a big achievement, as *P. pastoris* cells are known to settle very quickly in the bottom of wells that result in growth cessation. The developed high-throughput expression-screening platform allows us to screen more than 1000 transformants in one round. After developing high-throughput screening platform, we have evaluated the efficiency of 11 different signal sequences for the secretion of diagnostically important proteins. Ten out of 11 signal sequences were able to secrete the recombinant proteins in *P. pastoris* culture supernatant but the secretion efficiency differed heavily. Analysis of secreted proteins using N-term sequencing revealed that 3 out of 10 signals are not suitable for the secretion of recombinant antibodies as they leave extra amino acids on N-term of the recombinant protein. We have plans to evaluate the effect of different promoters, over expression of different chaperones, other folding and secretion aids on the secretion of recombinant antigens and antibodies. We are also in process to identify media additives, which help in the enhancement of recombinant antigens and antibodies secretion in *P. pastoris*. Till now we have developed the improved transformation protocol, developed high-throughput expression and screening method which allows screening of >1000 clones in a single round, and identified secretion signals suitable for the secretion of recombinant antibodies.

Diagnostic development for acute febrile illnesses

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Urpo Lamminmäki
University of Turku, Finland

Anti-dengue virus antibody detection for routine diagnosis and sero-surveillance: The team was involved in the development of an indigenous test for the detection of anti-dengue virus (DENV) antibodies with very high specificity using DENV specific chimeric antigen. Now this team is focusing on improving the sensitivity of DENV specific assay by identifying new DENV specific immunodominant epitope/antigenic fragment using phage display library based approaches. Some of the identified epitopes are being evaluated using dengue virus positive sera panel. The idea is to incorporate these sequences in the new generation assay to increase the assay sensitivity without compromising the specificity.

Dengue virus NS1 antigen detection for routine diagnosis and surveillance: The team was also involved in the generation of indigenous DENV NS1 antigen detection assay. The current efforts are being put on the generation of new pairs of monoclonal antibodies to increase the NS1 antigen detection window and also the generation of serotype specific anti-NS1 antibodies. In collaboration with University of Turku, several anti-NS1 antibodies have been isolated using human framework synthetic antibody libraries. Using subtractive panning approach, several unique antibodies clones have been isolated which recognize the NS1 from four different dengue serotypes in a serotype specific manner. Though, the serotype specific anti-NS1 antibodies do not have any significant value in routine diagnostics but these tools are required to develop an assay for epidemiological surveillance to know the serotype of the circulating dengue virus. Also, the serotype specific NS1 Ag assay can be used during large vaccine clinical trials. This kind of assay may also give information about co-infection of two dengue serotypes. Till now we have generated 18 pan dengue anti-NS1 binders (recognizing all the four dengue serotypes), 11 dengue-1 specific binders, 2 dengue-2 specific binders, 3 dengue-3 specific binders, and 4 dengue-4 specific binders. Usefulness of these binders is being evaluated and some of the clones are being affinity matured so that commercially viable assay can be developed. In a phased manner, the project will be extended to the reagents and assay development for other important febrile illnesses.

Developing an antigen detection based daignostic assay using typhi specifc immunodominant motifs

Investigator
Ashutosh Tiwari

Collaborators

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Navin Khanna
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Typhoid fever is a significant global health problem with highest burden on the developing world. The severity of typhoid is often underestimated, and currently available serological diagnostic assays are inadequate due to lack in requisite sensitivity and specificity. This underlines an absolute need to develop a reliable and accurate diagnostics that would benefit long-term disease control and treatment and to understand the real disease burden. Here, we have utilized flagellin protein of S. typhi that is surface accessible, abundantly expressed, and highly immunogenic, for developing immunodiagnostic tests. Flagellin monomers are composed of conserved amino-terminal and carboxy-terminal, and serovar-specific middle region. We have generated a panel of murine monoclonal antibodies (mAbs) against the middle region of flagellin,

Ashutosh Tiwari

purified from large culture of S. typhi to ensure its native conformation. These mAbs showed unique specificity and very high affinity toward S. typhi flagellin without showing any cross-reactivity with other serovars. Genetic analysis of mAbs also revealed high frequency of somatic mutation due to antigenic selection process across variable region to achieve high binding affinity. These antibodies also displayed stable binding in stringent reaction conditions for antigen–antibody interactions, like DMSO, urea, KSCN, guanidinium HCl, and extremes of pH. One of the mAbs potentially reversed the TLR5-mediated immune response, in vitro by inhibiting TLR5–flagellin interaction. We have also showed binding analysis of these mAbs to the S. typhi flagellin, by flowcytometry, which demonstrates the practical possibility of detection of native conformation of flagellin, present on different bacterial surface, characterized by higher specificity and no cross reactivity. Therefore, these mAbs may be used in the generation of rapid and real-time systems for the detection of flagellated bacteria in contaminated food and therefore prevent intestinal infections. Through the sandwich ELISA, we have also demonstrated the application of these anti-flagellin mAbs, in developing a test for detecting soluble flagellin in serum, using as capture antibody and detecting antibodies. This sandwich ELISA appears to be quite sensitive with a detection limit of about 15 ng/ml. In conclusion, we have generated a repertoire of robust monoclonal antibodies against S. typhi flagellin, which may be used in the development of improved diagnostics, particularly in terms of rapidity, sensitivity, and specificity, vis-a-vis the ones currently available.

Development of highly specific and sensitive diagnostic test for typhoid: identification of novel biomarkers secreted exclusively after infecting human cells

Investigator
Ashutosh Tiwari

Collaborators
Shinjini Bhatnagar
Susmita Chaudhuri
Niraj Kumar
Navin Khanna
ICGEB, New Delhi

S. typhi causes systemic infection, typhoid, almost exclusively in humans. The major problem with typhoid diagnostic is lack of suitable infection model as *S. typhi* is human specific patogen. To overcome this, we have established an *in vitro* model system for *Salmonella* infection using polarized intestinal epithelial cell (IEC) culture system that mimics infection of this pathogen in the gut. We have establish this model by setting up a polarized IEC cell culture system on transwell plates containing microporous filter inserts to investigate differences in host responses and to identify *S. typhi* specific unique proteins produced during infection. Caco2 cells are being used in this study to develop an in vitro infection model for typhoid. Increased IL-8 cytokine level is used to determine the effective salmonella infection in this model. The investigations in this proposal would include use of comparative protein expression profiling of IECs will be analyzed by advanced proteomics approach. The study should give valuable information about the dynamics of host-pathogen interactions during infection of humans with *S.typhi* and provide insights into possible specific biomarkers for certain diagnostic assays.

Using *in vitro* infection model, we have identified three *S. typhi* specific proteins that are secreted exclusively after infection to human cells. To validate the possibility of these proteins as specific biomarker for diagnostic assay, we have cloned, expressed and purified these proteins using *E.coli*

expression system. One of the candidate protein was purified from inclusion bodies under denaturing condition, and was dialysed to remove denaturants. This purified protein was then used in IgM serum ELISA performed with Widal positive serum samples along with healthy volunteers serum as control. The result indicated that this S.typhi protein could be used to detect antibody response as extremely specific to typhoid infection. Currently we are preparing a significant amount of this protein to make this protein available to our industry partner for further validation to use it as potential diagnostic candidate.

Generation of anti-preS1–specific human neutralizing antibodies as possible therapeutic and diagnostic modalities.

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Neutralizing monoclonal antibodies are being found to be increasingly useful in viral infections. In hepatitis B infection, antibodies are proven to be useful for prophylaxis. There are also indications of their possible use for improving therapeutic outcome. The preS1 region (21-47 a.a.) of the hepatitis B virion contains the viral hepatocyte-binding domain crucial for its attachment and infection of hepatocytes. Antibodies against this region are present in the serum of spontaneously recovered individuals and have a high neutralizing capacity. Such antibodies are best suited for the immune based neutralization of hepatitis B especially in view of their not recognizing decoy particles. We have generated a phage-displayed scFv library using circulating lymphocytes of such individuals. Four preS1 peptide-specific antibodies, with unique CDR and framework sequences that bound to the peptide from the region 21-47 a.a. were selected from this library. These also bound to blood-derived antigen as well as to full-length recombinant preS1 antigen (108a.a) showing that they bound to the naturally folded peptide. Modeling shows that the scFvs bound to different amino acids within the preS1 peptide region. Ability to prevent binding of the preS1 peptide to HepG2 cells was taken as a surrogate marker for neutralizing capacity. These antibodies inhibited preS1-hepatocyte interaction individually and even better in combination. Such a combination of potentially neutralizing recombinant antibodies with defined specificities could be used for preventing/managing HBV infections, including by possible escape mutants.

CHO cell line engineering for improved product yield and stability

Investigators
Niraj Kumar
Susmita Chaudhuri
Ashutosh Tiwari

The demand for complex recombinant proteins for therapeutic and diagnostics is increasing worldwide and hence improvements in the overall yield of such products from bioprocess are of great interest to make them affordable. Chinese hamster ovary (CHO) cells are the most commonly used cell lines for large scale production of such high-quality (human-like) recombinant protein products. Typically, product is released, along other secretory proteins, by the cell into the culture media. These secreted proteins may have significant impact on cell growth, product quality & quantity during the production culture and the designing of strategies for efficient product purification. In our

Niraj Kumar

laboratory, it is noticed that ~50% of the product gets lost during production process by the cells and/or the components present in the culture (Figure-1).

Besides, a significant amount of product gets degraded by the component present in the culture media during storage and product purification process. If this loss could be minimized, it would help in improving the overall yield of the product from such production cultures and contribute in making product affordable. This is only possible by improving our understanding for biomolecules present in culture media. However, only few efforts have been made todate to explore these secreted proteins (the "secretome"), although significant technological advancements have been witnessed in the field of proteomics during the last two decades. Even from these, the majority of studies have identified a high proportion of intracellular- and non-secretory proteins (upto ~ 88%) in culture supernatant, even with cultures maintaining significantly high viability (>95%). Since no published data are available, it could be presumed that the percentage of these intracellular and non-secretory proteins identified in the secretome might be derived from on-going cell death in the culture. Hence the percentage of proteins-potentially-contributed-by-cell-death (PPCD) in culture medium can be calculated by multiplying per-cell-protein content with the number of dead cells in the culture. A comparison of PPCD with the total amount of protein in the culture supernatant at any given time point may enable the early prediction of the percentage of such proteins among secreted proteins. However, in our laboratory we observed that the calculated concentration of PPCD is significantly higher than the total protein in the culture media at all-time points investigated (Figure-2). This suggests that high proportion of PPCD is being degraded by the biomolecules released by the cells and/or consumed by the viable cells present in the culture media, besides concurrent secretion of certain secretory proteins. Although if PPCD are degraded and/or consumed selectively and contribute to the identification of high number of intracellular proteins in the secretome is unclear, yet it is known that the composition of secretome modulates dynamically over time and affect cell growth & recombinant protein production in culture. However, the knowledge of genuinely secreted CHO proteins is very limited which could be possibly due to the unavailability of well-defined methodologies for sample collection & preparation for mass-spectrometry and/or followed data analysis using databases that contain less number of secretory protein and hence the potential of secreted proteins in regulation of recombinant protein production from CHO cultures largely remained unexplored.

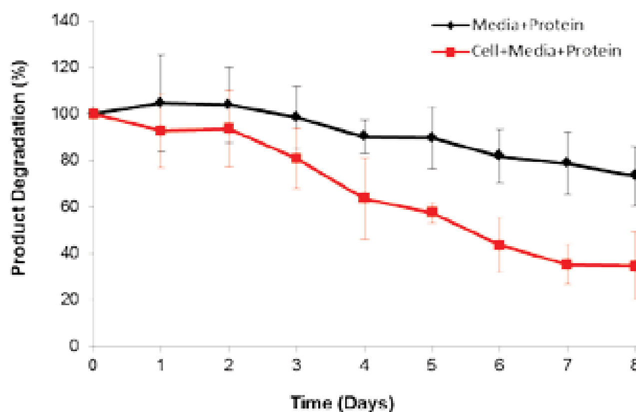


Figure 1. Degradation of recombinant protein product during CHO batch culture.

Therefore, the goal of this project is to outline importance of secreted proteins in improving recombinant protein production from bioprocesses and to identify potential ways for their efficient investigation using proteomic approaches. Using this knowledge, designing cell line engineering for higher yield and higher stability proteins will be done.

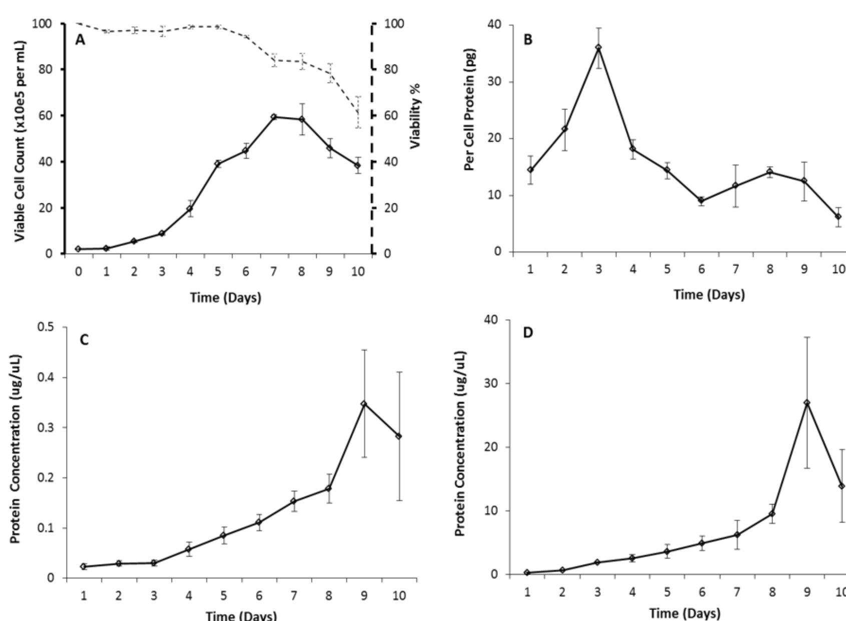


Figure 2. Estimation of protein-potentially-contributed-by-cell-death (PPCD) in culture medium. For this, CHO-K1 cells were grown in suspension culture in CD-CHO media (serum-free and chemically defined) and cell counts were performed everyday using trypan-blue dye exclusion method. Pre-decided amount of culture media was collected and centrifuged at 1000 rpm at 4 °C for 20 min. The supernatant was concentrated to known volume using 5 kDa molecular weight cut-off centrifugation filters to enable the calculation of protein content per milliliter (mL) in original sample. Known number of cells was sampled and lysed using urea-based lysis buffer. Protein concentration was estimated in concentrated media and cell-lysate using Bradford protein estimation method. Per-cell-protein content was calculated by dividing the total amount of protein with total number of cells in the sample. The concentration of PPCD was calculated by multiplying the number of dead cells/mL with per-cell-protein content in the sample at that respective time-point. A: cell growth; B: per-cell-protein content, C: protein concentration detected in spent-media and D: concentration of PPCD. Error bars represents standard deviation among three biological replicates.

Biomarker discovery and diagnostic development for efficient diagnosis of pneumonia

Investigators

Susmita Chaudhuri
Niraj Kumar
Ashutosh Tiwari

Susmita Chaudhuri

Pneumonia is a major cause of childhood mortality and morbidity, especially in resource-poor countries. It is primarily caused by bacterial and viral acute lower respiratory infection and is the single largest cause of deaths (27.5%) among children in post-neonatal period in India, with an incidence of 0.03-0.52 episodes per child per year. It is clinically diagnosed based on cough, difficulty in breathing, rapid respiratory rate, chest in-drawing and/or decreased level of consciousness/ danger signs. However, these clinical criteria over-diagnose bacterial pneumonia, as children with viral infections in lower respiratory tract will also be diagnosed as pneumonia. Even the chest radiography do not discriminate between various causes. Of the currently available diagnostics, nucleic acid based methods have shown highest sensitivity (70-90%) and specificity (60-90%). However, they have also have issues, such as –

- These tools use probes against only a few pathogens (1-2 most prominent pathogens), however pneumonia is caused by number of pathogens
- Even if the probes have been used for multiple pathogens, the test has not been clinically validated, contributing to large number of false positive or negative cases, reducing clinical sensitivity and specificity.

- Existing tools do not differentiate between bacterial and/or non-bacterial pneumonia and are not much useful in maximizing targeted antibiotic therapy and hence significantly reduce emergence of drug resistance.
- These tests do not differentiate between colonizing and invading pathogens and also do not provide information about the presence of the any drug-resistant pathogen
- The team at THSTI aims to identify and validate biomarkers that may differentiate between invasive bacterial and non-invasive non-bacterial pneumonia with information about the presence of any drug-resistance pathogen. The knowledge will be used to develop efficient pneumonia diagnosis.

Generation of high affinity DNA aptamers for the detection of tuberculosis

Investigators

Tarun Kumar Sharma
Jaya S. Tyagi

Tuberculous meningitis (TBM) is one of the most devastating manifestations of extra pulmonary tuberculosis (EPTB) and alone caused an estimated mortality of 1.5 per 100,000 population in India. Early diagnosis and timely therapeutic intervention are both essential for the effective management of TBM. However, the accurate laboratory diagnosis of TBM is very challenging owing to a low bacterial load and the paucity of cerebrospinal fluid (CSF), especially from pediatric participants. The current diagnostic tools suffer from one or other deficiency include to cite some specific example: inadequate sensitivity as in smear microscopy, adequate sensitivity but enormous delay in turnaround time as in culture, dependence on sophisticated instrumentation and proprietary reagents such as Gene Xpert. Therefore, to address the aforementioned challenges an accurate, rapid, low cost and simple tests are urgently needed for the diagnosis of TBM.

We have generated a panel of high affinity ssDNA aptamer against recently reported potent TBM marker HspX, an antigen that proved its utility to be used as potential TBM marker (Haldar et.al 2012). These ssDNA aptamer candidates were screened from a huge random DNA library (having ~10^{15} unique sequences) using subtractive SELEX (Systemic Evolution of Ligands by Exponential Enrichment) strategy. A number of novel aptamer candidates (30 in number) were obtained through SELEX. As obtained 30 aptamer candidates were evaluated to determine their binding ability for HspX. Further, cross reactivity of these aptamers were examined against six other mycobacterial antigens (ESAT-6, CFP-10, GlcB, Ag85complex, MPT-51 and LAM). Based on the results 6 aptamer candidates were selected and designated as star aptamers. These 6 star candidates demonstrated high specificity for their cognate target (HspX) and no cross reactivity was observed against other mycobacterial antigens tested so far. Further, limit of detection (LOD) was determined for selected aptamer candidates. Result unequivocally demonstrated that as low as 8ng antigen can be detected using developed aptamer candidates. Moreover, we have also compared the specificity of selected aptamer candidates with anti-HspX antibody and results explicitly demonstrated the superiority (no cross reactivity with non-target antigen) of aptamers over antibody. We have also determined the apparent dissociation constants (Kd) for selected aptamer candidates. The selected aptamer showed Kd in

low nanomolar range (11.7 to 123 nM). In order to decipher the site where an aptamer candidate interacts with HspX we have also performed molecular modeling followed by docking studies. Molecular modeling data suggests each aptamer candidate have unique site of interaction on HspX. A good diagnostic reagent is the one, which is ready to use so to test this we have used aptamers as such i.e., without heating and cooling (a necessary step to form a secondary structure of aptamers) and performed aptamer linked immunosorbent assay (ALISA). ALISA results evinces high affinity of aptamer against HspX thus we conclude that these aptamer candidates can be used as ready to use diagnostic reagents. Further result obtained also support target responsive structural change mechanism of aptamers. Current we are evaluating the apatmers performence in clinical samples.

Development of aptamer and nanomaterials based sensing platform for small molecules

Investigator
Tarun Kumar Sharma

Collaborator
Vipul Bansal
RMIT University, Australia

As a part of this project we have developed an aptamers-nanomaterial based sensing approach for the detection of variety of small molecules. As a proof of concept we have demonstrated a Turn-off/Turn-on assay by employing the peroxidase-like enzyme activity of gold nanoparticles (nanozyme) and high affinity of aptamers. Following this approach we have developed aptamer-nanozyme based assay for kanamycin (a model small molecule and pesticide. This work was highlighted on cover page of prestigious journals, ChemComm and Analytical Chemistry.

Synthesis, characterization and pharmacokinetic evaluation of a novel solid-lipid nanoparticle formulation for oral drug delivery of anti-TB drugs

Investigators
Subham Banerjee
Jonathan Pillai

Jonathan Pillai

A preliminary literature review of unmet needs in the anti-TB therapy space revealed that the commercially available standard dual-drug oral formulation of Isoniazid and Rifampicin has a highly variable rate of efficacy. This is primarily because reactions facilitated by the low pH in the stomach, leading to the formation of non-active metabolites that may cross-react with each other, further decreasing drug absorption through the gut. This leads to variable bioavailability and significant loss of efficacy, thereby severely compromising therapeutic outcomes. As a result, there is a need for stable oral formulations that can effectively protect the drugs.

We are exploring a novel formulation using solid lipid nanoparticles (SLNs) for these drugs. Encapsulating the drugs in a lipid matrix will ensure their survival under low pH conditions. Furthermore, meticulous selection of appropriate lipids and precise control over formulation conditions leads to nanoparticulate morphology optimized for further enhancing gut absorption. Overall this novel formulation is likely to provide significant advantages over the currently available commercial product. Synthesis and characterization of the SLNs is in progress, as is the development of novel methods of concurrently analyzing the dual-drug combination using High Performance Liquid Chromatography (HPLC).

Evaluation of outer membrane vesicles as drug-delivery vehicles

Investigators
Sapna Jain
Jonathan Pillai

Collaborators
Krishnamohan Atmakuri
VIDRC, THSTI

Multiple species of both Gram-negative and Gram-positive bacteria have been reported to secrete membrane vesicles. As these mostly spherical vesicles are spontaneously secreted in the 50-400 nm size range, and carry a number of distinct bacterial membrane and cytosolic proteins, they show great promise both as vaccine candidates as well as drug-delivery vehicles. While the biogenesis of these vesicles remains unclear, they have nonetheless been successfully formulated into vaccines for meningiococcal disease. A recent report has also indicated their potential for targeted delivery of miRNA in cancer. We are exploring the use of these membrane vesicles as delivery vehicles for therapeutic moieties other than intracellular proteins, including the possibility of encapsulating anti-bacterial drugs.

Over the past year, we have successfully optimized conditions for isolating and purifying outer membrane vesicles (OMVs) from non-pathogenic strains of mycobacterium. Furthermore, we have performed some preliminary studies on characterizing these in terms of their size, shape. Current work in progress includes characterization using high-resolution electron microscopy techniques for accurate determination of both surface and intra-vesicular morphology and contents. Furthermore, future work will focus on encapsulation of first-line anti-TB drugs inside non-pathogenic OMVs and testing them for efficacy against pathogenic strains of mycobacterium tuberculosis.

Evaluation of Medtech innovation practices in emerging economies

Investigator
Jonathan Pillai

Collaborators
Ashish Nimgaonkar
Johns Hopkins University, USA

Despite the strong local demand for medical technology in India, over 80% of all devices are imported. Historically, multi-national companies have marketed their high-end products in the Indian market, even though these products were originally and primarily developed for developed countries. As a result, a number of these products are either inappropriately designed or marketed at an unaffordable priced for the Indian market. More recently however, incentives for translational research and a push towards indigenous product development has inspired Indian innovators towards developing medical technology specifically designed for Indian patients. This new generation of products is not only effective in India, but is also relevant for patients in similar emerging markets like Bangladesh, South Africa, China, Brazil etc. Furthermore, since a lot of these products are affordably priced for emerging markets, they present as significant cost benefits even for developed economies facing increasing cost-pressures from their healthcare systems. Our collaborative work in this area includes identification of case studies of innovations that have been successfully introduced in parallel in both markets. Furthermore, the collaboration has led to the formation of an exchange program facilitating the visit of a team of students from JHU to India in 2015. The team will conduct a series of interviews with medtech innovators in India and identify key features of successful innovations relevant to both emerging markets and developed countries.

Peer-reviewed Publications

1. Chopra, A.; Shukla, R.; Sharma, T. K. (2014) Aptamers as an emerging player in Biology. Aptamers and Synthetic Antibodies , 1(1): 1-11.
2. Ghosh, I. N.; Sharma, T. K.; Shrivastava, S. K.; Pathania, R.; Navani, N. K. (2013) Synergistic action of cinnamaldehyde with silver nanoparticles against spore-forming bacteria: a case for judicious use of silver nanoparticles for antibacterial applications. International Journal of Nanomedicine, 8: 4721-4731.
3. Kulshreshtha P, Tiwari A, Priyanka, Joon S, Sinha S, Bhatnagar R (2015) Investigation of a panel of monoclonal antibodies and polyclonal sera against anthrax toxins resulted in identification of an anti-lethal factor antibody with disease-enhancing characteristics. Mol Immunol (in press).
4. Sharma C, Sankhyan A, Sharma T, Khan N, Chaudhuri S, Kumar N, Bhatnagar S, Khanna N, Tiwari A (2015) A repertoire of high-affinity monoclonal antibodies specific to S. typhi: as potential candidate for improved typhoid diagnostic. Immunol Res 62(3): 325-40.
5. Sharma TK, Ramanathan R, Mohammadtaheri, M.; Daima, H. K.; Shukla, R.; Bansal, V. (2014) Aptamer-mediated 'turn-off/turn-on' nanozyme activity of gold nanoparticles for kanamycin detection. Chemical Communications, 50: 15856-15859.
6. Sharma TK, Ramanathan R, Rakwal R, Agrawal GK, Bansal V (2015) Moving forward in plant food safety and security through NanoBioSensors: Adopt or adapt biomedical technologies? Journal of Proteomics 15:1680-1692.
7. Sharma, T. K.; Shukla, R. (2014) Editorial: Nanoscience and Aptamer Technology for Point of Care Diagnostics. Nanoscience and Technology 1:1-2.
8. Sharma, T. K.; Shukla, R. (2014) Nucleic acid aptamers as an emerging diagnostic tool for animal pathogens. Advances in Animal and Veterinary Sciences, 2: 50-55.
9. Weerathunge, P Ramanathan. R.; Shukla, R.; *Sharma, T. K.; Bansal, V. (2014) Aptamer-controlled reversible inhibition of gold nanozyme activity for pesticide sensing. Analytical Chemistry, 86: 11937–11941.

Patents

Ashutosh Tiwari

Inventors:	Ashutosh Tiwari, Chandresh Sharma, Anurag Sankhyan, Tarang Sharma, Shinjini Bhatnagar, Navin Khanna
Patent Title:	Monoclonal Antibodies specific to Salmonella typhi flagellin, and use thereof
Filed on:	13 March 2015
Application number:	683/DEL/2015
Inventors:	Ashutosh Tiwari, Tarang Sharma, Chandresh Sharma, Anurag Sankhyan, Shinjini Bhatnagar, Navin Khanna
Patent Title:	Production of recombinant ATB protein and its uses as diagnostic tool thereof
Filed on:	14 May 2015
Application number:	1350/DEL/2015

Inventors:	Ashutosh Tiwari, Anurag Sankhyan, Subrata Sinha
Patent Title:	Human monoclonal antibodies specific to preS1 domain of Hepatitis B virus, and use thereof
Filed on:	28 July 2015
Application number:	2291/DEL/2015

Seminars and conferences

Ashutosh Tiwari

Title of the talk:	Inhibition of HBV envelop-hepatocyte interaction by an array of recombinant human neutralizing antibodies from naturally recovered individuals
Name of the meeting:	Fundamental Immunology and Its Therapeutic Potential
Place and date:	Cold Spring Harbor Laboratory, USA, 14-18 April 2015.

Jonathan Pillai

Title of the talk:	A novel device for emergency management of upper esophageal bleeding
Name of the meeting:	IEEE Point-of-care Devices Conference
Place and date:	Seattle, USA. October 9-12, 2015
Title of the talk:	A New Model of Parallel Innovation in MedTech: A Tale of Two Worlds
Name of the meeting:	Technology Transfer Society (T2S) Annual Conference
Place and date:	Baltimore, USA. October 22-25, 2015

Tarun Kumar Sharma

Title of the talk:	Nucleic acid aptamer-based novel diagnostic tool for tuberculous meningitis .
Name of the meeting :	Tuberculous meningitis meeting
Place and date:	Vietnam 20-23 May, 2015.

Extra-mural Grants

Tarun Kumar Sharma

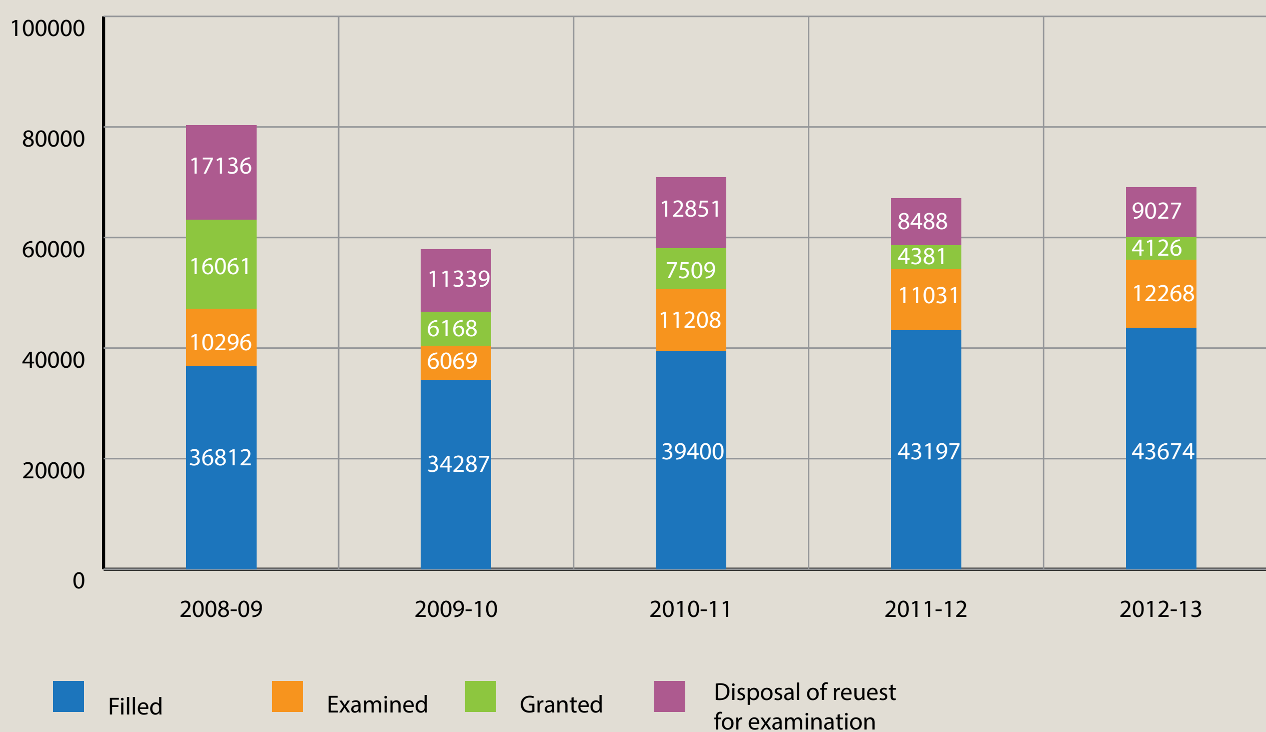
Funding agency- Commonwealth of Australia and Australian Research Council
Amount- Rs. 15,50,000
Duration: From March 2014 - September 2014.
Title of the grant- Development of aptamer and nanomaterials based sensing platform for small molecules

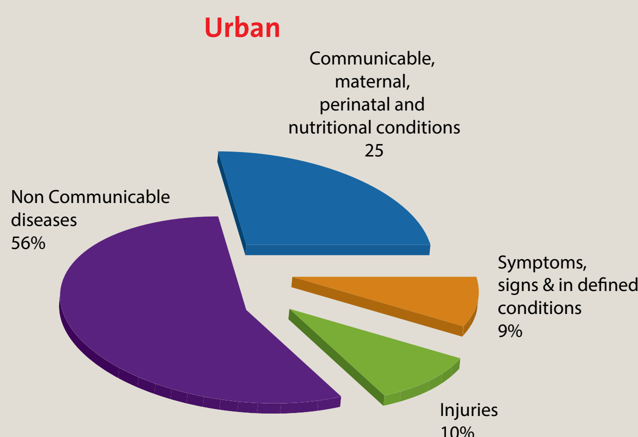
Honours and Awards

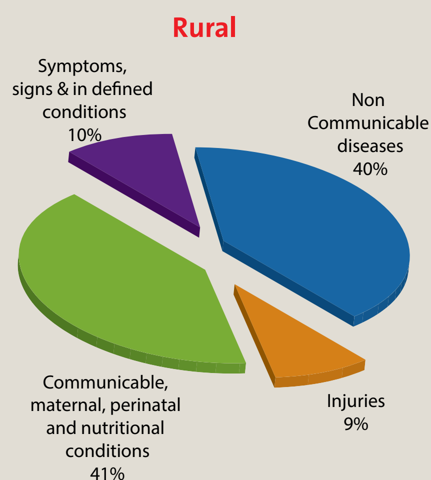
Tarun Kumar Sharma

Australian Endeavour Research Award (Commonwealth of Australia)
Indo-Australian Career Boosting Research Fellowship (DBT- Govt. of India)
Appointed as Associate Editor of Frontiers in Molecular Diagnostics journal

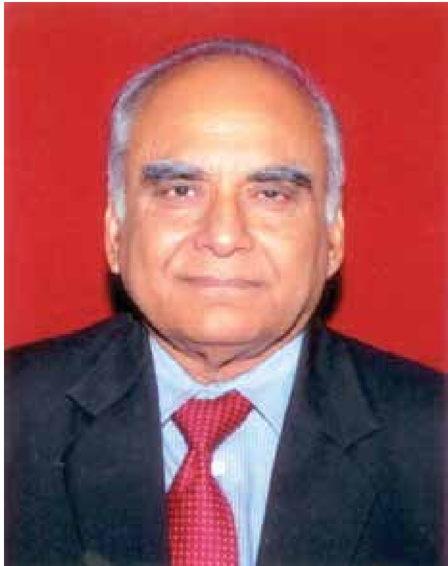
Policy Center for Biomedical Research







An Overview



N. K. Ganguly

The Policy center for Biomedical research was conceived to bridge the huge gap that exists between health researchers and those who implement and are impacted by that research. The center was envisioned to bridge this gap by providing technology analysis that could guide strategic planning and ensure the dual goal of meeting local heath needs and supply global health technologies. The center also conducts meetings, both national and international, in order to provide a platform for discussion and deliberation for various stakeholders for creation of locally useful technologies in terms of their design, purpose, price and user friendliness. It also provides novel strategies for use of affordable interventions that are available and have been found safe, effective and could have an impact on public-health issues in India, if introduced through the national program. With its communication partners, the center also actively participates in demand generation activities by way of meetings conducted across the country. It also engages in mapping of capacity for health research in the country, both human and infrastructure, such that the resources and optimally used especially when there is a health emergency.

Flagship program on point of care (PoC) diagnostics

Investigators
Bratati Mukhopadhyay
N.K. Ganguly

Bratati Mukhopadhyay

Under the Flagship program of the Center on Point of Care (PoC) Diagnostics for communicable diseases in India, a comprehensive analysis for choice of a Platform for PoC diagnostics for Tuberculosis has been created keeping in mind the high diseases burden of TB in the country and the absence of any PoC tests which is suitable for early diagnosis leading to definitive cure and reduce transmission. This will provide support to developers of indigenous technologies to identify relevant partners and to create a platform for interaction of multidisciplinary stakeholders which eventually will facilitate to create policy framework for integration of these diagnostics at various levels of point of care.

Comprehensive analysis of health technologies on PoC Tuberculosis diagnostics in regard to their performance, and challenges were carried out. Upon performing the whole exercise, two of the major ideas were identified. Partnership was created between the TB research laboratory at AIIMS, New Delhi and a New Delhi based renowned Microscopy Company with coordination from PCBR. Similarly, an attempt was made to create the partnership between Reametrix Pvt. Ltd. at Bangalore and the same microscopy Company which is facilitated and monitored by PCBR.

Based on the urgency to develop PoC diagnostics for TB, the investigators have initiated facilitation of assessment of the promising technologies and their validation through appropriate Industry - academia partnership which is interlinked with strategies to accelerate progress in reducing the disease burden by new PoC diagnostics for early diagnosis of TB.

Flagship program on Cholera

Investigators
Kaushik Bharati
Sanjukta Sen Gupta
N.K. Ganguly
G. B. Nair

Under this project, a cholera expert group in India has been formed to validate the collation and analysis of data for creation of a roadmap. This group, which has multidisciplinary stakeholders is being involved in creation of a roadmap for introduction of Cholera vaccine through a series of discussions and has made considerable progress. The roadmap was slated for discussion with international experts on Cholera in the IDEAsia meeting which was coordinated and organized by the Center in March 2015. Following the initiation of these activities, THSTI has also been given a membership at the Global Task Force for Cholera Control of the WHO. A meeting of regional and global experts in Cholera control and prevention was also organized under the Initiative against Diarrheal and Enteric Diseases in Asia banner from 30th March -2nd April 2015. The meeting resulted in exchange of ideas and plans adopted by other countries in the region and other continents to fights the scourge of cholera. It was also an opportunity of various stakeholders within the country from academia, government, industry, WHO (SEARO and country office), WaSH divisions of UNICEF etc. to come together and discuss a health problem that is often brushed under the carpet but carries a significant burden in the country. New partnerships are being forged with various stakeholders, WaSH division at UNICEF being one of them. The investigators have also partnered with Health Policy Division of the Institute of Economic Growth to carry out

economic analysis for cholera vaccine introduction (as well as for other health interventions)

Initiative on other vaccines in India

Investigators

Sanjukta Sen Gupta
N.K. Ganguly

Collaborators

International Vaccine Access Center (IVAC)
Global Health Strategies (GHS), New Delhi

IPV: The investigators were invited for a round table on advocacy for IPV vaccines organized by the collaborators. They were also invited to speak and contribute a paper at the 32nd Roundtable- conference on "Lessons from the Success of Polio Elimination" organized by Ranbaxy Science Foundation in October 2014. The paper submitted, will be published in the proceedings of the round table.

Pneumococcal vaccine: An international symposium is being planned in November 2015 in New Delhi in partnership with IVAC and GHS. Few planning meetings have taken place with the BMGF and IVAC.

Flagship program on leveraging global health technologies to promote maternal & child health in India: the current landscape & opportunities for advancement in low-resource settings

Investigators

Mona Duggal
Gautam Kumar Saha
Swati Verma
Nisha Arora
N. K. Ganguly

The landscaping on currently developed & available technologies pertaining to MCH that have made a sustainable impact on maternal and child health or have aided reduction in maternal & child deaths for India was done and their high advantages for the Public Health utilization were studied.

It is evident from the data that while Sri Lanka, Maldives reached international targets on infant mortality and under-five mortality, Bangladesh is moving favorably towards MDG4 goal. In India, even after introduction of NRHM and IMNCI, the drop is not so significant especially in some of the northern states. This could be because of non-integration of IMNCI in a national system and also due to lack of resources at the centers where it was implemented. The major deaths occurred as there were a large number of pre-term births, IUGRs and cases of septicemia, where the probable causes were not having the infrastructure for homecare and health systems for point of care. Factors like maternal nutrition, environmental factors like water, both availability and quantity; sanitation, indoor air quality also affect child survival and analysis of these factors revealed gaps and challenges in this area. The impact of Special Neonatal Care Units (SNCUs) set up at district level still needs to be analyzed. The other causes of death among children less than five years of age are diarrheal diseases and pneumonia. In Bangladesh, the deaths have come down appreciably, however in India, these have not been reduced to significant extent due to poor coverage of oral rehydration and zinc supplementation programs. Non-uniform distribution and improper access to clean water, both in terms of quality and quantity, also seems to correlate with the increase in the two syndromes. WaSH interventions in general, are resource intensive to implement as well as maintain.

Similarly, in the area of maternal health, the interventions that have improved

health of mothers in neighbouring countries like Bangladesh with similar demography and social conditions are being identified. Some countries that have done exceptionally well in the Region, like Maldives and Sri Lanka are also being looked at. Analysis of health systems and mechanisms of implementation of the maternal and child health programs in these countries, are in initial phases. These can serve as very good lessons for India to learn from.

Additionally, the technologies programs eg Family Planning, nutrition affecting disease prevalence, focus health scenario of respiratory/diarrheal diseases have also been analyzed.

Various aspects of nutrition and education of women of child bearing age and its impact on maternal and child health is proposed to be studied in the next year.

To evaluate India's current value chain in diagnostics landscapes, market, market shortcomings

Investigators
Bratati Mukhopadhyay
N.K. Ganguly

Despite the fact that cures starts with actual diagnosis, only 25% people get the chance of diagnostics. Based on that fact, the Policy Center for Biomedical Research attempted to create a "Dashboard for diagnostics in India" to evaluate the value chain for some of the most common Communicable Diseases. This exercise can contribute in identifying highest priorities for Research and Development (R&D) intervention in diagnostics for the country. This is also likely to bridge the gap between product development, validation and for creating appropriate policies for their uptake.

A detailed landscaping of the diagnostics of importance and priority was carried out. The global giants were scrutinized for their current products available in the market. The promising platform technologies developed has been carefully examined in the aspect of value addition to the market. The pipeline products identified from the global Market diagnostics landscape as well. Upon analysing, the priority and needs for the infectious diseases diagnostics was created. An attempt was made for a project to develop on Diagnostics dashboard based on the priority infectious diseases of India in collaboration with IMS health, Gurgaon. The future directions: Collaborative project may be initiated.

Strengthening regional framework for research and developing action plan for health research in South East Asia (SEA)

Investigators

Sanjukta Sengupta
Bratati Mukhopadhayay
Kaushik Bharati
Gautam Kumar Saha
Swati Verma
Radhika Gigras
Nisha Arora
G. B. Nair

Collaborators

Manisha Sreedhar
WHO SEARO
Madhur Gupta
WCO, New Delhi

Sanjukta Sengupta

An analytical report on a Study to suggest methodologies to develop norms and standards for classification of Health R & D needs of Developing Countries was created. A concept and matrix developed by the centre in was discussed and evaluated by experts from the SEA Region held in New Delhi in Nov, 2014. The exercise carried out provides a research action plan for the Region for the next biennium, achieve tangible results through innovations in health research. Three Demonstration projects were also generated on the following areas: Tuberculosis diagnostics and anti-tubercular drugs; Pneumococcal vaccine, and Fever diagnostics by Center for Biodesign.

As per the Central Advisory Board of PCBR recommendation on generation of big data sets, concept for a National Health Research Observatory was created for analysis of financial flows and norms and standards for health R&D. This was discussed with major Government stakeholders and WHO country office in a consultative meeting in March 2014. All experts in the meeting agreed in principle for the proposal of NHRO. WCO is of the opinion that this can be on major pillar for Global Observatory proposed by WHO. A matrix was created by the scientists at PCBR for data input related to funding of R&D in health and has been evaluated and validated with a sample set of projects from ICMR and DBT. Validation of two matrices developed and emerged from the Bangkok CEWG consultation meeting in order to collect, analyze, and monitor R&D resource flow using the case studies of Indian research funding agencies was created which need to be used by all SEA countries in order to develop a proposal for a National R&D observatory (NHRO). The report is pending with WHO for publication.

Assessment/next steps for global strategy and plan of action on public health, innovation and intellectual property for all 8 elements of GSPA WHA61.21

Investigators

Bratati Mukhopadhayay
Mona Duggal
Kaushik Bharati
Sanjukta Sengupta
Gautam Kumar Saha
Swati Verma
Radhika Gigras
Nisha Arora
Nilanjana Bhattacharya
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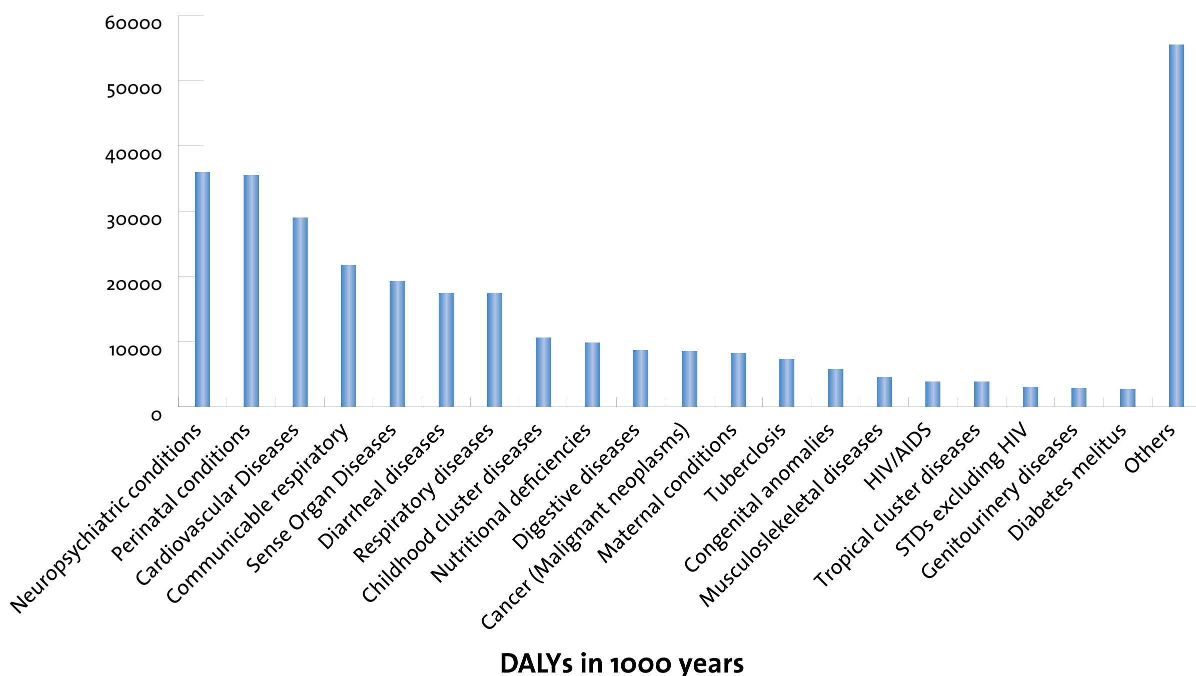
Madhur Gupta
WCO, New Delhi

The center was approached by WHO through its India Country Office, for creating a situation analysis for India on this topic. It included analysis on: Prioritizing R&D Needs (Element 1) and Promoting R&D (Element 2) in Indian context, based on the new, innovative technologies developed in the last decade and by commitments /investments in the health R&D. Mapping and analysis of the health policies and priorities of international vis-a-vis national agencies; the status of traditional medicine; major organizations involved in R&D in India and the knowledge sharing mechanisms/structures etc. have also been included (Elements 1&2). A mapping, analysis and identification of gaps in "Building and Improving Innovative Capacity" for health interventions including various government policies and financing mechanisms/budgets for supporting these activities, was also done (Element 3). Analysis of the trends in Transfer of Technology for health interventions in India by various Ministries, Departments, Major funding agencies and the key institutions under them was carried out for the last 5 years (2008-2013) (Element 4). The

patents filed and granted by the Indian institutions was analysed by their respective funding agencies, ministries, country of filing, in the area of drugs and biotechnology. Patents granted to major pharmaceutical companies in the years 2010-2013 was also mapped and analysed (Element 5).

Similarly, listing and analysis of health care infrastructure, both public & private, quantum and trends of healthcare spending, review of different avenues and available models of financing that are used to "prioritize delivery and access" in India also created (Element 6). Innovative mechanisms for financing of R&D for health products relevant for the control and treatment of the diseases prevalent in developing countries was analysed, as were the new mechanisms used to improve sustainability of funding that is essential to support a long-term R&D efforts. Recently introduced innovative funding for supporting entrepreneurs & industry for product development, various models of infrastructure & manpower-sharing and improving effective utilization of available resources and the most serious gaps in financing for health products and R&D in India, were also documented (Element 7). Through scrutiny of census data, reports published by WHO and related publications, an analysis of the health problems in the demographics context, progress in bioethics, existing surveillance systems, gaps in monitoring mechanisms and challenges in health R&D was created. This was done to evaluate access and impact of interventions in different economic strata of the society, to determine long term health problems, emerging threats from infectious diseases and to safeguard of ethical practices, both in public health and R&D in post TRIPS era (Element 8). Recommendations to strengthen prioritization mechanisms in R&D, tech transfer & IPR, sustainable financing, capacity building, program evaluation and monitoring systems for public health were also provided. The report will be discussed with stakeholders in August, 2015 and finalized, which will be presented in the upcoming meeting of WHO –SEARO.

TWENTY DISEASE TYPES RESPONSIBLE FOR 80% DALYS BURDEN OF INDIA



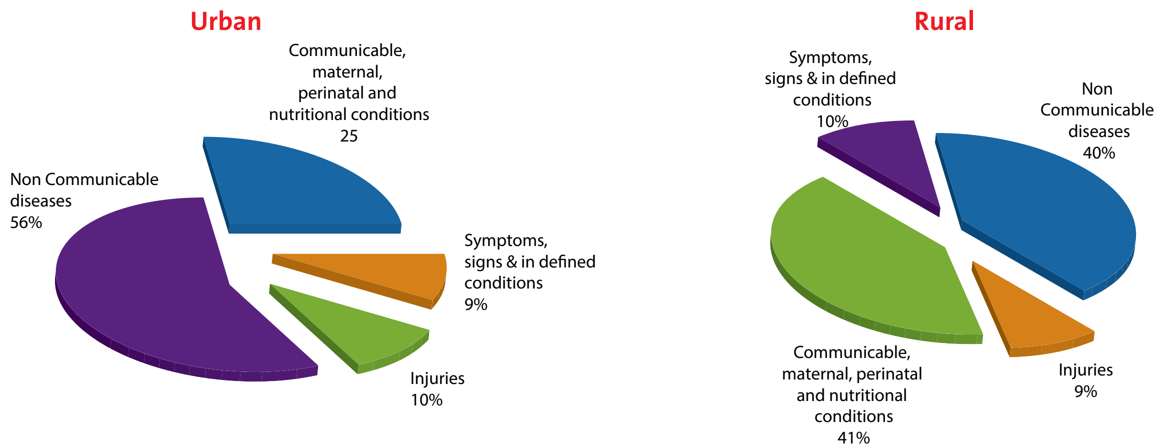


FIGURE: TRENDS IN PATENT APPLICATIONS OVER THE LAST 5 YEARS IN INDIA

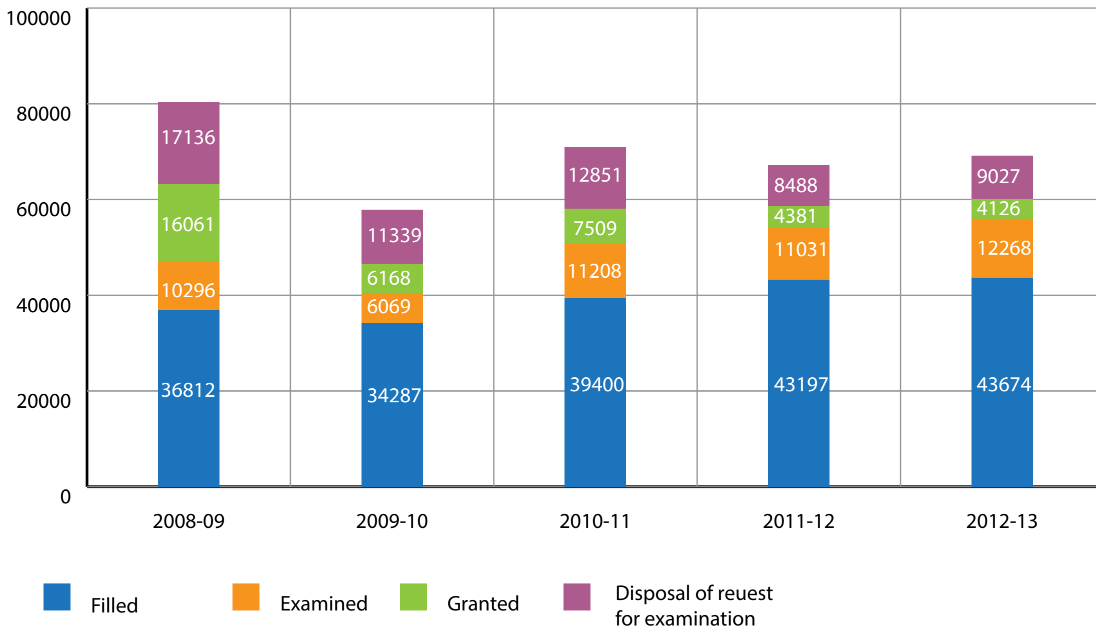


FIGURE: HEALTHCARE SPENDING IN PUBLIC AND PRIVATE SECTOR IN 2010

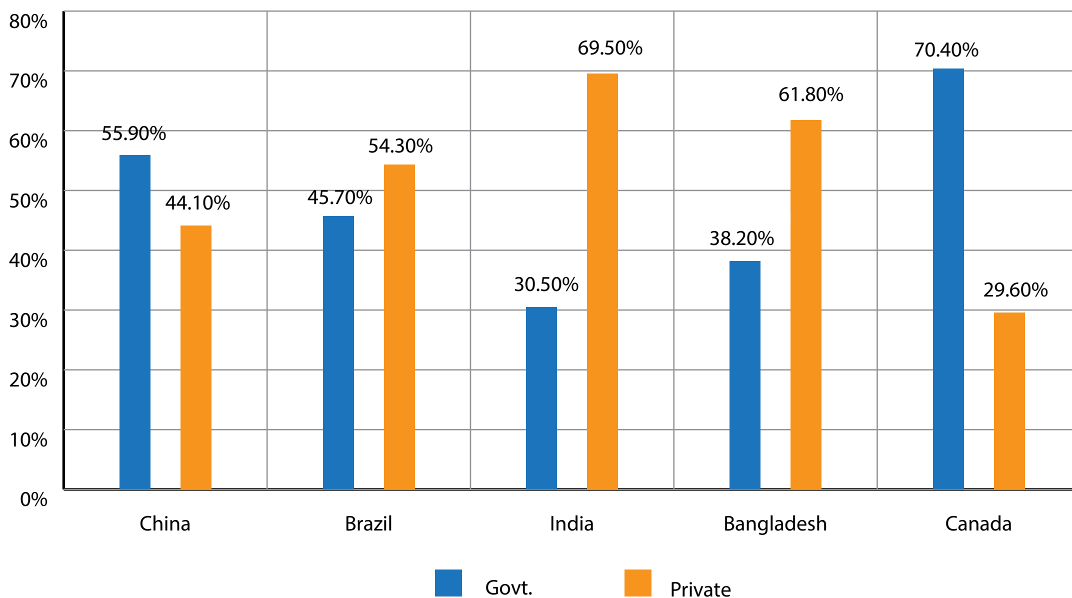
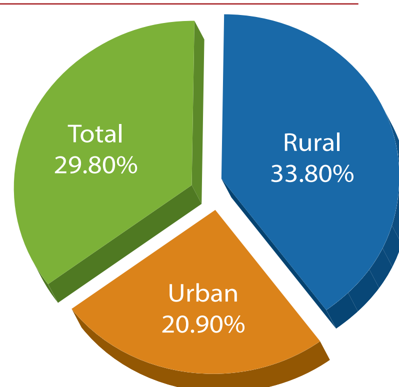


TABLE: STATE WISE LOCATION OF TELEMEDICINE PROJECTS

State	Medical Institutes	Medical Colleges	Corporate Hospitals
J & K (12)	✓	✓	NA
Punjab (5)	NA	✓	NA
Himachal Pradesh (27)	✓	✓	NA
Haryana (2)	NA	✓	NA
Rajasthan (44)	NA	✓	✓
Maharashtra (4)	✓	✓	✓
Karnataka (30)	NA	✓	✓
Lakshadeep (5)	NA	✓	NA
Kerala (29)	✓	NA	✓
Tamil Nadu (13)	✓	NA	NA
Pondichery (5)	NA	NA	✓
Andaman & Nicobar (6)	NA		NA
Andhra Pradesh (16)	NA	NA	✓
Chhatisgarh (19)	NA		NA
Madhya Pradesh (1)	NA		NA
Uttar Pradesh (3)	✓	✓	NA
Uttaranchal (3)	NA	✓	✓
Delhi (4)		NA	NA
Orissa (8)	NA	✓	NA
Jharkhand (1)	NA	✓	✓
Bihar (1)	NA	✓	NA
West Bengal (9)	NA	NA	✓
Sikkim (1)	NA	✓	NA
Meghalaya (1)	NA		NA
Andhra Pradesh (1)	NA		NA
Nagaland (3)	NA		NA
Manipur (1)	NA		NA
Assam (3)	NA		NA
Tripura (7)	NA		NA
Mizoram (2)	NA		NA
North East States (21)	NA		NA

PERCENTAGE OF POPULATION BELOW POVERTY LINE (NHP 2011)



Percentage of Population

Identification of gaps to strengthen health R & D in India and comparison of health sector within the region for priority setting to achieve health research goals

Investigators
Gautam Kumar Saha
N. K. Ganguly

Collaborator
Triono Soendoro
WHO, SEARO, New Delhi

Gautam Kumar Saha

Promoting innovation in the country is also to make India a leader in the region for bringing health to all. As we live integrated global and regional economy, the threat of infectious looms large. Steadily NCDs are on the rise due spread of fast food culture and sedentary lifestyle. In the presence of large population with vast economic disparities, limited resources, the major priority health sector in India and region have been identified. Series of consultative meeting led to development of concept to address the major research issues and it was important to know how the India and the region have progressed in the promoting health R&D and how the region can progress by coordinated efforts.

A concept for the promotion of R &D was prepared and further discussed in an Inter Country, Regional meeting of SEAR nation in New Delhi and comprehensive series of recommendations were obtained: strengthening R & D initiatives to address local priorities by ensuring inter-country collaboration with adequate and sustained funding, especially in smaller countries in the region, to help in appropriate policy-making and program management. Work on advocacy and community support to strengthen the review processes, enhance capabilities and provide sustained funding for health research, Create a consortium of South East Asian Regional industries and institutes to develop and implement large multi-centric research studies on locally relevant priority areas in world-class ethical framework. This will facilitate strengthening multi-sectoral and collaborative approach to achieve Universal Health Coverage (UHC) and MDG goals which significantly improves the quality, standards and access to health systems.

Rural health in India and its relation to household air pollution

Investigators
Gautam Kumar Saha
Bratati Mukhopadhyay
Kaushik Bharati
Verma
Radhika Gigras
NIsha Arora
N. K. Ganguly

Collaborator
Kirk Smith
University of California, USA

The major areas covered were: a. Analysis of the impact on health of biomass (dung, crop residues, and wood) and coal used as cook fuel in Indian households: Trends in usage, health evidence, need for clean alternatives, Landscape of potential interventions in India with History of improved biomass stove programs; b. Current status of advanced biomass stoves in India; Potential for clean liquid and gaseous fuels from biomass and solar cooking; potential for expanding LPG and natural gas connections; c. Potential for electric cooking alternatives – induction cooking and specialized cooking appliances; d. Cost-effectiveness analysis of alternatives; Demonstration projects that can be proposed using new technologies that lead to insights on how large-scale programs could be organized. The development and manufacturing of pollution monitors targeting cost and sensitivity along with durability of such devices to be used in India was also taken into account.

The focus was on how the technology transfers issues can be resolved and mechanism for local production to make products available and in a cost effective manner. Framing of the policies in regard to the above and its

implementation pathways were also considered. Potential for clean liquid & gaseous fuels from Solar Cooking where the benefits of using different types of solar cookers were brought to light and new advocacies were highlighted.

Laboratory mapping on public health, bio-surveillance and global security in order to prevent and tackle disease outbreaks

Investigators

Bratati Mukhopadhyay
Gautam Kumar Saha
Swati Verma
Nisha Arora
N. K. Ganguly

Collaborators

Shah Hossain
Mayank Dwivedi
CDC India

India has rich resources and laboratory networks with public health competencies. Whereas those labs in the public health networks are accessed, the capacity available outside the network is rarely used in a public health event. The purpose of this mapping exercise is to create a database of al laboratories having potential and capacity for public health purpose. The basic information regarding the capacity and function of the labs is planned to be created so that the strengths are captured and lay a basis for more intensive survey to detect and address gaps and quality assurance.

The information generated from this and further surveys would have a governance dimension which will lead to enhancement of capacity, optimization of resources, and a more regulated, biosafe and biosecure lab network that may ultimately lead to a change in policy in the country. This will be a useful tool for tackling the sudden disease outbreaks as well. Additionally, this would also enhance the competence of the country to attend to the obligations under IHR and also be able to provide service to the region. Series of interactive meetings, deliberations were made for the creation of initial protocol and a survey questionnaire and partnership with Manipal University, Bangalore for carrying the mapping exercise, was explored.

A pan India exercise on the Laboratory mapping on public health, bio surveillance and Global security has been initiated in partnership with the Manipal Center for virus research. A survey questionnaire made and list of all Research and Development Institutes carrying out work on infectious diseases, all the Medical Colleges, NABL accredited Laboratories involved in serology and Microbiology, Public health Laboratories have been created. The survey has been sent to the organizations and responses are being received. Based on the inputs received, visit to the places of importance will be arranged, the Northern part will be covered by the PCBR and Southern part by the Manipal University. An analysis on the gaps and challenges in the area and way forward for strengthening the capacity will be done. A database will be developed. A meeting is proposed to be held in September, 2015 to disseminate the report to the concerned stakeholders.

Publications

1. Kumar R, Sharma YP, Thakur JS, Patro BK, Bhatia A, Singh IP, Rana SK, Chakraborti A, Dhanda V, Sapru S, Sharma M, Shah B, Ganguly NK, (2014) Streptococcal pharyngitis, rheumatic fever and rheumatic heart disease: Eight-year prospective surveillance in Rupnagar district of Punjab, India. Natl Med J India, 27(2):70-5.
2. Ganguly NK, Croft S, Singh L, Sinha S, Balganesh T, (2014) Biomedicine and biotechnology: public health impact. Biomed Res Int.
3. Lagrange PH, Thangaraj SK, Dayal R, Deshpande A, Ganguly NK, Girardi E, Joshi B, Katoch K, Katoch VM, Kumar M, Lakshmi V, Leportier M, Longuet C, Malladi SV, Mukerjee D, Nair D, Raja A, Raman B, Rodrigues C, Sharma P, Singh A, Singh S, Sodha A, Kabeer BS, Vernet G, Goletti D , (2014) A toolbox for tuberculosis (TB) diagnosis: an Indian multi-centric study (2006-2008); evaluation of serological assays based on PGL-Tb1 and ESAT-6/CFP10 antigens for TB diagnosis. PLoS One, 9(5).
4. Mukhopadhyay, B. and Ganguly, N.K., (2014) The Unexplored Role of Probiotics on the Parasitic Pathogens. Food and Nutrition Sciences, No. 22, 2177-2184.
5. Bratati Mukhopadhyay and Nirmal Kumar Ganguly, (2014) The Unexplored Effect of Probiotics on Protozoan Parasite. Probiotic Association of India Newsletter, Vol. 1, Iss. 6.
6. Hajela N, Nair GB, Ramakrishna BS, Ganguly NK, (2014) Probiotic foods: can their increasing use in India ameliorate the burden of chronic lifestyle disorders?. Indian J Med Res., 139(1):19-26. Review.
7. G-FINDER Report - 2014 Neglected disease research and development: Emerging trends -(Dr. Gautam Kumar Saha)
8. The G-FINDER Report 2013: Neglected disease research and development: The public divide (Dr. Gautam Kumar Saha)
9. Lagrange PH, Thangaraj SK, Dayal R, Deshpande A, Ganguly NK, Girardi E, Joshi B, Katoch K, Katoch VM, Kumar M, Lakshmi V, Leportier M, Longuet C, Malladi SV, Mukerjee D, Nair D, Raja A, Raman B, Rodrigues C, Sharma P, Singh A, Singh S, Sodha A, Kabeer BS, Vernet G, Goletti D, (2013) A toolbox for tuberculosis (TB) diagnosis: an Indian multicentric study : Evaluation of QuantiFERON-TB gold in tube for TB diagnosis. PLoS One, 8(9):e73579. doi: 10.1371/journal.pone.0073579. eCollection .
10. Gupta SS, Nair GB, Arora NK, Ganguly NK, (2013) Vaccine development and deployment: opportunities and challenges in India. Vaccine, 31 Suppl 2:B43-53, Review.
11. Bharati K, Ganguly NK,(2013) Tackling the malaria problem in the South-East Asia Region: need for a change in policy? Indian J Med Res, 137(1):36-4.
12. Bharati K, Ganguly NK, (2013) Does India need an indigenous HPV vaccine and why? J Public Health Policy, 34(2):272-87. doi: 10.1057/jphp.2013.4. Epub.
13. Mukhopadhyay B, Ganguly NK, (2013) Tuberculosis research in India. Curr. Sci , 105(5): 594-596.

Books and Book Chapters Published

1. Book Chapter : Status of Biotherapeutics Development in India : Kaushik Bharati and N K Ganguly: Book: Biotechnology Volume:7 Drug Discovery
2. Book: Omics for Personalized Medicine: Publisher Springer; Chief Editor: Prof. N K Ganguly, Book chapter 27 . Pharmacogenomics and Personalized Medicine for Infectious Diseases: Prof. Nirmal Kumar Ganguly and Dr. Gautam Kumar Saha

Edited Book

Studies on Respiratory Disorders: Oxidative Stress in Applied Basic Research and Clinical Research Editors: N K Ganguly , S. K. Jindal, S.Biswal, P.J. Barnes, R. Pawankar

Invited Talks

1. Demonstration Project on TB diagnostics at SEARO, Sept. 2013 by Dr. Bratati Mukhopadhayay
2. Demonstration project on Pan-serotype pneumococcal vaccine at SEARO - Dr.Sanjukta Sen Gupta)
3. TB project in WCO, Sept. 2013, by Dr. Bratati Mukhopadhayay
4. Conceptualizing WHO ELEMENTS for the meeting at WHO SEARO, New Delhi on 16th July, 2014
5. Conceptualization of the Biosurveillance project in NCDC, Sept. 2014 by Dr. Bratati Mukhopadhayay

Meetings attended

1. Consultation meetings for Review the Antimicrobial Resistance conducted by PHFI. March 2015: Dr. Gautam K. Saha
2. Yakult India Microbiota and Probiotic Science Foundation Symposium– From Bench to Community, March 2015: Drs. Sanjukta Sen Gupta and Gautam K Saha
3. 4th Advanced Course on Tuberculosis Diagnostics Research at McGill University, Montreal, July 7-11, 2014: Dr. Bratati Mukhopadhyay
4. Symposium on Public Health and Intellectual Property Rights in India conducted WHO July 2014: Dr. Gautam K. Saha
5. TB diagnostics and management at NITRD, New Delhi, on 23. 3. 2015 by Dr. B. Mukhopadhyay
6. Symposium on public health and intellectual property rights in India on 2nd July, 2014 at Imperial Hotel
7. Policy Forum on antimicrobial resistance and stewardship in hospitals, 28 November IHC New Delhi
8. Antimicrobial Resistance: India's role in tackling the global crisis Meeting schedule on March 5, 2015 at Gulmohar Hall, India Habitat Centre, New Delhi

Meetings conducted

1. Cholera and Typhoid at India Habitat Center (IHC), April, 2013
2. Seventh Asian Conference on Lactic Acid Bacteria, India Habitat Center, New Delhi, September 6-8, 2013.
3. Consultation on "National R & D Observatory in India" with THSTI, BIRAC and WHO-India Country Office with Theme of the meeting "Conceptualizing the National R & D Observatory in India: The Way Forward. at NII on March 14, 2014
4. Symposium on Dengue Control and Prevention, at IHC, September 29, 2014
5. Inter-country meeting for Strengthening Regional Framework and for Developing Research Action Plan" in partnership with WHO SEARO at Le Meridien, on October 14-16, 2014, New Delhi.
6. Fourth Meeting of Initiative against Diarrheal and Enteric Diseases in Asia at Hotel Grand, New Delhi from March 30-April 2, 20152

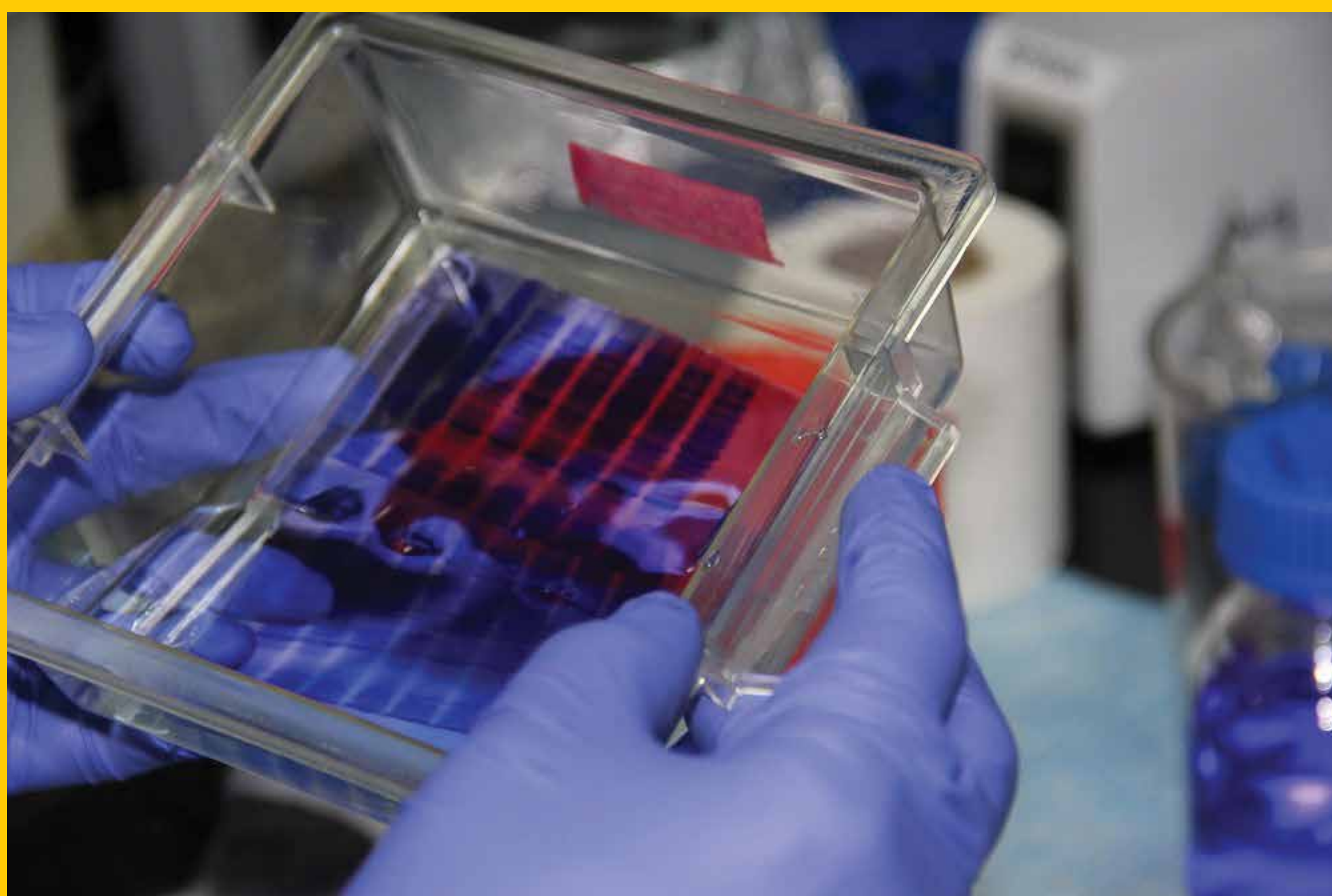
Trainings/Workshops attended

1. Medical Writing, at PHFI, Gurgaon, May 27-30, 2014
2. Sample size calculation and Statistical techniques, at PHFI, Gurgaon, July 15-18, 2014
3. SAS Fundamentals in Clinical Research, at ICGEB, New Delhi, Nov 24-28, 2014
4. BIRAC CDSA regulatory Workshop on October 16, 2014, at India Habitat Center, New Delhi
5. Indo-US Workshop on "Challenges of Emerging Infections and Global Health Safety" on November 18-20, 2014 at INSA, New Delhi

Members of Boards and awards

1. Member, Regional Task Force on Diseases Targeted for Elimination-constituted by Regional Director SEARO
2. Member of the Board of Trustees, the INCLEN Trust International
3. Chairman, Vallabhbhai Patel Chest Institute - Stem Cell Committee.
4. Advisory Committee to the Director's Global Work Group at CDC, Atlanta, USA
5. Dr. D Sundaresan Memorial Lecture Award on 11th February 2015 at 10.00 AM at NDRI, Karnal: Topic of Lecture : Probiotics and Vaccine
6. Helmholtz International Fellow, 2015, Helmholtz Association of German Research Centres, Helmholtz Center for Infection Research, Germany
7. Member, Board of Trustees, ICDDR,B
8. Dr. Bratati Mukhopadhyay was awarded full support of registration fee to attend Advanced TB Diagnostics Course at McGill University, Montreal, Canada; 7-11th July, 2014 by McGill University.

Drug Discovery Research Centre



An Overview

Kanuri V. S. Rao

The past decade has seen a radical transformation in our approach towards examination of biological systems, and dissection of the regulatory mechanisms involved. This is a result of the ongoing explosion of new technologies that facilitate, on the one hand, generation of a more global perspective on biological processes, while also providing for improved molecular resolution on the other. While high-throughput approaches reinforce the notion that the molecular components of a cell are organized into a large network composed of functional modules, analysis of the resulting features underscores that biological systems represent complex systems that exhibit non-linear dynamical behavior. Thus both homeostatic and perturbed functioning in disease represents emergent properties that derive from such behavior. The DDRC was founded on the belief that a better appreciation of the network structure of biological systems, and the methods that have emerged to study its properties, now provide a unique opportunity for developing new and more effective strategies for therapy.

The mission and functional organization of DDRC: In keeping with its intent, the DDRC has been designed as a multi-disciplinary research centre that integrates basic with translational research in the field of drug discovery. Its overall mission is to integrate diverse disciplines to generate a robust and versatile pipeline for drug discovery research. Convergence between varied disciplines such as physics, mathematics, computational sciences, engineering and biology is required for interrogating network properties and identification of regions of sensitivity. Seamless linkage of this activity with downstream capabilities in high-content screening, chemistry and pharmacology then anchors a facile workflow from target identification to lead development and optimization. The Centre is still in the process of becoming fully established and this is expected to be complete by early 2016. At that time DDRC will be functionally defined as a platform consisting of the following domains of expertise:

- *Assay Development and High-content screening:* Development and standardization of robust, sensitive, and reproducible platforms for high content, medium-throughput screening.

- *Synthetic and Medicinal Chemistry:* Strong capabilities for organic synthesis and SAR optimization, coupled with medicinal chemistry expertise.

- *Cell and Molecular Biology:* Expertise in developing new tools and approaches for analyzing the basis of disease-specific phenotypic perturbations in cellular systems. Research emphasis on integrating high-throughput experimental approaches with the tools of systems biology to delineate disease-specific networks.

- *Structural Biology:* Cloning, expression and purification of proteins/ protein complexes. Biochemical characterization through activity and kinetic assays. Biophysical characterization by SPR, ITC, co-elution, GPC and SAXs. Structure determination of protein, protein-ligand, and protein-protein complexes.

- *Pharmacology and Analytical Biochemistry:* Provide the downstream analysis for a candidate drug once it has been identified. This includes evaluation of pharmacological properties such as PK/ADME *in vivo* and *in vitro*, and tissue distribution of compounds using rodent models. Strength in mass spectrometry for quantitative analysis of the cellular proteome, lipidome and metabolome.

- *Computational and Mathematical Biology:* Incorporate and develop expertise in all aspects of high-throughput data analysis, network biology, and mathematical modeling of network dynamics. Capabilities for data handling extends across all molecular components and processes of a cell, complemented by strong expertise in the modeling of complex systems behavior. The latter is synergistically approached from both network-based and purely mathematical strategies. Additional areas of expertise include chemoinformatics, bioinformatics, and *in silico* drug design.

Research focus at DDRC: The present area of focus of DDRC is the metabolic syndrome (MetS). MetS is a chronic progressive disorder that has become a global public health concern, in addition to emerging as an epidemic of 21st century India. This disease essentially represents a disorder of energy utilization and storage, and is diagnosed by a co-occurrence of three out of five of the following medical conditions: abdominal (central) obesity, elevated blood pressure, elevated fasting plasma glucose, high serum triglycerides, and low high-density cholesterol (HDL) levels. The etiology of this syndrome is complex as is its pathophysiology. In addition to genetics, the contributory factors include aging, diet, low physical activity, stress, disrupted chronobiology/ sleep, mood disorders, excessive alcohol use, and smoking. Importantly, while MetS was traditionally thought to afflict the elderly, recent years have seen an increasing incidence in the younger population. This is largely a result of lifestyle changes where the effect of reduced physical activity is compounded by the increased consumption of high calorie diets. It is the emergence of this latter phenomenon that warns of an impending public health catastrophe for India.

The operative paradigm at DDRC is that a better understanding of MetS can only be achieved by taking both its temporal features and its multivariate character into account. Given that MetS finds differential expression - in terms of clinical symptoms - in the human population, it is our view that capturing the underlying variables and defining their relationship to the eventual outcome will be central to eventually gaining a grip on the problem. Broadly, therefore, the aim is to extract the dynamical network that captures

initiation, progression, and development of MetS; and then exploit this data for the purposes of vulnerable target identification and subsequent drug development. To this end, research at DDRC is presently being conducted under four broad themes. These are:

- *Integrating a platform for disease interrogation and drug target identification*
- *Lead discovery and development*
- *Early Translation*
- *Discovery Research*

It is emphasized that research under each of the above themes combines both experimental and theoretical approaches that converge through the collaborative participation of multiple investigators. Although collaborative in nature each program is, however, intellectually driven by a specific Team Leader. Additionally, the Team Leader is also responsible for maintaining focus and momentum of the project.

Integrating a platform for disease interrogation and drug target identification.

Team Leaders
Samrat Chatterjee
Kanury Rao

Investigators
Sanjay Banerjee
Shilpa Jamwal

Samrat Chatterjee

Use of model trajectories to understand the regulatory mechanisms underlying MetS

The broad aim of this project is to generate quantitative molecular profiles of MetS development in both humans and in animal models, and then employ the data to generate network models that capture the disease development process. It is hoped that analysis of such networks by using mathematical tools will reveal the vulnerable nodes, or node combinations, that are key to either disease development or maintenance. To generate the methodologies for such complex analysis we have been employing microarray data from the mouse model of diet induced MetS that was previously generated in the laboratory of Kanury Rao at ICGEB. In these earlier experiments C57/Bl6J mice were kept either on normal diet (ND), or on a diet containing high levels of fat and sucrose (HFHSD) for a period of eighteen weeks. Periodic monitoring of these animals revealed that, in contrast to the former group, mice in the latter developed the classical symptoms of MetS. This included a marked gain in weight along with the development diabetes, dyslipidemia, and chronic systemic inflammation. Starting from 24 hrs after initiation of the feed, and stretching up to the 18-week time point, different tissues (including liver, adipose tissue, skeletal muscle, hippocampus etc) were periodically collected from these mice. RNA was extracted from these tissues and the gene expression pattern determined through a microarray analysis. We at DDRC have first taken the liver-specific gene expression data for developing methods that capture disease initiation, progression and establishment.

A preliminary analysis was first conducted in order to reduce noise from the data. Each probe has three replicate samples and their respective geometric mean with p-value. The gene expressions in the list were given in log2-scale ratio between different groups with ND group. A gene was said to be significantly regulated if its expression was greater than 1 (up-regulation) or less than-1 (down-regulation) i.e., if the expression was two folds up or down with respect to the control. Expression value between -1 to 1 then the gene was taken to represent insignificant perturbation. Further, to remove any kind of noise from the data, and to maintain consistency, we selected only those genes that showed the same regulation pattern throughout all the three replicates. This exercise led us to a table with significantly perturbed genes and their temporal expression.

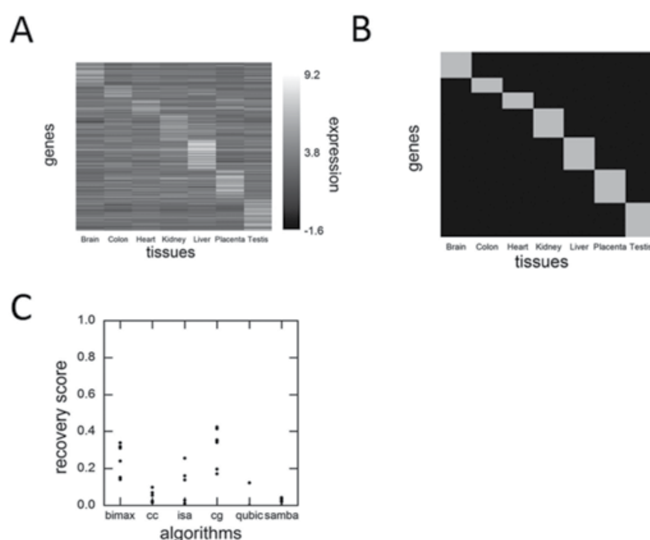


Figure 1. Tissue-wise significantly perturbed genes are presented in a matrix form

Next, taking this list of significantly perturbed genes we developed a novel cluster analysis method to group the genes based on their temporal pattern and then look for the functional classes or the pathways shown by those clusters. Iterative rounds of this exercise have now identified a cluster of 50 highly perturbed genes showing perturbation for a significant time period of disease progression (figure 1). Importantly, all of these genes were found to be closely associated with the pathways that are linked to obesity and diabetes. A subsequent network analysis then further refined this cluster to identify 6 genes that functioned as key master regulators of these pathways. Efforts are now underway to experimentally validate these findings.

Improving the resolution of protein-protein interaction (PPI) networks

Team Leader
Samrat Chatterjee

Investigators
M. Irudayaraj
Shailendra Asthana
Amit Yadav
Kanury Rao

Essential for our investigation of disease-specific networks is the availability of high-resolution PPI networks. While PPIs databases exist there are, however, deficiencies in these that preclude their useful exploitation for analysis of network function, and consequent drug target discovery. Notable among these is the lack of detail with regards to the sub-cellular localization (e.g. cytoplasm, mitochondria, nucleus etc) of the individual proteins, and also the lack of directionality to the links. To fill these gaps we have embarked on an exercise to build networks consisting of more biologically relevant information that can be further used in interpretation of experimental data and building mathematical models. As a first step to this end, we have focused on incorporating the spatial dimensions to the PPI network and this process is now near completion. Subsequent to this our aim will be to incorporate directionality to the individual links. Since this will involve a large-scale literature curation exercise, we propose to achieve this through a national network that will link with students and faculty across many institutions. An App that will enable this exercise is currently under development.

In concert with the above exercise our goal also includes structural characterization of post-translational modifications (PTMs), to aid a better understanding of interface sites of PPIs. PTMs facilitate PPIs, and, because of their reversible nature, often act as molecular switches that govern the interactions. PTMs can induce a change in protein conformation and some of these (e.g. phosphorylation and acetylation) can function as a docking site for interacting biomolecules; thereby regulating the formation of complexes. Therefore, structural characterization of PTMs can aid a better understanding of PPIs, by turning abstract system representations into reliable 3D models that more accurately reflect biological reality at the level of atomic detail.

Team Leader
Samrat Chatterjee

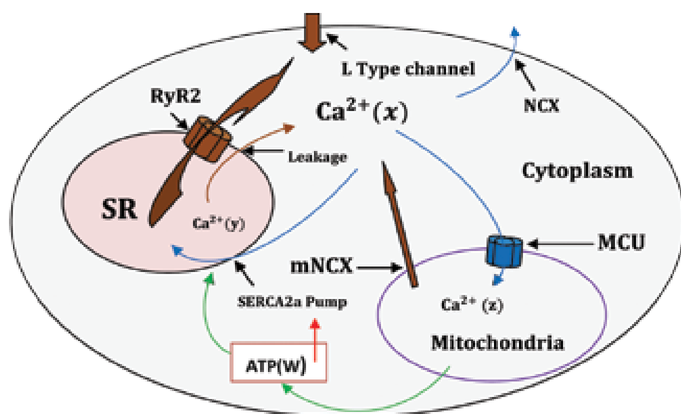
Investigator
Kanury Rao

Collaborator
Nandadulal Bairagi
Jadavpur University, Kolkata

Development of small-scale mathematical models to resolve dynamics of cellular processes

In complex biological systems, mathematical models are emerging as important tools for analysis. Large-scale models help to examine the whole system and identify points of interest using tools such as flux balance analysis. On the other hand, small-scale models help to capture the local mechanisms that are hidden in a particular cell process. Over the last year we have built few small-scale models to understand the underlying mechanisms involved. One such model involves calcium signaling in cardiac cells. This is important when we study obesity and diabetes as it leads to cardiac cell dysfunction. By recalibrating model parameters we demonstrated how mitochondria cope with functionally impaired processes and accumulate calcium (figure 2). A strong coupling of the $[Ca^{2+}]_m$ oscillation with the ATP synthesis rate ensured a robust calcium cycling and mitigated progression to heart failure (figure 3).

We are presently building additional such models for integration in our results obtained from systems biology approaches to dissect the local interactions, and capture their dynamics. Such models should prove extremely useful when dissecting regulatory modules of the MetS-specific network.



$$\frac{dx}{dt} = \alpha + \delta y + \eta z - \xi x + \frac{\gamma x y}{b^2 + x^2} - \frac{p x^2 w}{q_1^2 + x^2} - \frac{\zeta x^2}{q_2^2 + x^2},$$

$$\frac{dy}{dt} = \frac{p x^2 w}{q_1^2 + x^2} - \frac{\gamma x y}{b^2 + x^2} - \delta y,$$

$$\frac{dz}{dt} = \frac{\zeta x^2}{q_2^2 + x^2} - \eta z,$$

$$\frac{dw}{dt} = \rho + \mu z - \sigma w,$$

Figure 2: Mathematical model for capturing cellular dynamics

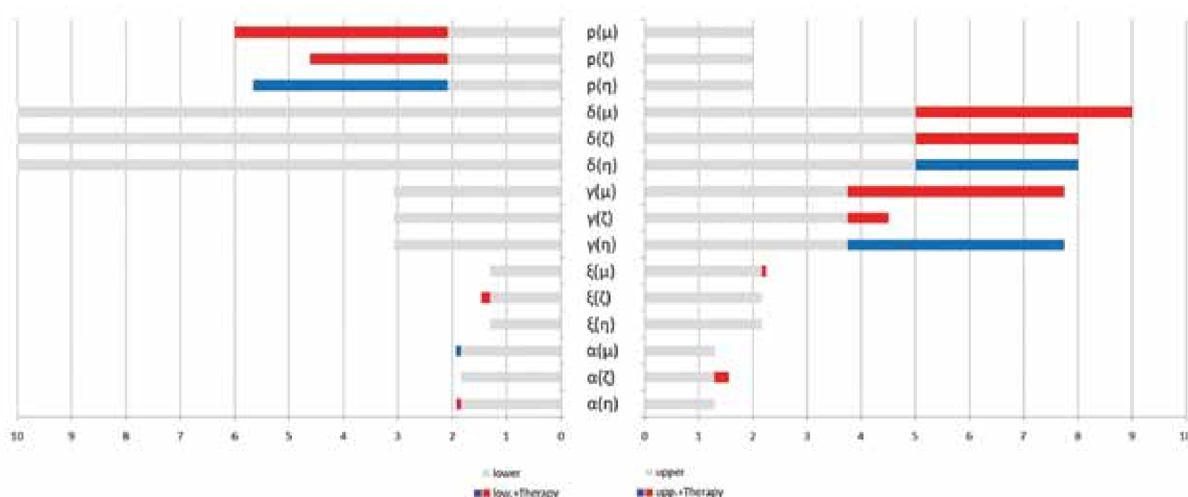


Figure 3: The oscillatory ranges of the parameters involved in the heart dysfunctioning were expanded through parameter calibration. The longer the bar means more effective is the calibration.

Team Leader
Kanury Rao

Investigators
Mukul Midha
Amit Yadav
Samrat Chatterjee

Mass spectrometry based methodologies for interrogating disease-specific phenotypic perturbations

Understanding disease development dynamics, as a prerequisite to drug target identification, hinges on the ability to generate high-resolution quantitative – and time resolved – data on the phenotypic perturbations that characterize this process. And given that phenotypic perturbations are normally expressed through changes in proteome and metabolome composition, our focus at DDRC has been to develop/standardize mass spectrometry based methods for a quantitative analysis of these components. Complementing this are efforts to incorporate the resulting information into proteo-metabolome networks for Flux Balance Analysis (FBA).

Using test systems, several methods for quantitative proteome analysis have been standardized. These include the iTRAQ protocol, an isobaric labeling method that allows for simultaneous comparison of protein levels in multiple samples, and the SILAC approach that provides for *in vivo* incorporation of a label into proteins for MS-based quantitative proteomics. Importantly, we have also been able to combine both of these approaches in order to increase the multiplexing capability up to 24 samples. This latter exercise proved a challenge from the standpoint of data analysis, but we have successfully developed a new computational method for this (see Project 1.5). We have also successfully adopted the new label free technique SWATH-MS, which is based on data-independent acquisition (DIA). We plan to extensively use this for quantitating proteome changes in animal model tissue, as a function of disease progression. To this end experiments are currently under way to experimentally generate an in-depth draft map of the rat proteome by using high-resolution mass spectrometry (HRMS). This exercise will be completed within the next six months, and the goal is to use the reference map for discovery-driven experiments. Thus, generated SWATH maps from relevant tissue, at a given stage of disease, will be mined using a targeted data-extraction strategy, to profile perturbations at the level of pathway-specific proteins. Time-dependent changes in proteome will be integrated with a parallel analysis of the metabolome through a dynamic network analysis, and subsequent interrogation through the use of Ordinary Differential Equation (ODE) based models is expected to reveal regions of sensitivity in the disease development network. Finally, we have also standardized the BONCAT approach for detecting newly translated proteins in perturbed cells.

For metabolome analysis we have optimized two analytical, mass spectrometry-based, platforms. One is an untargeted platform that can detect up to 6000 molecular ions in serum/plasma samples. Using this method we have initiated a study that monitors changes in serum metabolome, as a function of MetS disease progression in humans. For this study we are collaborating with Prof. Nikhil Tandon of AIIMS, Delhi, and Prof. Chittaranjan Yajnik of the Diabetes Unit, KEM Hospital, Pune. Dr. Tandon has been conducting a longitudinal study for the past 7 years and about 4000 samples have been collected over this period, with the blood glucose and HbA1c levels being characterized. Dr. Yajnik has been studying fetal programing of diabetes for nearly the past two decades. One such study is a longitudinal study, extending over a period of

18 years, on a cohort that includes mothers, fathers and their offspring. Our analysis focuses on samples from both of these studies. The larger aim here is to characterize the temporal modulations in serum marker composition, as a function of disease progression, through ODE models to define the relationship between disease dynamics and the individual parameters. By then employing principles of the Chaos theory, we hope to eventually extract a relationship that will predict outcome simply based on the knowledge of either the initial or an intermediate condition.

The targeted platform analyzes metabolome composition in cells and tissues. It detects and quantifies a set of about 200 metabolites that are collectively representative of all of the major biochemical pathways of a cell. This method is now being used to monitor temporal changes in metabolic flux, in different rat tissues, as a function of disease development. As discussed earlier, this data will be integrated with the corresponding proteomics analysis of the same tissue to generate proteo-metabolome networks for FBA.

Computational mass spectrometry: Developing new tools for analysis

Team Leader
Amit Yadav

Investigators
Mukul Kumar Midha
Shilpa Jamwal

Amit Yadav

The focus here is on computational mass spectrometry, proteomics and post-translational modifications. This involves large-scale identification of modifications in blind mode from high-resolution quantitative proteomics data to map their role in driving the protein-protein interactions, cellular localization and tissue specific roles in normal development, and in metabolic disease progression. We also develop new statistical algorithms, software, pipelines and visualization tools to enable data analysis and interpretation for investigating the next generation proteomics data. PTMs are the hidden players in regulation of cellular pathways in a rapid manner and insights into their functioning and crosstalk can yield a goldmine of potential drug targets. Integrated together with structural insights and mathematical modeling, we can discover and fine-tune biological regulation which may open up a new paradigm in drug discovery and development. Specific programs undertaken here are:

Hyperplexing enables the study of temporal dynamics of newly translated proteins

A limitation with proteomics using mass spectrometry is its limited multiplexing capacity. Using a model system, we have employed several sets of SILAC (3-plex) with iTRAQ (6-plex) to increase the multiplexing capacity to 18-plex, termed hyperplexing. These sets of the 18-plex data were run on high resolution ABI 5600 triple-TOF mass spectrometer. We have developed analysis pipelines and iTRAQ analysis tool to deal with such multifarious data (figure 4). Our tool shows high correspondence with ProteinPilot (R^2=0.99) and iTRAQAnalyzer (R^2=0.99) on independent public datasets. We are currently mapping the temporal profiles of newly synthesized proteins in differently perturbed cells. Hyperplexing is a generically applicable pipeline that will enable large scale monitoring of samples in high throughput with substantially reduced instrument time. The in-house developed analysis

pipeline together with mzJSON for novel interactive data visualizations will make it a routine process with the help of high-end compute infrastructure. This technique can then be applied and integrated with other teams of DDRC utilizing quantitative proteomics for example – understanding ubiquitin degradation system as a potential for drug target exploration (with Sameena Khan).

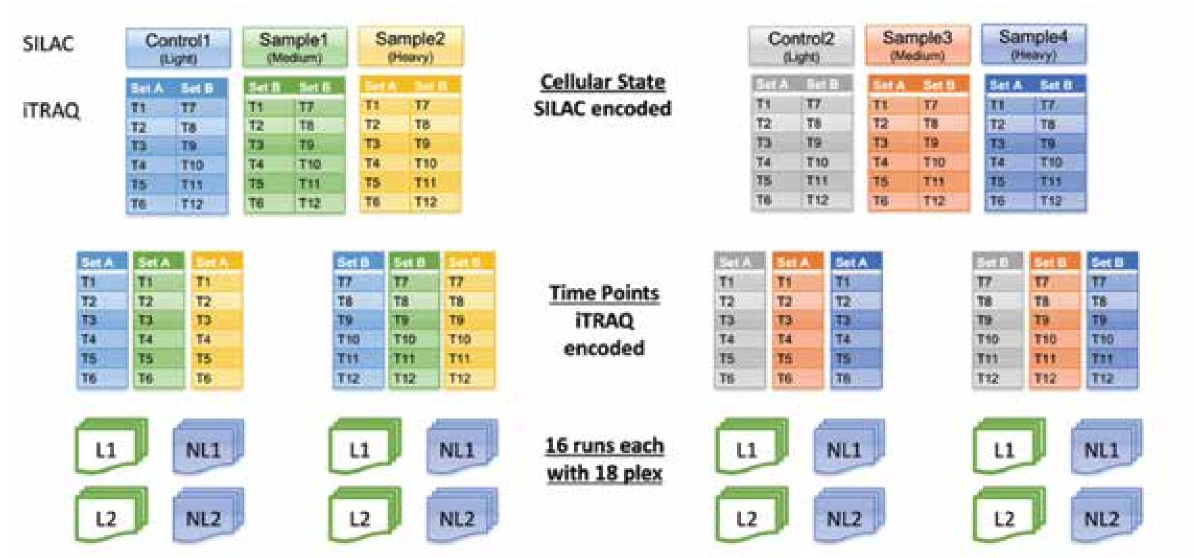


Figure 4: Hyperplexing enables the study of temporal dynamics of newly translated proteins.

Emergent properties of the human disease PTM-ome

We are mining the human proteome utilizing the highly curated neXtProt database to map the association between diseases and post-translational modifications (PTMs). We have found multiply modified proteins to have high disease incidence and most of such proteins are non-housekeeping in nature. We defined - **Functional diversity index (FDI)** of a protein (based on biological processes, protein-protein interactions (PPIs) and PTM frequency), which is an indicator for disease susceptibility of a human protein (figure 5). We are currently exploring if domains and disorder also have a disease prediction propensity.

Other DDRC members are exploring this data for leads into role of PTMs in diabetes and other metabolic disorders (Shilpa Jamwal and Sanjay

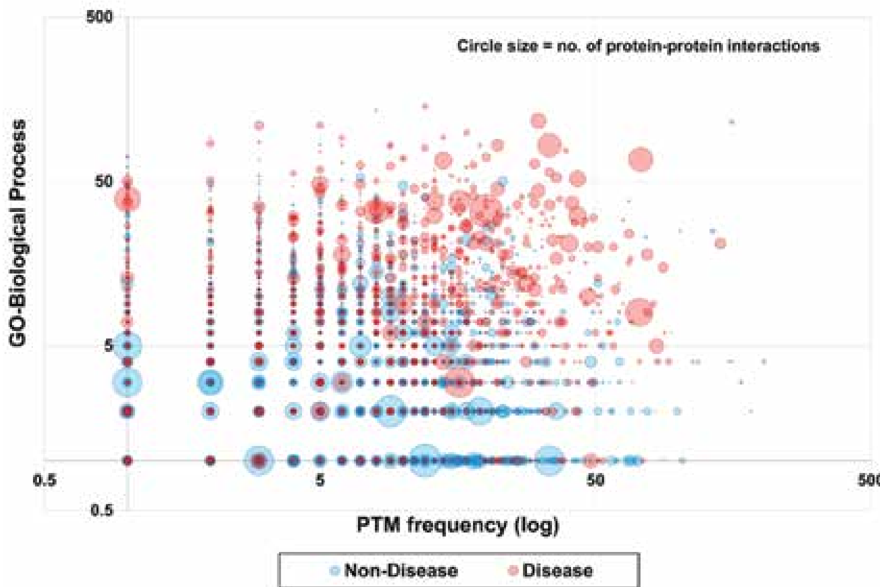


Figure 5: Functional diversity index of a protein is an indicator for disease susceptibility of a human protein

Banerjee). Shailendra Asthana will explore this data for structural insights into PTM for their role in PPI in interaction interfaces of binding pockets and active sites by docking and simulations. Sameena Khan will be exploiting this information for ubiquitination and protein degradation.

mzJSON-a data standard for browser based interactive visualization for proteomics data

Data visualization is an important step in analysis and interpretation of large-scale proteomics studies. Standard XML data formats for raw, identification results and quantitation data are continuously becoming burgeoning and unmanageable. We have proposed **mzJSON**, a data format based on JavaScript Object Notation (JSON) for data exchange and browser based dynamic, interactive visualizations using applications developed in JavaScript. Since these applications can run in any modern web browser, data visualization becomes easy and platform independent and any web developer can exploit JavaScript libraries like D3 for creating their own custom applications. We have developed some applications from mzJSON format and these are in use for data analysis by our mass spectrometry group. We can extend these functionalities in future for specific analysis needs of DDRC group.

Delineating the cellular and molecular events that initiate MetS

Team Leader
Shilpa Jamwal

Investigators
Mukul Midha
Shailendra Asthana
Samrat Chatterjee

Shilpa Jamwal

Along with strategies for therapeutic intervention, DDRC also recognizes the value of developing new approaches that either prevent initiation of MetS, or mitigate its progression. For this purpose we are making use of the diet-induced rat model of MetS to define the early perturbations that characterize initiation of disease. Our first focus in this direction is the perturbations induced in visceral white adipose tissue (VWAT), as the free fatty acids (FFAs) liberated from this site represent at least one of primary events that initiates/drives MetS. Additionally, adipocytes also link between obesity and diabetes, with adipocyte-derived factors playing a critical role in regulating insulin sensitivity of other peripheral tissues (figure 6).

The animal experiments have been conducted, and serially collected VWAT over multiple time points has been generated. Additional tissues collected include liver and skeletal muscle, and the first objective is to analyze each of these to determine the time point at which insulin resistance first appears. Taking this time point as the window for establishment of the process towards MetS (i.e. pre-diabetic condition), a detailed temporal analysis of VWAT will be performed. Parameters to be studied will include immunocyte infiltration, biochemical changes, and modulations in the proteo-metabolome flux. While providing a framework for subsequent analysis of the disease initiation process, these findings are also aimed at generating an assay platform for developing and testing lead compounds capable of inhibiting/suppressing MetS development.

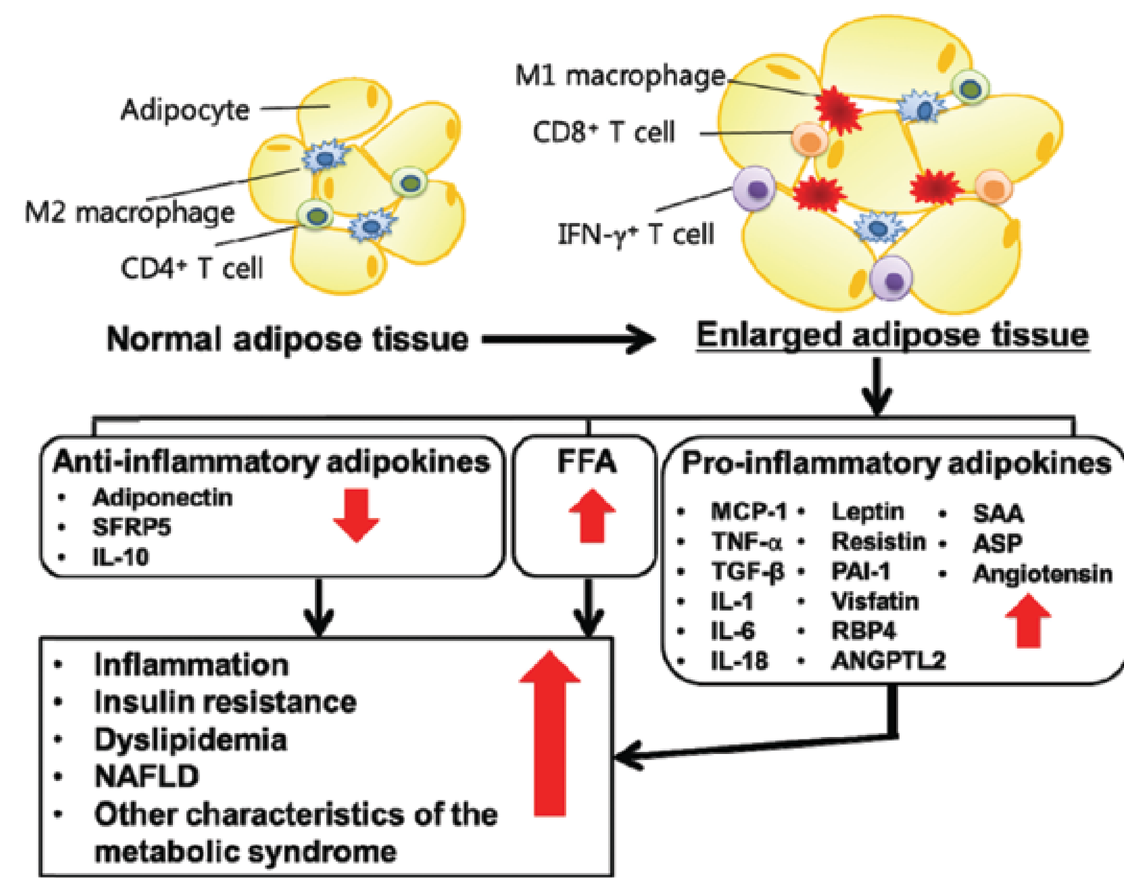


Figure 6: Secretion of inflammatory adipokines from adipose tissue in obese state. In obese state, the enlarged adipose tissue leads to dysregulated secretion of adipokines and increased release of free fatty acids. The free fatty acids and pro-inflammatory adipokines get to metabolic tissues, including skeletal muscle and liver, and modify inflammatory responses as well as glucose and lipid metabolism, thereby contributing to metabolic syndrome. In addition, obesity induces a phenotypic switch in adipose tissue from anti-inflammatory (M2) to pro-inflammatory (M1) macrophages. The red arrows indicate increased (when pointing upward) or decreased (when pointing downward) responses to obesity. ANGPTL, angiopoietin-like protein; ASP, acylation-stimulating protein; IL, interleukin; MCP-1, monocyte chemotactic protein; NAFLD, nonalcoholic fatty liver disease; PAI-1, plasminogen activator inhibitor-1; RBP4, retinol binding protein 4; SAA, serum amyloid A; SFRP5, secreted frizzled-related protein 5; TGF-β, Transforming growth factor-β; TNF-α, tumor necrosis factor-α.

Delineating master regulatory mechanisms of the functional modules of a cell

Team Leader
Sameena Khan

Investigators
Shilpa Jamwal
Shailendra Asthana
Amit Yadav

Our earlier studies, on network analysis of gene transcription data capturing MetS progression in tissues obtained from the mouse model (Project 1.1), revealed an intriguing aspect of this disease. These results indicated that multiple functional modules of the cells of the target tissue were perturbed even in the very early stages of disease. Importantly, this feature was retained throughout the course of disease progression and establishment. This observation underscored a challenge from the standpoint of effective drug development since it implied that combinations of inhibitors targeting the different modules would need to be employed. On the other hand, however, these results also highlighted that all of these modules were in fact linked together through some key regulatory molecules, many of which were components of the ubiquitination machinery. The intriguing possibilities offered by this observation then prompted us to focus on a better understanding of the Ubiquitin proteasome system (UPS), and its

regulation of cellular functions. Support for this effort was also provided by related observations from mass spectrometric analysis, which suggested that turnover rates of proteins may also be involved in fine-tuning functional responses of a cell.

Selective Ubiquitin proteasome system (UPS) degradation is executed indistinctly in three steps, recognition of the protein degradation signal sequence (degron), marking this target protein for degradation via ubiquitin (Ub) conjugation, and finally degradation by the proteasome. UPS mediated pathway confers constitutive and conditional metabolic instability on a protein by temporal control and selective degradation. The destabilizing N-terminal residues, called N-degrons are a class of protein degradation signals that dictates the *in-vivo* half-life of proteins. Substrates of this pathway are often generated by proteolytic cleavage or N-end modifications. UPS pathway known to control the levels of key proteins regulates almost all of the cellular activities including cell cycle progression, DNA replication and repair, transcription, protein quality control, immune response, and apoptosis. UPS has been explored for its link to cancer, autoimmune disorders and the age related, neurological disorders and metabolic disorder. Details of the typical mammalian N-end UPS is presented in figure 7.

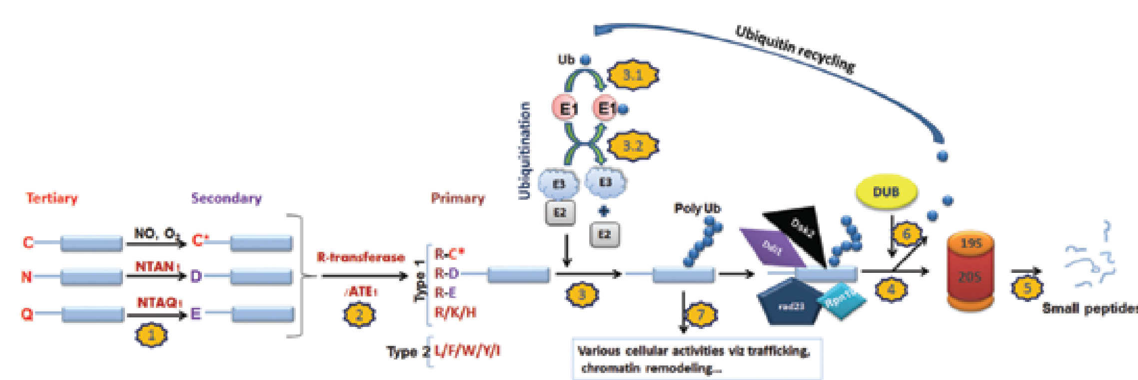


Figure 7. Map of Ubiquitin mediated degradation machinery in humans is depicted. Only N-end mediated UPS pathway is shown and single letter code is used for amino acid description. 1 Tertiary destabalising residues N, Q and C on the N-terminal of substrate protein (blue rectangle) are modified by NTAN1 (N-terminal N-amidase) and NTAQ1 (N-terminal Q- amidase) enzymes to yield secondary destabilizing N-terminal residues D and E. Oxidized Cys derivatives (C*) also serve as secondarydestabilizing residues in mammals. 2 Secondary destabilizing residues D, E and oxidized Cysteine derivatives (C*) on the substrate protein N-terminal are conjugated by Arg-tRNA transferase (R-transferase) to R (a primary destabilizing residue). 3 Destabilizing N-terminal basic (R, K, H) and bulky hydrophobic (L, F, Y, W, I) residues are bound directly by N-recognin (also called E3 Ubiquitin (Ub) ligase), which mediate substrate selectivity by recognizing N-terminal signal. A complex of E3 Ub ligase and E2 ubiquitin-conjugating enzyme (E2) transfers Ub molecule to Lys residue on substarte protein. 3.1 Ubiquitin is activated by E1 ubiquitin-activating enzyme (E1) and 3.2 is transferred to E2. 4 Polyubiquitinated substrate can be targeted to the proteasome via shuttle proteins (Dsk2, Ddi1, rad23, rpn10) and 5 destroyed by complex 26S proteasome machinery (20S core + 19 S regulatory particle). Finally, 6 deubiquitinating enzymes (DUB) recycle ubiquitin proteins. 7 Poly or monoubiquitylation can also be an activation/repression signal that modulates the substrate activity in several cellular processes such as trafficking or chromatin modeling.

We have initiated investigation of the role of UPS in metabolic syndrome. The larger aim is to decrypt the fine balance between ubiquitination and deubiquitination processes, and probe how this fine-tuning regulates diverse cellular functions. In this context our first goal is to identify the E3s and DUBs that regulate TNF -induced apoptosis of cardiomyocytes, and subsequently delineate those that crosstalk with the anti-apoptotic, insulin-dependent

signaling pathway. We are also interested to see the pattern of ubiquitinated proteins in the normal cardiomyocytes and next then to link it with the E3 and DUBs. The regulatory roles of E3 ligases and DUBs are being studied through a multi-disciplinary approach that combines biophysics and structural biology, bioinformatics and network analysis, mass spectrometry, and various tools of biochemistry and cell and molecular biology. The larger aim in this specific program is to integrate structural biology with the tools of systems biology for drug development efforts.

Lead discovery and development

Although the current efforts at DDRC focus on developing strategies for extracting and analyzing disease-specific networks we are optimistic that this exercise will soon yield novel targets that can then be exploited for drug development. To prepare for this, we are in the process of developing the following capabilities:

Team Leader

Shilpa Jamwal

Developing screening platforms for in vitro models of MetS

To support our lead-to-drug development activity DDRC intends to set up a high-content screening platform with a medium throughput. While we hope to establish this platform soon we are, in the meanwhile focused on developing and optimizing *in vitro* systems that can assay for the various cellular perturbations that typify MetS. Once standardized for both robustness and sensitivity, these systems will be employed for target validation, as well as for lead molecule discovery and development. Our model systems include the different cell types that relevant for MetS. These include hepatocytes, adipocytes, macrophages, smooth muscle cells, cardiomyocytes and pancreatic beta-cells. These models are intended to replicate and create the molecular dynamics and phenotypic profile of tissues representing the pathology of disease in focus.

Broadly, the cellular properties being assayed in these models are listed below, although the exact range used will depend on the cell type.

- Adipokine secretion
- Pro/Anti Inflammatory cytokine secretion
- Insulin receptor sensitivity
- Glucose uptake
- Balance between glycolysis and gluconeogenesis
- Lipid turnover and accumulation
- Mitochondrial Stress
- Oxidative Stress
- Endoplasmic Reticulum Stress

While the panel above may appear to be large it, however, takes into account the diverse etiologies and multivariate complexity of MetS. Insulin receptor

sensitivity is being monitored in the terms of the phosphorylation status of IRS1/2 and JNKs, whereas monitoring levels of PEPCK, G6P, and GHK approach gluconeogenesis. Lipid turnover is measured through levels of DAG's and TAG's, and commercial kits are available for the remaining assays listed above.

Team Leader
Rajkumar Halder

Rajkumar Halder

Synthetic chemistry for lead molecule development and optimization

We are presently handicapped in this activity because our Chemistry laboratory is not yet ready. However, work on this is almost complete and we expect to be able to occupy it soon. This group will primarily focus on complementing the ongoing exercise of interrogating disease-specific perturbations through PPI networks by developing strategies for pharmacophore generation against 'non-druggable' targets. We strongly believe that the strategy of interrogating disease-specific mechanisms through high-resolution PPIs, and then exploiting this for drug development by focusing on the relevant non-druggable target space will - with time - define a unique global niche for DDRC.

Early translation

While our more long-term programs continue, we have also initiated an activity with the more immediate objective of developing novel leads against known disease-relevant protein targets. In this exercise both druggable and non-druggable sites on the proteins are being explored through advanced computational techniques. Leads generated from this exercise will be experimentally validated, and further developed by the teams associated with the Lead discovery and development component of DDRC (Theme 2). The projects that are currently underway are described below.

Team Leader
Shailendra Asthana

Sodium Glucose Transporter 2 (SGL-2s): A target for the control of hyperglycemia in type 2 diabetes

The treatment of diabetes has been mainly focused on maintaining normal blood glucose concentrations. Insulin and hypoglycemic agents have been used as standard therapeutic strategies. However, these are characterized by limited efficacy and adverse side effects, making the development of new therapeutic alternatives mandatory. Inhibition of glucose reabsorption in the kidney, mediated by Sodium glucose transporter-2 (SGLT-2), represents a promising therapeutic approach. Therefore, our aim is to develop new modulators against SGLT-2 by using a drug-discovery scheme. For this purpose a structure-based computational approach is employed, which encompasses homology modeling of SGLT protein, molecular dynamics, virtual screening, pharmacophore- ligand- structure- and fragment based screenings, to identify novel inhibitors with high potency and affinity (figure 8). Several promising hits have been identified. These molecules await synthesis for experimental validation and further development.

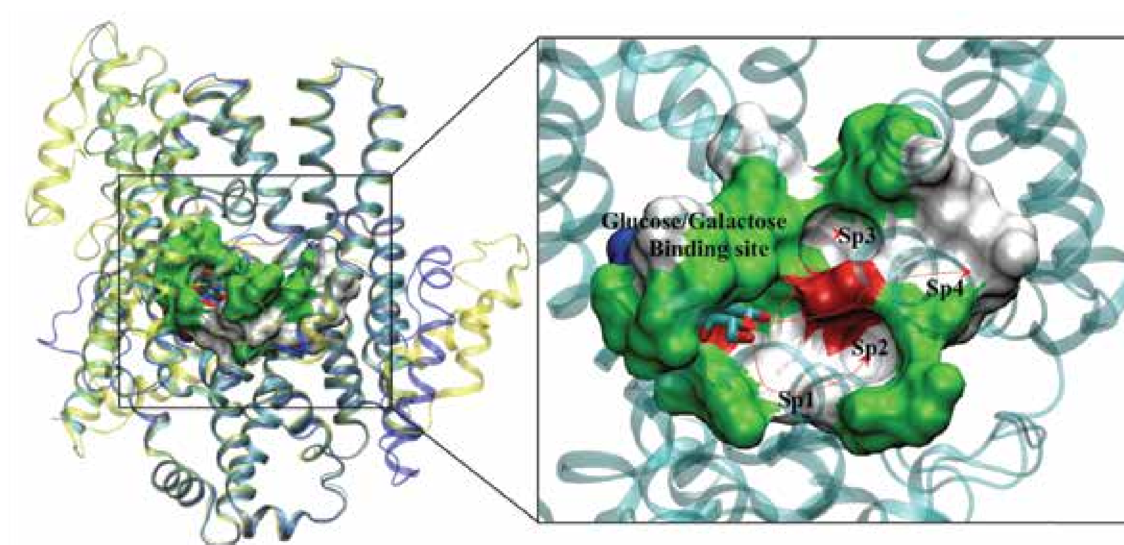


Figure 8: Superimposed modeled structure of SGLT1 and SGLT2. The glucose/galactose binding site is shown. Newly identified neighboring, small pockets (Sps) of glucose binding site are used for structure based drug discovery approach.

Team Leader
Shailendra Asthana

Investigator
Sanjay Banerjee

SGLT-1, a target for cardioprotection

The Discovery Research component of DDRC (Theme 4) had previously observed that protein Kinase C (PKC) mediates activation of SGLT1 in precondition-induced cardioprotection. However, whether PKC physically interacts with SGLT1 remained unknown. Therefore, an extensive protein-protein, rigid- and flexible-docking was carried out to identify the interface site. The strong affinity predicted for the PKC-SGLT1 complex, and the juxtapositioning of the phosphorylation site with the interface site supports that PKC mediated SGLT1 activation, and consequent cardio-protection, is likely mediated through a direct physical interaction (figure 9).

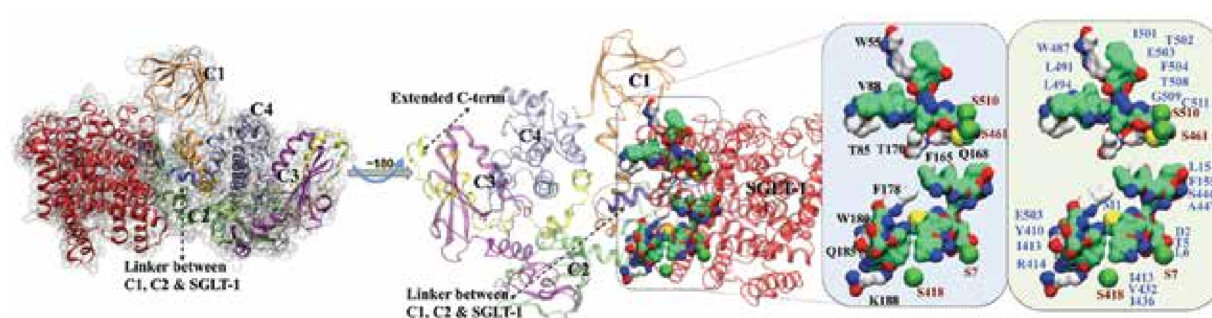


Figure 9: Protein-protein docking: Domain-wise rendering of PKC, C1: orange, C2: lime, C3: purple, C4: ice-blue and extended C-terminal: yellow, while SGLT1: red. The linker part is in blue. The atom wise VdW surface rendering of interface residues as C: green (SGLT1) and white (PKC), O: red, N: blue and S: yellow. The inset view is showing the interacting PKC and SGLT1 residues, separately.

Team Leader
Shailendra Asthana

Investigator
Shilpa Jamwal

Structure-based discovery of novel modulators through different interconnected metabolic routes that trigger sirtuins

Sirtuins have been implicated in metabolic diseases and are considered as attractive drug targets. Mammalian sirtuins are a family of nicotinamide adenine dinucleotide (NAD+)- dependent protein deacylases implicated in a wide range of cellular process. It is known that sirtuins could all catalyze long chain deacylations, but with varying degree of specificity and efficiency. The mechanistic basis underlying these unique deacylation profiles was not investigated in detail at the structural level. The link between NAD+ dependence and the nature of the acyl group are unclear. NAD+ metabolism is known to affect the cellular function of sirtuins. Structural characterization of sirtuins can provide a more complete understanding of the mechanisms that control deacylase activity of sirtuins. In addition these results may aid in the interpretation of cellular studies and allow for the design of rational small molecule regulators of sirtuin activity. Activating sirtuins is a big challenge. Recent reports claim that small molecules such as SRT1720 and resveratrol activate human SIRT-1 and its orthologs. We are studying sirtuin modulation through a novel strategy that employs advanced computational methodologies of drug development by targeting the binding site of SIRTs (either substrate binding site, NAD^+ site, inhibitor and/or activator site) (figure 10). The major hurdle in developing sirtuin modulators is the in-complete structures of sirtuins, where disordered and flexible regions likely play a crucial role in enzymatic activation. We have now developed robust models of SIRTs for use in structure- or ligand-based approaches for virtual screening. This activity is currently underway. Hits identified will be chemically synthesized and the efficacy will be experimentally validated. To further enrich our analysis, we are also structurally mapping the sequentially curated in-house data of PTMs, with a particular emphasis on acetylation and phosphorylation. Since most PTMs do not exist alone in the structural environment, and the combination of their states reinforce one another, we believe that incorporation of such data could provide more insights into the regulatory features of sirtuin biology.

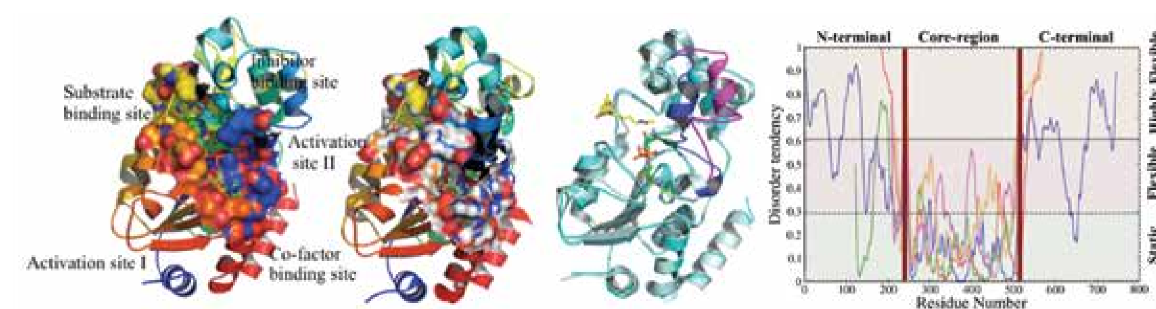


Figure 10: Sirt1 structure: one protein can be inhibiting via multiple route, by targeting substrate, co-factor, or allosteric site. The regions of target in sirtuins are shown by surface view.

Team Leader
Shailendra Asthana

Collaborator
Jamuna Subramaniam
IIT-Chennai

Shailendra Asthana

Identification of transporters and G-protein coupled signaling cascades involved in lifespan extension

The antihypertensive drug reserpine is known to extend lifespan in C. elegans. In addition to increased longevity, reserpine also induces stress tolerance and improved locomotion up to a late age, thereby improving quality of life. Importantly, high quality lifespan extension also confers protection against cancer and other diseases, as well as resistance against infection. Recent studies have shown that reserpine does not target the known lifespan extension pathways. Instead it modulates activity of the neurotransmitter acetylcholine (ACh). This activity of reserpine was mediated through binding to the vesicular acetylcholine transporter (VAChT). However, reserpine also binds and inhibits, in an irreversible manner, another transporter, the vesicular monoamine transporter (VMAT), and this binding causes adverse side effects. As the two transporters seem to be the main target of reserpine, an exercise aimed at gaining structural insights into the recognition process, and subsequent identification of novel modulators through a computational biological approach will be extremely helpful. Homology modeling of the vesicular acetylcholine transporter (VAChT), the transporter that loads ACh into the synaptic vesicles (similar to VMAT), and virtual compound library screening to identify the specific modulators of VAChT by docking on the 3D structures were carried out (figure 11). Due to limited structural information about this transporter, an extensive computational approach was performed to generate models of VAChTs. This modeling is ongoing and, subsequent to this, our aim will be to identify potential modulators for lead screening by combining Virtual and Fragment based approaches.

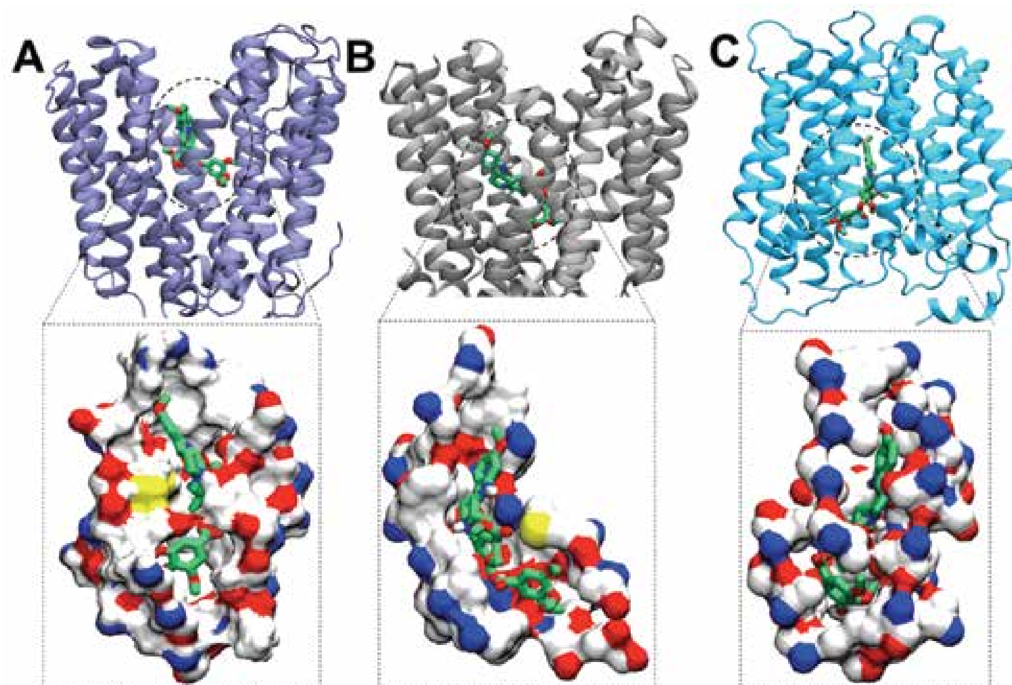


Figure 11: Reserpine binding site identified in three systems under study. A. VAChTele, B. VAChTh and C. VMAT. The protein are rendered in cartoon and reserpine is shown in licorice and colored according to atom type C:green, O:red, N:blue and H:white. The binding cavity (shown by dotted circle) of reserpine is shown by surface view.

Discovery Research

In concert with the above programs, we conduct a few basic research programs that are aimed at exploring both experimental and theoretical aspects of biological processes related to MetS. The objective here is to generate mechanistic insights into such processes, which may then support our translational activities. The projects under this theme are as follows:

Delineating the molecular mechanism of Diabetic Cardiomyopathy

Team Leader

Sanjay K Banerjee

Sanjay K. Banerjee

Diabetic patients have almost twice the rate of mortality because of congestive heart failure than non-diabetic individuals. Both hyperglycemia and insulin resistance increase the risk of cardiac complications. Alteration of cardiac muscle in diabetes has led to the recognition of a new cardiac disease *termed* **'***diabetic cardiomyopathy***'** (DCM). Several etiological factors like hyperglycemia, metabolic abnormalities, and redox imbalance (oxidative stress) may affect the physiology of the diabetic heart. The overwhelming effect of all these factors leads to cardiac abnormalities including myocardial dysfunction, myocardial hypertrophy, impairment of contractile proteins, accumulation of extracellular matrix proteins and decreased left ventricular compliance. Several mechanisms such as overproduction of mitochondrial reactive oxygen species (ROS), increased glucose auto-oxidation, altered myocardial energy metabolism and increased synthesis of advanced glycation end products (AGE's) have been proposed for hyperglycemia-induced oxidative damage in the diabetic heart. The goal of this project is to capture the disease progression and find novel targets against diabetic and hypertrophic cardiomyopathies.

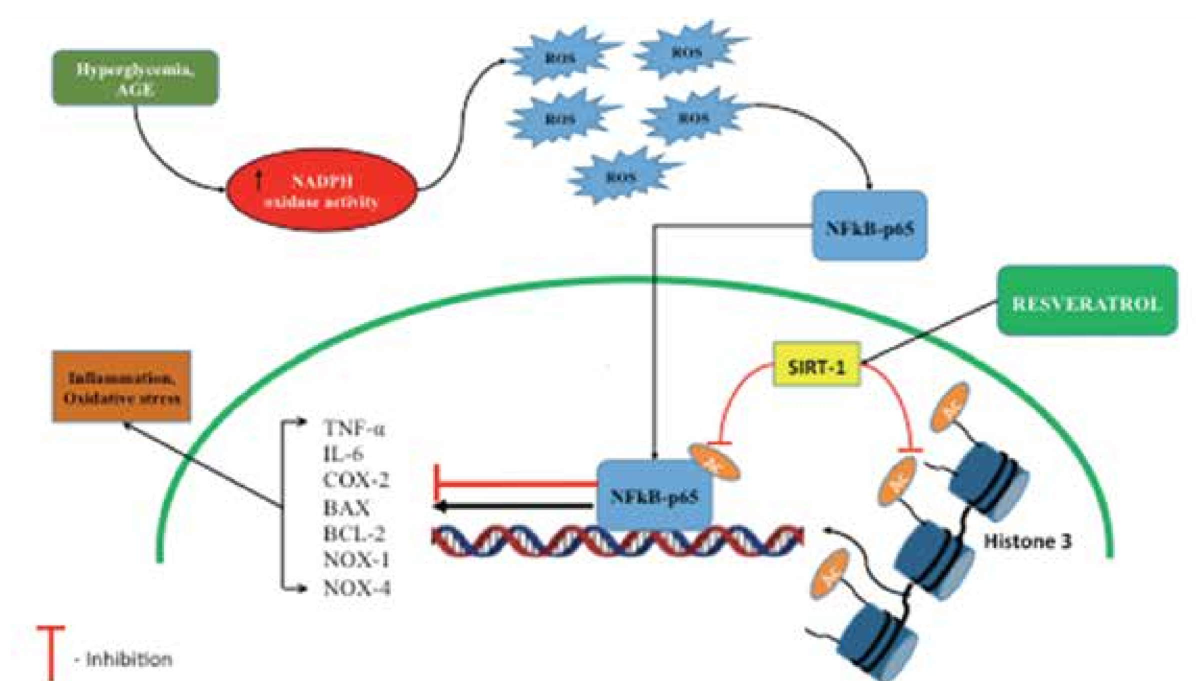


Figure 12: Effect of Sirt1 activation by resveratrol on NFkB activation and inflammatory gene expression in diabetic heart

Recently, the involvement of posttranslational modification in disease biology has gained wide interest and focus in our center. Acetylation of histone and non-histone proteins is considered to be one of the important post-translational and epigenetic modifications. The role of acetylated protein in disease biology is still in its infancy and its role in malfunctioning diabetic heart is poorly understood. We have evaluated the acetylation of one such important signaling protein, i.e. NFkB-p65 which plays a crucial role in the progression of cardiomyopathies. Acetylation plays a prominent part in governing the nuclear action of NFkB. Acetylation of discrete lysine residues in p65, a subunit of NFkB modulates distinct functions of NFkB, including transcriptional activation, DNA binding and assembly with its inhibitor IkBα. Acetylation of lysine at 310 and to a lesser extent at lysine 221 residues play a key role in the overall transcriptional activity of NFkB. Increased NFkB activity through acetylation is controlled by a feedback mechanism, i.e. deacetylation of NFkB. Histone deacetylases (HDAC's) are the enzymes that cause deacetylation of histone proteins, whereas sirtuins, class III HDAC's deacetylates histone as well as non-histone proteins. Acetylation/deacetylation events of p65 likely represent important regulators of NFkB activity.

We hypothesized that activation of SIRT-1 could be useful to inhibit NFkB activity in diabetic heart and thus could be a potential therapeutic intervention for diabetic cardiomyopthy. We have explored the role of SIRT1 in fructose-fed diabetic heart and demonstrated the therapeutic potential of resveratrol through its SIRT-1 modulation property. The beneficial effect of resveratrol has been linked to sirtuin-1 (SIRT-1) activation (figure 12). We have demonstrated that SIRT1 activation ameliorates cardiac hypertrophy, electrocardiographical abnormalities and oxidative stress in the fructose fed diabetic rat heart. Mechanistic studies revealed that fructose feeding to Sprague Dawley (SD) rats over a period of eight weeks leads to cardiac hypertrophy and increased oxidative stress through increased activity of NADPH oxidase (NOX) and ROS production. We found increased activity of nuclear factor kappa B (NFkB) p-65 along with decreased SIRT-1 activity in the diabetic heart. Resveratrol activates SIRT-1, which deacetylate NFkB-p65 at lysine 310 and histone 3 (H3) at lysine 9 position. SIRT1 activation leads to decreased binding of NFkB-p65 to DNA, and attenuated cardiac hypertrophy, oxidative stress through reduced transcription of NADPH oxidase subunits. *In-vitro* analysis also revealed that SIRT-1 activation by resveratrol is associated with decreased NFkB-p65 activity and NOX transcription. Similarly, knock-down or inhibition of SIRT1 in H9C2 cells increased NFkB-p65 K310 and H3K9 levels (figure 13). Our data demonstrated that SIRT-1 activation by resveratrol leads to deacetylation of both NFkB and H3, thereby attenuating cardiac oxidative stress and complications in diabetes. Other proteins regulated by sirtuins are also being identified by co-IP followed by mass spectrometry. The aim here is to generate a more integrated

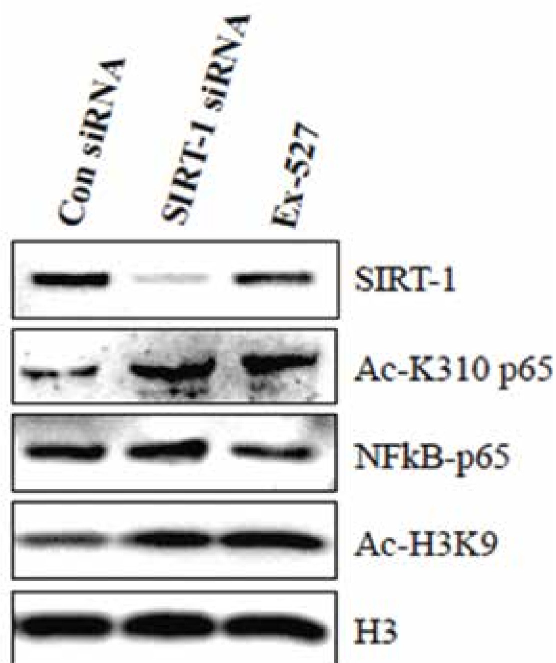


Fig 13: Effect of sirt1 knock-down on p65 and H3 acetyltion in H9C2 cells.

perspective on regulation of SIRT-1 activation through a network analysis.

Another related objective is to identify active metabolites from garlic. Garlic is rich in sulfur compounds but most of them not stay intact in-vivo. With the help of LC-MS, we have identified active sulfur compounds that may be responsible for beneficial effect of garlic against metabolic disorder. We have also characterized them in in-vitro cellular system. With the help of chemistry group of DDRC, we plan to synthesize several stable sulfur compounds from these active metabolites.

Team Leader

Samrat Chatterjee

Identifying points of sensitivity in biological networks undergoing random perturbations

Therapeutic strategies that target key molecules have not fulfilled expected promises for most common malignancies. Major difficulties include the incomplete understanding and validation of these targets in patients, and the use of single-pathway targeted approaches that are proving to be not so effective therapies for human malignancies. Signaling pathways are not linear pathways but it includes molecular crosstalk. To understand this we need to consider and analyze the system as a complex network of interacting components. Such departure from the traditional paradigm of studying single pathway to more global approach will aid the design of novel therapeutics and will help to overcome the shortcomings of the existing therapeutic strategies. Here, we use mathematical models to dissect the network to understand the importance of motif structure in determining the cellular function in presence of noise and their distribution in a signaling network. This will help us to quantitatively measure the sensitivity of a motif in the signaling network under noise and thus enable us to develop a formula that will rank the nodes according to their presence in different motifs in a biological network. This ranking could then be used to identify potential drug candidates.

The motivation for the present study came from an early work where we studied the sensitivity dependence of a node on its position in the network architecture. We used a mathematical model for three-node feed-forward loops and identified that the organization of motifs in specific manner within the network serves as an important regulator of signal processing. Further, incorporating a systemic stochastic perturbation to the model we proposed a possible design principle, for higher-order organization of motifs into larger networks in order to achieve specific biological output. The design principle was then verified in a large, complex human cancer cell signaling network (taken from literature). Further analysis permitted us to classify signaling nodes of the network into robust and vulnerable nodes as a result of higher order motif organization. We observed that the distribution of these nodes within the network at strategic locations then provides for the range of features displayed by the signaling network (figure 14). These preliminary results underscored the potential of this work and, therefore, motivated us to pursue large-scale analyses in this direction. While our initial work was restricted to three node FFLs, we recognized that other categories of motifs were also present in the signaling network. These included Feed Back Loops, Four-node FFLs, and Bifans among others. Consequently, an extension of the

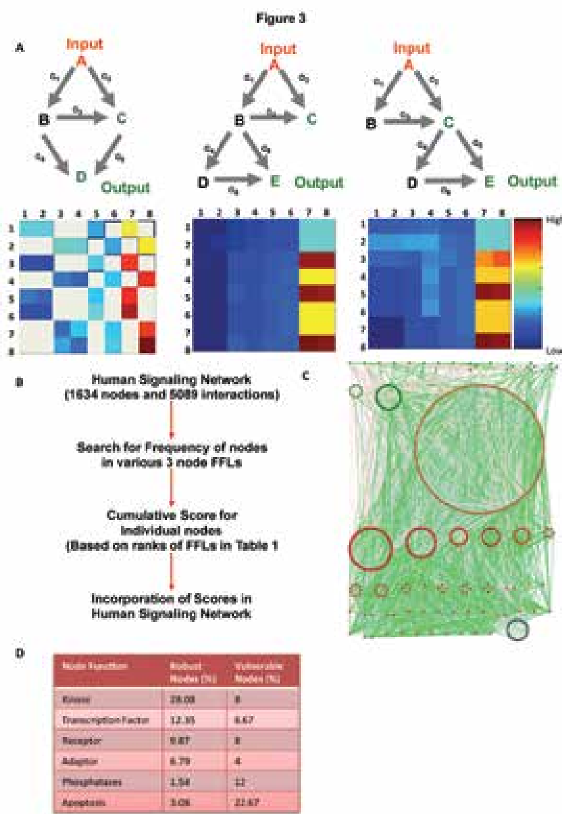


Figure 14: Different kinds of three node feed forward loops attached to each other. The sensitivity of the nodes are given in form of a matrix where tow and coloumn represents each motifs. Algorithm showing how the sensitive and robusts nodes are selected.

analysis described here to incorporate all such motifs can clearly be expected to provide many additional insights into the regulatory aspects of signal transduction.

In the last year, we extend the study on different motif structures by performing analysis on two node motifs with all possible combination. Our initial study focused on the deterministic systems, which once understood will be extended to the stochastic version. Two node structures are the smallest possible structure but studying those structures give us initial insights for selecting motifs that showed the richest dynamics (like bistability, oscillation etc), for subsequent evaluation at higher dimension. The various levels of interpretation achieved in this project will be experimentally validate by the relevant teams at DDRC, thus providing for an iterative scheme to delineate the weak links (i.e. possible drug targets) in an intracellular regulatory network.

Peer-reviewed Publications

1. Singh, V., Kaur, C., Chaudhary, VK., Rao, K., Chatterjee, S., M. tuberculosis Secretory Protein ESAT-6 Induces Metabolic Flux Perturbations to Drive Foamy Macrophage Differentiation. Scientific Reports. (in press).

2. Chatterjee, S., Pessani, D., Venturino, E., Harvesting strategies for ``bianchetti" and ``blue fish" in the Ligurian Sea (North Mediterranean). Appl. Math. Infortn Scis. (in press).

3. Pedruzzi, G., Rao, K., Chatterjee, S., (2015). Mathematical model of mycobacterium-host interaction describes physiology of persistence. Journal of Theoretical Biology 376: 105-117

4. Biswas, S., Chatterjee, S., Chattopadhyay, J., (2015). Cannibalism may control disease in predator population: Result drawn from a model based study. Mathematical methods in the Applied Sciences 38: 2272-2290.

5. Sinha, N., Negi, S., Tikoo, K., Sharma, S., Tripathi, P., Kumar, D., Rao, K V S., Chatterjee, S., (2014). Molecular signatures for obesity and associated disorders identified through partial least square regression models. BMC System Biology 8:104.

6. Mehrotra, P., Jamwal, S., Saquib, N, Md., Sinha, N., Siddiqui, Z., Manivel, V., Chatterjee, S., and Rao, K, V S., (2014). Pathogenicity of Mycobacterium tuberculosis is expressed by regulating metabolic thresholds of the host macrophage. PLOS Pathogens 10 (7): e1004265. doi:10.1371/journal.ppat.1004265

7. Mehta J, Asthana S, Mandal CC, Saxena S. (2015) A molecular analysis provides novel insights into androgen receptor signaling in breast cancer. PLoS One. 17;10(3).

8. Asthana S, Shukla S, Ruggerone P, Vargiu AV. (2014) Molecular mechanism of viral resistance to a potent non-nucleoside inhibitor unveiled by molecular simulations. Biochemistry 11:6941-53.

9. Caboni P, Liori B, Kumar A, Santoru ML, Asthana S, Pieroni E, Fais A, Era B, Cacace E, Ruggiero V, Atzori L. (2014) Metabolomics analysis and modeling suggest a lysophosphocholines-PAF receptor interaction in fibromyalgia. PLoS One. 19;9(9).

Invited Book Chapters

1. Aggarwal S, Yadav AK (2015) Dissecting the iTRAQ Data Analysis. Statistical Analysis in Proteomics, (Methods in Molecular Biology, Springer) (in press)

2. Aggarwal S, Yadav AK (2015) False Discovery Rate Estimation in Proteomics. Statistical Analysis in Proteomics, (Methods in Molecular Biology, Springer) (in press)

Patents

Patent title:	*Drug Targeting.*
Inventors:	Rajat Anand, Srikanth Ravichandran, Samrat Chatterjee
Filed on:	09.01.2015
Application No.:	78/DEL/2015 (Indian application)

Patent title:	*Computer Software for Drug Targeting*
Inventors:	Rajat Anand, Samrat Chatterjeee
Filed on:	20.08.2014
Application No.:	52951/2014-CO/SW (Indian, copyright application)

Patent Title:	*Novel Autophagy-inducing Compounds*
Inventors:	Amit Sharma, Manickam Yogavel, Kanury Rao, Varshneya Singh
Filed on:	17.04.2014
Application No.:	1055/DEL/2014 (Indian application)

Patent Title:	*Compounds for Induction of Autophagy*
Inventors:	Amit Sharma, Manickam Yogavel, Kanury Rao, Varshneya Singh
Filed on:	17.04.2014
Application No.:	1056/DEL/2014 (Indian application)

Patent Title:	*Novel Compounds as anti-Tubercular Agents*
Inventors:	Sundeep Dugar, Dinesh Mahajan, Kanury Rao, Varshneya Singh
Filed on:	30.05.2014
Application No:	1431/DEL/2014 (Indian Application)

Centre for Human Microbial Ecology

An Overview

G.B. Nair

CHME was created as a niche Centre of THSTI on 26th July 2013 through an SFC grant from DBT. The main objective of the Centre was to explore the striking alliance between microorganisms and the human host and attempt to understand the role and effect of microorganisms in human health and disease. The main focus of CHME research is directed to investigate the role and impact of human microbiome in certain health disorders which are directly linked to microbial richness, dynamics and functional repository of the microbial genome. The major emphasis will be to take an ecological approach that considers the microbial community as a whole rather than as individual organisms as in the case of microbial pathogens. The processes include factors like: the richness and dynamics of the microbiome in human health and disease, Inter-relationships within the microbiome and its interaction with the host and importance of microbial metabolites in microbial and host physiology.

While the culture independent exploration of microbial communities and their functional repository provide the platform to predict microbial interactions and contribution of microbial functions to host physiology, high resolution candidate gene approaches, also known as functional metagenomics, provide the clue to what extent do the microbial functions influence host health and disease states. To explore the microbiome structural insights, the centre has developed an in-house next generation DNA sequencing facility within a year of receiving financial assistance from DBT. Functional metagenomics, the most promising part of human microbiome research, is emerging as highly important system that can be used to better understand the impact of microbial genomic repertoire on maintenance of healthy states.

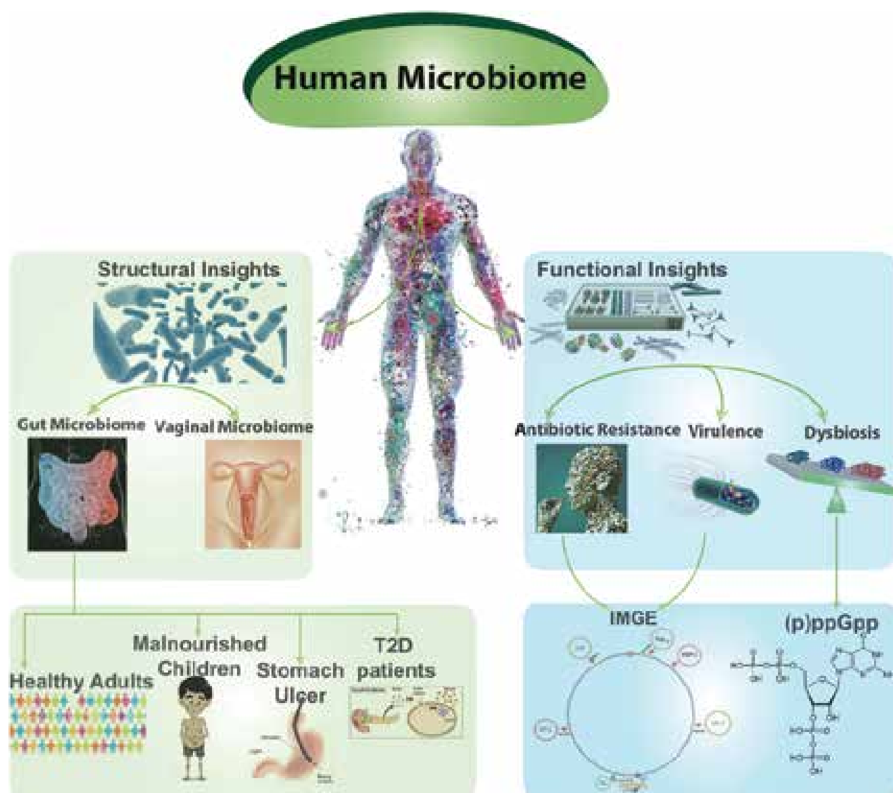
In CHME, structural and functional metagenomics are integrated to understand human microbiome and explore three basic questions; Who is in there? What are they doing? How are they doing it? During the first year of the establishment of CHME, laboratory infrastructure needed for the Center was put in place and the Scientific Advisory Committee to monitor and evaluate the performance of the Centre was prepared. The work at CHME spanned three major disease-areas, which include malnutrition, Type 2 Diabetes and Pre-term Birth. Each of the research projects has made substantial progress and are in various stages of progress. The first research study initiated in the centre was on the “Gut microbiome of Indian children of varying nutritional status”. The findings led us to design an extended study, where the gut

microbiome of 170 severe acute malnourished children during disease state and recovery are being explored. This project incidentally has the largest number of samples sequenced as of now at CHME.

We also recognize that it is extremely important to understand the richness and diversity of gut microbiome of healthy Indians. A study is ongoing with 50 adult Indian and 47 adult Japanese subjects and later on 150 additional Indian subjects will be included. A spin-off of this study is the finding of pathogenic enteric bacteria in the gut microbiota of healthy children and this is being studied in great details. We have also initiated a study to explore the gut microbiome of Type 2 Diabetic and pre-diabetic Indian subjects. Gut microbiome of around 300 subjects belonging to either category is completed. The third areas where work has just been initiated is on the large multidisciplinary program on Pre-Term Birth.

The study on functional metagenomics has also made significant progress. The initial emphasis was directed on bacterial small molecule signaling systems and antibiotic resistance traits of enteric pathogens. The metabolic pathway of guanosine penta- and tetraphosphate, a nucleotide derived small signaling molecule, was extensively studied in different human gut bacteria to combat microbial dysbiosis. More than 2000 enteric pathogens of different origins were also screened to test susceptibility against 20 different antibiotics. The complete genome of multidrug resistance human pathogen, *Providencia rettgeri*, was sequenced and 31 genes were predicted for antibiotic resistance. The functional validation of all the resistance traits is going on.

The innate and acquired immune systems in host intestine can differentiate pathogenic and commensal microbes and defend host against enteric pathogens. Perturbation of intestinal residents is associated with intestinal inflammation and can lead to several inflammatory diseases. The CHME has also initiated investigation on host immune system to understand the role of TGF-β3 in the induction and generation of pathogenic Th17 cells and how pathogenic Th17 cells are regulated in intestinal inflammation.



Integration and excision mechanisms of integrative mobile genetic elements essential for *Vibrio cholerae* pathogenicity

Investigators
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Bhabatosh Das

Bhabatosh Das

Vibrio cholerae, the etiological agent of diarrhoeal disease cholera, harbors large numbers of Integrative Mobile Genetic Elements (IMGEs), which notably contributes in bacterial pathogenesis and provide fitness factors to the pathogen that help the bacterium to compete with other bacteria in natural environment (**Fig.1**). Vibrio pathogenicity islands-1 (VPI-1), a 41-kb DNA segment physically linked to a tmRNA gene (*ssrA*), flanked by two nearly identical repeat sequences, is a well known IMGE and present in all epidemic *V. cholerae* isolates. VPI-1 is essential for *V. cholerae* pathogenesis and disease development. Most IMGEs encode a single transposase or integrase to mediate their site-specific integration in the host chromosome. In this regard, VPI-1 is particularly interesting since it carries two putative recombinases (Int_{vpi} and VpiT) and both can mediate excision reaction independently. Sequence analyses of the two putative recombinases indicate that they are quite different from each other. The Int_{vpi} contains the conserved R-H-R-Y signature motif of the tyrosine recombinases, while the similar motif is not distinct in VpiT. This raised a considerable interest for the understanding of the different molecular mechanisms driving the integration and excision of VPI-1.

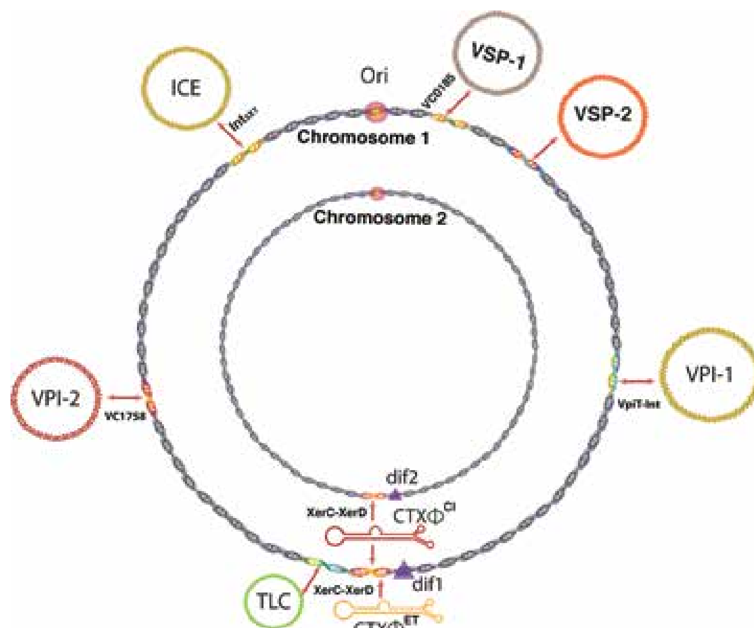

Figure 1: Schematic representation of the Integrative Mobile Genetic Elements (IMGEs) presents in the chromosome 1 (large) or 2 (small) of V. cholerae. Except CTXϕ, all other IMGEs have unique attB site either in large or small chromosome. IMGEs present in the dif region exploit host-encoded recombinases for integration while the genome of other IMGEs encode their own recombinases for integration.

First, VPI-1 was tagged with a selection (*cat*) and counter selection (*sacB*) markers by allele exchange method using conditionally replicating vector pDS132. The reporter strain carrying *sacB-cat* allele in *tcpA* locus of VPI-1 was confirmed by PCR and sequencing. Precise rate of VPI-1 excision from N16961 chromosome was measured. VPI-1 deleted N16961 derivative strain was isolated from selection plate supplemented with 15% of sucrose. The $attP_{VPI}$ and $attB_{VPI}$ regions of VPI-1 were PCR amplified, cloned and sequenced by Sanger sequencing methods. The precise sequences for the regions were determined.

A *V. cholerae* reporter strain carrying *lacZ-attP* allele in place of $attP_{VPI}$ was constructed to dissect the insights of VPI-1 integration. Integration module of VPI-1 carrying Int_{vpi}-*attP-VpiT* region was cloned in a TS vector. Both the integrases (Int_{vpi} and VpiT), crucial for VPI-1 integration, were cloned and over-expressed in homologous and heterologous host.

Guanosine penta- and tetraphosphate metabolisms in bacteria and implications in gut microbiota homeostasis

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Collaborator
G. Balakrish Nair

For an organism to survive in a complex ecology and compete with closely or distally related multiple species for niche and resources, it is indispensible to sense the surrounding environment and harmonize the cellular functioning accordingly. Guanosine penta - or tetraphosphate, collectively known as (p)ppGpp, the key intracellular small signaling molecule in bacteria, directly participates in the modulation of cellular physiology, upon sensing unfavorable environmental signals (**Fig. 2**). Quantitative differences in the intracellular concentration of (p)ppGpp determines the precise pattern of gene expression in wide range of bacterial species. Much of the fundamental work of (p)ppGpp metabolism were performed in *Escherichia coli*. Recent reports in Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria, the four dominant microbial phyla in human gut, indicate there are fundamental differences in sensing environmental clues and regulation of (p)ppGpp metabolism. We are investigating the mechanistic insights of (p)ppGpp metabolism in gut microbiota including *Prevotella copri, Faecalibacterium prausnitzii, Bifidobacterium adolescentis, Escherichia coli* and *Vibrio cholerae* to understand how the (p)ppGpp level of bacteria influence the microbial homeostasis, and whether the (p)ppGpp synthetase/hydrolase could be used as potential target to develop therapeutic agents to fight microbial dysbiosis and restore homeostasis in gut like complex ecosystem (**Fig. 3**).

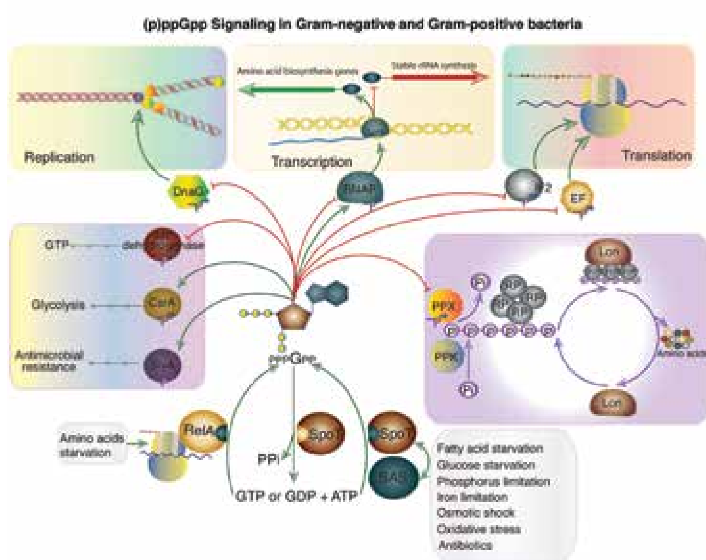

Figure 2. Basic outline of the (p)ppGpp metabolism and the cellular process differentially regulated by the (p)ppGpp. DnaG, Primase; RNAP, RNA polymerase; IF, Initiation factor; EF, Elongation factor; PPX, Polyphosphatase; PPK, Polyphosphate kinase; RP, Ribosomal protein, SlyA, Transcriptional regulator; CsrA, Carbon storage regulator; IMP dehydrogenase, Inosine-5'-monophosphate dehydrogenase; SAS, Small alarmone synthetase.

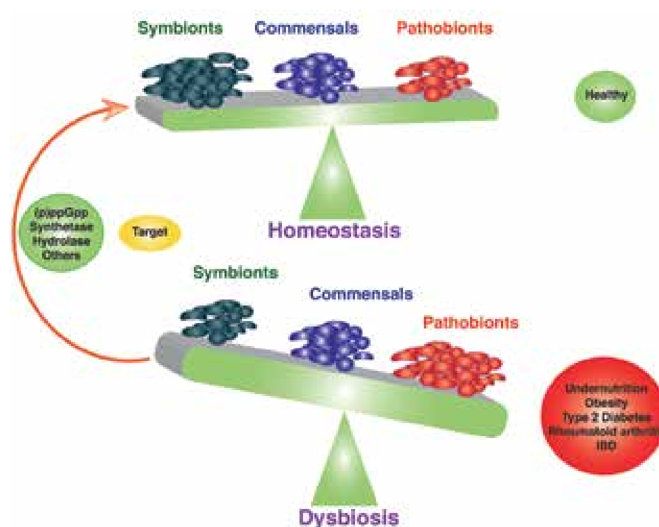

Figure 3. Level of (p)ppGpp, a hyper phosphorylated derivative of GTP/GDP, inversely related to the multiplication rate of bacteria. The (p)ppGpp metabolic pathway could be a potential target to fight dysbiosis related health disorders including malnutrition, T2D, IBD.

Nine (p)ppGpp synthetase and/or hydrolase genes from six different bacteria *(Prevotella copri, Faecalibacterium prausnitzii, Bifidobacterium adolescentis, Mycobacterium smegmatis, Escherichia coli and Vibrio cholerae)* belonging to four different phyla were cloned in two different cloning and expression vectors. Functional characterizations of each of the genes were done in defined medium in heterologous genetic backgrounds. Several gene-knockouts have been done in *V. cholerae* to develop *relA*-, *relA-spoT*-, *relA-spoT-relV* deleted strains. All the strains were confirmed by PCR and sequencing. (p)ppGpp° phenotypes of *relA-spoT-relV* were confirmed by several biochemical assays.

Two important reporter strains carrying beta-galactosidase in place of small alarmone synthetase (RelV) and (p)ppGpp synthetase/hydrolase (SpoT) genes were developed to dissect the signaling pathways that induced SAS and/or SpoT. Role of (p)ppGpp in *V. cholerae* persistency was evaluated. Induction of (p)ppGpp synthetase/hydrolase transcription in presence of different classes of antibiotics were evaluated.

Multidrug resistance in enteric pathogens: Molecular insights into resistance traits

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Rise of antibiotic resistance in pathogenic bacteria poses major health concern world-wide, but the problem is particularly worrying in India, where hospital standards are inconsistent and antibiotics are readily available over the counter. In India, three classes of antibiotic-resistant pathogens are emerging as major threats to public health. First and most importantly, multidrug resistant (MDR) and pandrug-resistant (PDR) Gram-negative bacteria pose the great threat of infections that are truly untreatable. Strains of *Acinetobacter baumannii, Vibrio cholerae, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi* and *Pseudomonas aeruginosa* are resistant to some or all antibiotic classes commonly used to treat Gram-negative bacteria: penicillins, cephalosporins, carbapenems, monobactms, quinolones, aminoglycosides, tetracyclins and polymixins. Several mechanisms can confer antibiotic resistance in bacteria, but the most common mechanism of resistance in enteric pathogens is the enzymatic inactivation of the antibiotic by hydrolysis or by formation of inactive derivatives. Such resistance determinants are acquired by pathogenic bacteria from a pool of resistance genes in other microbial genera. The resistance genes are either integrated into autonomously replicating mobile genetic elements or could lysogenised into host chromosomes by site-specific recombination using tyrosine or serine recombinases. An understanding of resistant determinants circulating in bacterial pathogens will provide information not only about resistance frequencies but also to identify new mechanisms that may contribute to eradicate resistance determinants from the existing resistant pathogens.

Nearly 2000 human pathogens including *Vibrio cholerae, Vibrio fluvialis, Vibrio parahaemolyticus, Escherichia coli (ETEC/EHEC), Klebsiella pneumonia, Salmonella typhi, Pseudomonas aeruginosa* and *Providencia rettgeri* has been screened against 15 different antibiotics, which interfere DNA replication, RNA transcription, protein synthesis, cell wall synthesis and different metabolic pathways (**Table 1**). An extensive drug resistance *P. rettgeri* was characterized

	Aminoglycoside		Quinolone		Glycopeptide		B-lactam		SA	Pyrm.	Mac.	Amph.	TetC	Rif
Organisms	Str.	Kan	Nal	Cip	Zeo	Van	Amp	Imp	Sul	Tri	Ery	Chl	Tet	Rif
E. coli (ETEC)	S	R	R	R	S	R	R	S	R	R	R	R	R	R
V. cholerae	R	R	R	R	R	R	R	S	R	R	S	R	R	R
V. parahem.	R	R	R	R	R	R	R	S	R	R	S	R	R	R
V. fluvialis	R	R	R	R	R	R	R	S	R	R	S	R	R	R
S. enterica	R	R	R	R	R	R	R	S	R	R	R	R	R	R
K. pneumonia	R	R	R	R	R	R	R	S	R	R	R	R	R	R
P. aeruginosa	R	R	R	R	R	R	R	S	R	R	R	R	R	R
P. rettgeri	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Table 1: *Antibiotic resistance traits detected in different human pathogens. Nearly 2000 pathogenic bacterial isolates were screened for five different classes of antibiotics.*

R = Resistant; S = Sensetive. SA=Sulfonic acids; Pyrz. = Pyrazinamide; Mac = Macrolide; Amph = Amphenicol; Tet = Tetracycline. Str. = Streptomycin; Kan. = Kanamycin; Nal. = Nalidixic acid; Cip.; Ciprofloxacin; Zeo. = Zeocin; Van. = Vancomycin; Amp. = Ampicillin; Imp. = Imipenem; Sul. = Sulfamethoxazole; Tri. = Trimethoprim; Ery. = Erythromycin; Chl. = Chloramph Str. = Streptomycin; Kan. = Kanamycin; Nal. = Nalidixic acid; Cip.; Ciprofloxacin; Zeo. = Zeocin; Van. = Vancomycin; Amp. = Ampicillin; Imp. = Imipenem; Sul. = Sulfamethoxazole; Tri. = Trimethoprim; Ery. = Erythromycin; Chl. = Chloramphenicol; Rif. = Rifampicin.

biochemically. The complete 16S rRNA gene sequence for the same was determined by capillary sequencer. The complete genome of *P. rettgeri* has been sequenced in our laboratory using 454 GS FLX + pyrosequencer. The complete genome was assembled and annotated. Thirty genes encoding resistance against five different classes of antibiotics have been identified. Subset of resistance genes including extended spectrum beta-lactamase, with or without endogenous promoter were cloned in different cloning and expression vectors. Resistance functions in heterologous genetic backgrounds were also confirmed.

Gut microbiome and gut inflammation in severe acute malnourished Indian children

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Malnutrition, characterized by symptoms like delayed growth, deficiencies in vital nutritional components etc., is a global health concern and affecting more than 300 million children worldwide. It is one of the major health concerns in India since around 50% of children below the age of five suffer from various forms of malnutrition. In developing countries like India, malnutrition cannot be attributed to food insecurity alone. Efficient digestion of complex food ingredients and adsorption of nutrient are also crucial for normal development. The human gut microbiome, collective genomes of all the microbes residing in the gastrointestinal tracts, provides several metabolic functions that are not encoded in our own genome and plays important role in nutrient pre-processing, assimilation and energy harvest from ingested food particles. Consequently, dysbiosis of the gut microbiota has been implicated in malnutrition.

Currently, we are investigating gut microbiome and level of three different gut inflammatory markers (Neopterin, Calprotectin and Myeloperoxidase) in the fecal samples of 170 severely acute malnourished (SAM) children. Samples were collected from two different time points in a cohort of SAM children;

during enrollment and after treatment with antibiotics and ready-to-use therapeutic food followed up weekly upto 16 weeks or recovery, whichever is earlier. Gut inflammatory markers were measured by ELISA using commercially available kits. The level of Neopterin was detected by competitative ELISA wehere, as the levels of Calprotectin and Myeloperoxidase were detected by sandwich ELISA. 16S rRNA based targeted metagenomics approach was adopted to investigate the gut microbiome of 340 samples collected from 170 SAM subjects.

Community DNA from 340 fecal samples was extracted using our laboratory-developed community DNA extraction method. Bacterial 16S rRNA genes of all the isolated samples were amplified with adaptor and bar code tagged composite primers, specific for C1 and C5 regions. Purified samples were used for metagenomic sequencing using in-house 454 GS FLX+ pyrosequencer. All the stool samples were used to measure the levels of Neopterin, Calprotectin and Myeloperoxidase, three well-characterized biomarkers for gut inflamation. Levels of calprotectin and myeloperxidase were determined by sandwiched ELISA methods whereas; neopterin level was determined by competitive ELISA using commercially available kits.

MicrobDiab - Studies of interactions between the gut Microbiome and the human host biology to elucidate novel aspects of the pathophysiology and pathogenesis of Type 2 Diabetes

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The incidence of Type 2 Diabetes (T2D) increases at a pandemic scale and is accompanied by severe organ damages, which results in enormous costs on the health care systems and lowers the quality of life and life expectancy of millions of people in India. Recent research indicates that altered gut microbiota composition and function may be involved in the pathogenesis of T2D and its co-morbidities. Therefore, there is a strong rationale to explore whether interactions between the gut microbiota as evaluated at the collective microbial genome level (the microbiome) and the host biology can provide novel insights into the pathophysiology and pathogenesis of pre-diabetes and T2D. The overall objective of the ongoing project is to identify gut microbiome signatures in Indian and Danish study participants which associate with pre-diabetes and T2D thereby enabling development of novel biomarkers for early diagnosis of people at high risk of progression to overt T2D.

Currently, we are (**a**) performing extensive phenotypes of 150 glucose tolerant individuals, 150 persons with pre-diabetes and 150 T2D patients from India and Denmark, respectively; a total of 900 individuals, and (**b**) metagenomic approaches were adopted to identify phenotype-specific gut microbiome profiles at microbial taxa and functional levels, (**c**) characterization of both common and ethnic specific gut microbiome patterns are going on to examine how they associate with the glucose tolerance state, insulin sensitivity, insulin secretion, inflammation markers, blood metabolomics, circulating microbial non-coding RNA and blood group markers and (**d**) attempts will also be taken to develop and validate microbiome markers that discriminate between individuals having the various degrees of glucose tolerance.

Fecal and blood samples were collected from 270 Indian study subjects from three different groups with similar number of representation from glucose tolerant, pre-diabetes and T2D patients. Fecal DNA from 270 samples were extracted using laboratory developed community DNA extraction method. Bacterial 16S rRNA genes of all the isolated DNA samples were amplified with adaptor and bar code tagged composite primers, specific for C1 and C5 regions. Purified samples were used for metagenomic sequencing using in-house 454 GS FLX+ pyrosequencer. Subsets of samples were used for determination of most abundant bacterial species in the stool samples. For the same, complete 16S rRNA gene was amplified, cloned and sequenced. 16S rRNA based metagenomic sequencing of 220 subjects is completed.

The effects of human intestinal microbiota on immune responses

Investigators

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Collaborators

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“Microbiome” refers to the ecological community of commensal, symbiotic, and pathobiont microorganisms living in a specific ecosystem. The distal gastrointestinal tract is the largest reservoir of microbes in the humans, and accommodates more than 1×10^{14} anaerobic and facultative aerobic microbial cells belonging to around 1000 distinct bacterial species. It is becoming increasingly evident that the gut microbiome has a role in defining states of health and disease. Furthermore, while a healthy subject typically harbors a distinct but fluctuating composition of microorganisms in the gut, the composition may be dramatically altered depending on the food habit, environmental exposure and host genotypes. Recent advances in the ability to conduct genomic analyses of microbiome by community DNA sequencing, along with access to large databases have opened up the prospect for conducting pioneering studies in this field.

Currently, we are exploring the prokaryotic and eukaryotic microbial communities of the gut of healthy adult Indian and Japanese study subjects with the following objectives (**a**) Determine the prokaryotic and eukaryotic microbial community structures in 50 healthy Indians and equal number of Japanese study subjects. (**b**) Determine the difference in gut microbiomes in Indian and Japanese individuals and define possible “core” microbiome with certain degree of variations within Indian and Japanese healthy people. (**c**) Based on the microbial composition predicts target population susceptible to enteric pathogens. (**d**) Study possible interactions in the gut microbiome. (**e**) Determine the presence of enteric pathogens in the gut of healthy subjects using highly efficient nested PCR approach. (**f**) Determine role of gut microbiome in host immune maturation.

Fecal samples of 50 healthy adult Indians residing in the NCR region were collected. Communities DNA from all the collected samples were isolated. Bacterial 16S rRNA genes of all the isolated DNA samples were amplified with bar coded primers. Targeted metagenomic sequencing has been done for 97 samples projecting sequence variations in the V1-V5 regions. Distinct pattern of gut microbiota, both prokaryotic and eukaryotic, between Indian and Japanese subjects were observed. Presence of low level of enteric pathogens, including *Escherichia coli* (ETECC), toxigenic *Vibrio cholerae* and *Shigella* sp. in

the gut of subset of healthy Indian subjects were determined. Sequences of O1 and O139 specific antigen encoding genes were detected in *ctxAB* positive stool samples.

Inter-institutional program for maternal, neonatal and infant sciences: a translational approach to studying preterm birth

Investigators
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Nitya Wadhwa
G. Balakrish Nair
Partha P. Majumder
NIBMG, Kalyani
Dinakar M. Salunke
Tushar Kanti Maiti
RCB, Faridabada

Preterm birth (PTB) is a consequence of complex molecular process that triggers the quiescent uterus to expel the fetus before 37 weeks of gestation. Globally more than 10% babies are born preterm resulting 15 million preterm births annually. In India, the incident of PTB is around 13% that lead to 3.6 million babies being born preterm amongst the total 27 million born annually. Some commonly recognized precursors for preterm labor include age at pregnancy, spacing, multiple pregnancy, gestational age, infection or inflammation, decidual hemorrhage, uterine thrombosis, stress or uterine over distension, underlying maternal nutritional chronic medical conditions and psychological health, lifestyle and socio-economic factors. While all risk factors are possibly important, we have identified some risk factors that appear to us to be more important than others and regarding which there are critical gaps in knowledge. Multidisciplinary approaches, including metagenomics, genomics and proteomics are adopted to identify authentic risk factors and precise pathway that instigate PTB. In our laboratory, we adopted culture independent metagenomics approaches to elucidate diversity and dynamics of vaginal and gut microbiome in pregnant women. We are collecting high vaginal swabs (HVS) from pregnant women in four different time points and exploring diversity, dynamics and functions of bacterial component. Our initial focus will be determined the difference in vaginal microbiomes in term and preterm birth women and define possible "core" microbiome with certain degree of variations within preterm birth women.

Extraction of community DNA from HVS was standardized. Quality, quantity and suitability of isolated DNA for downstream applications were verified. Subset of isolated community DNA was used for PCR amplification using barcoded and adaptor tagged primers, specific for C1, C3, C5 and C9 regions of 16S rRNA gene.

The complete 16S rRNA genes amplified from fifteen HVS DNA samples were cloned into high copy number cloning vector. Complete sequences of 16S rRNA genes from subset of samples were determined by using universal M13F and M13R primers. Most abundant bacterial species present in the vagina of reproductive age Indian were determined.

Interplay between effector and regulatory T cells in the pathogenesis of intestinal inflammation

Investigators
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Collaborators
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Amit Awasthi

It is clearly and convincingly recognized that both pathogenic and nonpathogenic Th17 cells exist. Naïve T cells can be induced into Th17 cells in the presence of TGF-b1 plus IL-6 to become Th17 cells. These cells produce signature cytokines of Th17 cells like IL-17A, IL-17F, IL-21 and IL-22. In addition, Th17 cells induced by TGF-b1 and IL-6 also produce lot of IL-10 and IL-9 as well. Strikingly, these Th17 cells are non-pathogenic and unable transfer diseases like colitis and EAE in mouse models of IBD and multiple sclerosis indicating that the non-pathogenic nature of these cells, though they produce IL-17. However, once sensitized with IL-23, these non-pathogenic Th17 cells become pathogenic and induced high degree of tissue inflammation in colitis and EAE. Interestingly, IL-23-sensiized Th17 cells not only enhances the pathogenic markers of Th17 cells but also suppresses regulatory potential of Th17 cells by suppressing IL-10 production. Moreover, both IL-23- and IL-23R- deficient mice are completely resistant to developing EAE and colitis suggesting that IL-23 is not only required for inducing pathogenic Th17 cells but also critical for initiation of tissue inflammation in colitis and EAE (Figure 4).

To understand the role IL-23 in the induction of pathogenic Th17 cells as part of this project, we have performed microarray analysis of wild type Th17 cells and compared them IL-23R-/- Th17 cells, we have identified many genes which were differentially expressed between these two groups. We also identified the differential expression of TGF-β3 induced by IL-23 in Th17 cells suggested that this could be once of several genes essential for the development of pathogenic Th17 cells. We have further validated our results by various methods to identify the role of TGF-β3 in the induction and generation of pathogenic Th17 cells.

To further delineating the role of TGF-β3 in the induction and development pathogenic Th17 cells, we differentiated the Th17 cells in the presence of TGF-β3 plus IL-6 and compared them with TGF-β-induced Th17 cells. We found that both of these cell equally induced IL-17A and IL-17F genes. In addition, both of these cells induced similar level of RoRc, a master transcription of Th17 cells.

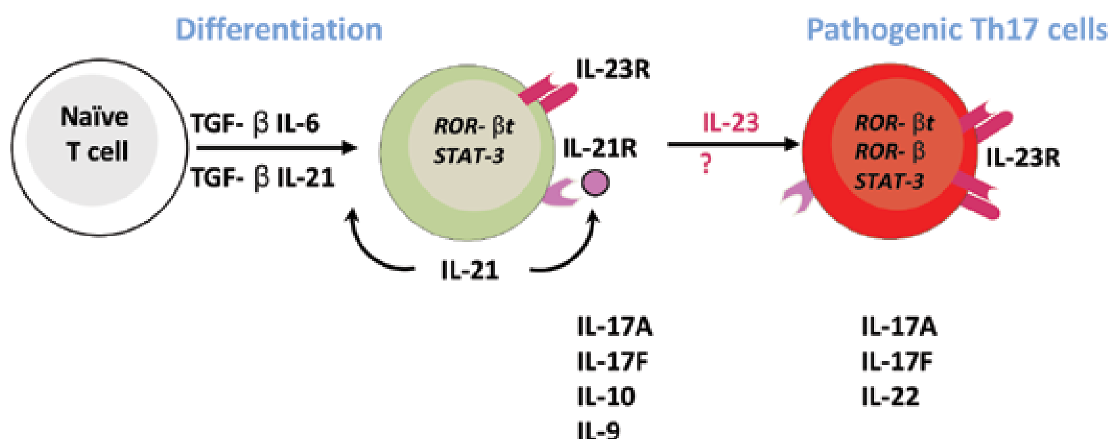

Figure 4. Understanding the mechanism of induction pathogenic TH17 cells. The antigenic stimulation of naïve T cells in the presence of TGF-β and IL-6 initiate the TH17 differentiation pathway. Exposure of IL-23 to TH17 cells not stabilizes phenotype of these cells but also induces the pathogenic feature of these cells. However, the mechanism by which IL-23 induces the pathogenic Th17 cells is not clearly understood.

However, TGF-β3-induced Th17 cells expressed very level of IL-23R expression as compared to TGF-β1-induced Th17 cells. This clearly suggested that TGF-β3-induced Th17 cells might be more pathogenic as compared to TGF-β1-induced Th17 cells as they express high surface expression IL-23R. To further validate our findings, we have compared both pathogenic and non-pathogenic Th17 cells by microarray analysis. Microarray analysis identified the gene signature which are differentially expressed between TGF-β3 and TGF-β1 induced Th17 cells (Figure 5).

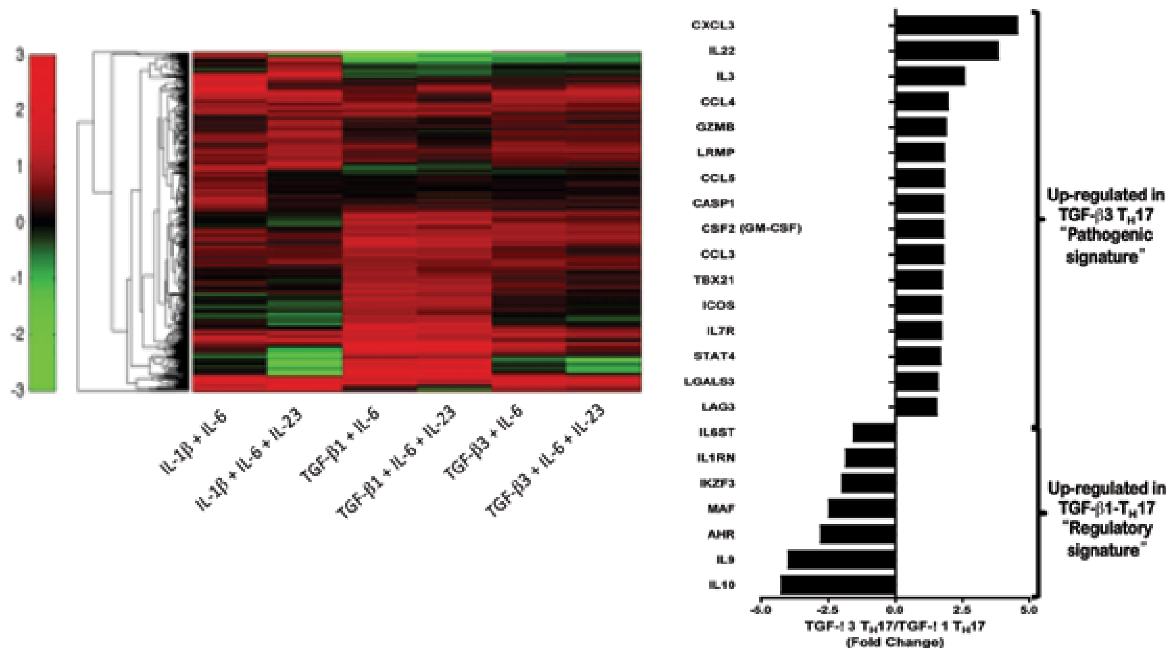

Figure 5: (a) Microarray analysis of TGF-β1/IL-6, TGF-β3/IL-6 and IL-1β/IL-6 with or without IL-23 treated sorted naïve CD4+ T cells. Expression of differentially expressed genes among these groups were shown, (b) differentially expressed genes in pathogenic vs non-pathogenic Th17 cells.

IL-27 dependent regulation of Th17 and regulatory T cells

Investigator

Sakshi Malik
Suyasha Roy
Amit Awasthi

Multiple mechanisms exist for IL-27-induced regulation of Th17 cells. IL-27 can suppress Th17 cells by directly targeting the expression of RoRγt. Moreover recently IL-27 was shown to induce the expression of PDL1 on T cell, which inhibits the development of Th17 cells through PD1-PDL1 interaction. These observations clearly indicated the existence of multiple mechanisms by IL-27 exerts its inhibitory functions on Th17 cells and Th17 cells associated tissue inflammation. To thoroughly study other additional mechanisms by which IL-27 regulated Th17 cells and the IL-23R expression, we performed a microarray analysis of T_H17 cells in the presence or absence of IL-27. Comparing the two cell populations, we observe an overall high level of similarity (data not shown; Pearson $r^2=0.99$, $p<10^{-10}$) between the two treatment groups. Focusing on outliers, we observed that only a small fraction of the genes (~2%) showed a significant (>2-fold) change (either increase or decrease). These included *Il17a*, *Rorα*, *Ccr6*, *Il12rb2* and *Tbx21*. Our microarray data revealed that IL-27 induced expression of IL-12Rβ2 on Th17 cells, which could be essential for the suppression of Th17 cells development. Using IL-12Rβ2-deficient mice model, we further validated our findings that IL-27-inudced IL-12Rβ2 expression is

essential for suppression of Th17 cells. In addition to T-cells, gd T-cells also play a critical role in inducing tissue inflammation in EAE (Figure 6).

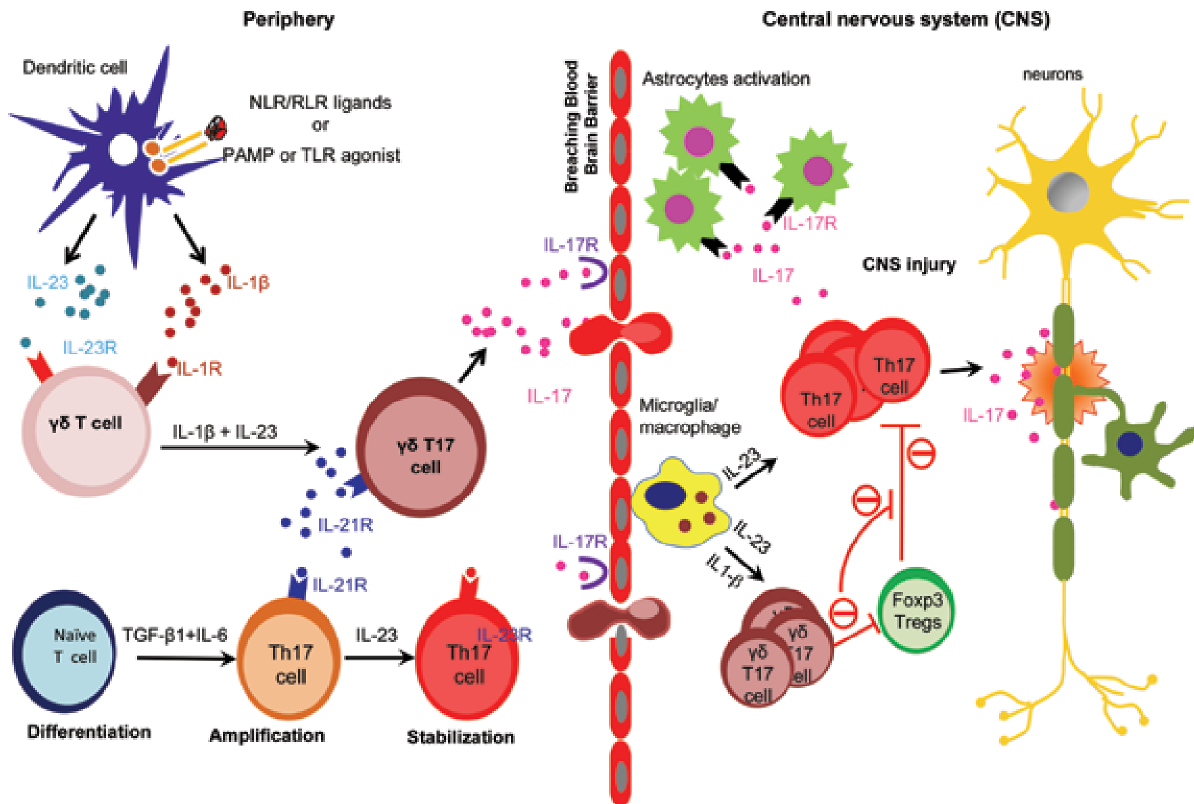

Figure 6. Peripherally primed γδ T cells execute their effector functions in the CNS. TLR and NLR ligands activate dendritic cells (DCs), which produce pro inflammatory cytokines IL-23 and IL-1β. IL-23 and IL-1β sensed by IL-23R expressing γδ T cells which in turn produce early burst of IL-17. On the other hand DCs produced IL-6 together with TGF-b induce Th17 cells differentiation. γδT17 cells can also secrete IL-21 which in turn further amplify their own IL-17 secretion and also amplify Th17 cells. Both differentiated γδT17 and αβ Th17 cells can breach the blood brain barrier to execute their effector functions in the CNS. Both γδT17 and αβ Th17 cells further amplified in the CNS by IL-23 produced activated microglia/macrophages. Inflammatory γδT17 cells further amplify the effector functions of αβ Th17 cells to induce CNS injury while inhibiting the regulatory functions Tregs cells in CNS during EAE.

Vitamin A is the "micro-environmental cue" for triggering disease activity in patients with Inflammatory Bowel Disease (Ulcerative colitis, Crohn's Disease)

Investigators

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Amit Awasthi

In this project we proposed to determine the following objectives: a) Does retinoic acid generates a significant inflammatory state in the human colonic mucosa? b) What are the micro-environmental conditions in human colonic mucosa, which nudge retinoic acid an active metabolite of Vitamin A to play a sustained pro-inflammatory role? c) Is it possible that Vitamin A induces an autoimmune response in humans?

We have found that Retinoic acid significantly enhances the generation pro-inflammatory T cells like Th17 cells and Th1 cells in healthy human subject. We first determined as to how Retinoic acid enhanced or suppressed human CD4+ T cells differentiation into Th1 and Th17 cells as these are the two major effector T cells play are dominant role in inducing tissue inflammation in IBD.

Culturing sorted naïve T cells into Th1 or Th17 culture condition in combination with Retinoic acid resulted enhanced Th1 and Th17 cells development.

We further tested as to how the Retinoic acid-treated dendritic cells (DCs) influence T cells differentiation into Th1 or Th17 cells. Interestingly, we found that Retinoic acid treated-DCs showed immature DCs phenotypes as compared to the DCs, which were treated with LPS alone. These RA-treated DCs enhanced the differentiation of Th1 and Th17 cells. After the determining the initial functions of Retinoic acid in T cells differentiation and DCs functions, now we will determine how the serum concentration of vitamin A in IBD pateints and its co-relation with inflammation, T cells differentiation and disease pathogenesis.

In addition to Th17 cells, other non-conventional T cells also produces IL-17, which is very crucial for inducing tissue inflammation in autoimmunity like IBD, RA and MS. $\gamma\delta$ T cells is one of the subset we are proposing to have inflammatory in tissue inflammation in EAE, a mouse model of human multiple sclerosis. We have discussed the role these $\gamma\delta$ T cells in our recently accepted review article (Fig. 6)

Peer-reviewed Publications

1. Bag S, Das B, Dasgupta S, Bhadra RK (2014) Mutational analysis of the (p)ppGpp synthetase activity of the Rel enzyme of *Mycobacterium tuberculosis*. Arch Microbiol 196(8):575-88.

2. Barman S, Koley H, Nag D, Shinoda S, Nair GB, Takeda Y. 2014. Passive immunity with multi-serotype heat-killed Shigellae in neonatal mice. Microbiology and Immunology. Aug;58(8):463-6.

3. Das B (2014) Mechanistic insights into filamentous phage integration in *Vibrio cholerae. Front. Microbiol. 5:650*. DOI: 10.3389/fmicb.2014.00650.

4. Das B, Banerjee R, Nair GB, Basak S (2014) Dynamics in genome evolution of *Vibrio cholerae. Infect Genet Evol* 23:32-41.

5. Das B, Kumari R, Pant A, Sen Gupta S, Saxena S, Nair GB (2014) A Novel, Broad-Range, CTXΦ-Derived Stable Integrative Expression Vector For Functional Studies. *J. Bacteriol* 196(23):4071-80.

6. Das B**,** Midonet C, Paly E, Barre FX (2014) XerD initiates the integration of TLCΦ into the *Vibrio cholerae* genome independently of FtsK. *Proc Natl Acad Sci USA* 111 (47): 16848-16853 .

7. Das B, Nair GB, Bhadra RK (2014) Acquisition and dissemination mechanisms of CTXΦ in *Vibrio cholerae*: New paradigm for *dif* residents. *World J Med Genet* 4: 27-33.

8. Ghosh TS, Sen Gupta S, Bhattacharya T, Yadav D, Barik A, Chowdhury A, Das B, Mande SS, Nair GB. (2014). Gut Microbiomes of Indian children of varying nutritional status. PLoS One 9:e95547.

9. Hajela N, Ramakrishna BS, Nair GB, Abraham P, Gopalan S, Ganguly NK. 2015 . Gut microbiome, gut function, and probiotics: Implications for health. Indian J Gastroenterol. Mar;34(2):93-107.

10. Kanungo S, Lopez AL, Ali M, Manna B, Kim DR, Mahapatra T, Holmgren J, Dhingra MS, Weirzba TF, Nair GB, Bhattacharya SK, Clemens JD, Sur D. 2014. Vibriocidal antibody responses to a bivalent killed whole-cell oral cholera vaccine in a phase III trial in Kolkata, India. PLoS One. May 6;9(5):e96499.

11. Kanungo S, Sen B, Ramamurthy T, Sur D, Manna B, Pazhani GP, Chowdhury G, Jhunjhunwala P, Nandy RK, Koley H, Bhattacharya MK, Gupta S, Goel G, Dey B, M T, Nair GB, Ghosh A, Mahalanabis D. 2014 . Safety and immunogenicity of a live oral recombinant cholera vaccine VA1.4: a randomized, placebo controlled trial in healthy adults in a cholera endemic area in Kolkata, India. PLoS One. Jul 1;9(7):e99381

12. Kim EJ, Lee D, Moon SH, Lee CH, Kim SJ, Lee JH, Kim JO, Song M, Das B, Clemens J, Pape JW, Nair GB, Kim DW (2014) Molecular insights into the evolutionary pathway of *Vibrio cholerae* O1 atypical El Tor variants. *PLoS Pathogens* 0(9):e1004384.

13. Kim JE, Lee HC, Nair GB, Kim WD. 2015. Whole-genome sequence camparisons reveal the evolution of Vibrio cholera O1. Trends in Microbiology. Apr 23. 1-11.

14. Lopez AL, Gonzales ML, Aldaba JG, Nair GB. 2014. Killed oral cholera vaccines: history, development and implementation challenges. Ther Adv Vaccines. Sep; 2(5):123-36

15. Malik S, Muzamil Want, Awasthi A (2015) Emerging role of gamma-delta T cells in tissue inflammation in EAE. Frontiers of Immunology 2015 (in press)

16. Tyagi RK, Garg NK, Jadon R, Sahu T, Karare OP, Awasthi A, Marepally SK (2015) Elastic liposome mediated transdermal immunization enhanced the immunogenicity of P. falciparum surface antigen, MSP-1. Vaccine (in press)

17. Yamasaki E, Yamada C, Jin X, Nair GB, Kurazono H, Yamamoto S. 2015. Expression of *marA* is remarkably increased from the early stage of development of fluoroquinolone-resistance in uropathogenic *Escherichia coli*. Journal of Infection and Chemotherapy. Feb;21(2):105-9.

Seminars and Conferences

Amit Awasthi

Induction and development of pathogenic Th17 cells and their regulation. Invited speaker at Massachusetts General Hospital, Harvard Medical School, May 13, 2014.

Induction and development of pathogenic Th17 cells and their regulation. May 15, 2014 Invited speaker at Oakland University, Michigan Role of pathogenic Th17 cells in Autoimmunity. March 27, 2015 Invited speaker at Biosparks, School of Life Sciences, Jawaharlal Nehru University (JNU). New Delhi

Bhabatosh Das

Title of the talk:	Guanosine penta- and tetraphosphate metabolisms in bacteria and implications in gut microbiota homeostasis
Name of the meeting:	International workshop on applications of systems and mathematical biology in public health.
Place and date:	NISER, Bhubneswar; 22-24 February 2015
Title of the talk:	Functional Metagenomics: Dissemination of Antibiotic Resistance Traits in Bacterial Pathogens
Name of the meeting:	Indo-German Bilateral Workshops on "Diagnostics of Translational Genome Sequencing in Clinical and Public Health Microbiology"
Place and date:	The Madras Medical Mission, Chennai, India; 19-21 March 2014

G B Nair

Title of the talk :	Oral cholera vaccine
Name of the meeting :	Global Task Force for Cholera Control
Place and Date:	Chavannes-de-Bogis, Switzerland, 26-27th June, 2014
Title of the talk :	The gut microbiome of undernourished Indian children
Name of the meeting:	The Gut, Its Microbes and Health
Place and Date:	ILSI SEA Region conference, Singapore, 8th- 9th October, 2014

Title of the talk :	A metagenomic perspective of undernourished children in India
Name of the meeting:	4th AFSLAB International Symposium
Place and Date:	Philippine Society for Lactic Acid Bacteria Philippines, 23rd-24th October,2014
Title of the talk :	Review of Lab Sciences Division
Name of the meeting:	Scientific Advisory Group Meeting
Place and Date:	International Centre for Diarrhoeal Disease Research, Bangladesh, 11th-13th November, 2014
Title of the talk :	From genomics to public health
Name of the meeting:	Indo-US Workshop on Challenges of Emerging Infections and Global Health Safety
Place and Date:	Indian National Science Academy, New Delhi , 18th-19th November, 2014
Title of the talk:	Demonstration projects
Name of the meeting:	Global Technical Consultative Meeting on Identification of Health R & D Demonstration Projects meeting
Place and Date:	World Health Organisation, Geneva, Switzerland, 3rd -5th December, 2014
Title of the talk :	Gut microbiota in children of varying nutritional status
Name of the meeting :	Microbiome Forum: Asia
Place and Date:	Kuala Lumpur, Malaysia, 19-20th January,2015
Title of the talk:	4th Idea Asia meeting
Name of the meeting :	IDEA Asia meeting
Place and Date:	The Grand, Vasant Kunj, New Delhi, 31st March, 2015
Title of the talk:	IPBS Doctoral fellowships for India
Name of the meeting :	IPBS 2015 Advisory Board meeting
Place and Date:	Osaka University, Japan 21-22nd April, 2015.

Honors and Awards

Amit Awasthi

Member of American Association of Immunologist (AAI)

Extramural Grants

Amit Awasthi

Funding agency:	Innovative Young Biotechnologist Award, DBT, Govt. of India
Amount:	38.81 lacs
Year:	2012-2015
Title:	IL-27 dependent regulation of Th17 and regulatory T cells
Funding agency:	Intermediate fellowship, DBT-Wellcome Trust India Alliance

Amount:	3.5 Cr
Year:	2012-2017
Title:	Interplay between effector and regulatory T cells in the pathogenesis of intestinal inflammation

Funding agency:	Department of Biotechnology, Govt. of India
Amount:	26.62 lacs
Year:	2013-2016
Title:	Vitamin A is the "micro-environmental cue" for triggering disease activity in patients with Inflammatory Bowel disease (Ulcerative colitis, Crohn's Disease).

Funding agency:	Department of Biotechnology, Govt. of India
Amount:	46.10 lacs
Year:	2014-2019
Title:	Human gastrointestinal immunology translational program (Glue grant).

Bhabatosh Das

Funding agency:	Dept. of Science & Technology, Govt. of India
Amount:	17 lakhs
Duration:	From July 2013 To June 2016
Title of the grant:	Integration and excision mechanisms of integrative mobile genetic elements essential for Vibrio cholerae pathogenicity

Funding agency:	Osaka University, Japan
Amount:	15 lakhs
Duration:	From April 2013 -March 2016
Title of the grant:	The effects of human intestinal microbiota on immune response

Funding agency:	Dept. of Biotechnology, Govt. of India
Amount:	759.58 Lakhs
Duration:	From July 2013 -June 2017
Title of the grant:	MicroDiab: Studies of interactions between the gut microbiome and the human host biology to elucidate novel aspects of the pathophysiology and pathogenesis of type 2 diabetes

Funding agency:	Dept. of Biotechnology, Govt. of India
Amount:	4885.32 Lakhs
Duration:	From December 2013- November 2018
Title of the grant:	Inter institutional program for maternal, neonatal and infant science- A translational approach to studying preterm birth

G B Nair

Funding agency:	Osaka University, Japan
Amount:	15 lakhs
Duration:	From April 2013- To-March 2016

Title of the grant:	The effects of human intestinal microbiota on immune response
Funding agency:	Dept. of Biotechnology, Govt. of India
Amount:	759.58 Lakhs
Duration:	From July 2013- To-June 2017
Title of the grant:	MicroDiab: Studies of interactions between the gut microbiome and the human host biology to elucidate novel aspects of the pathophysiology and pathogenesis of type 2 diabetes
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acer

Population Science Partnership Centre

An Overview

Nita Bhandari

The Population Science Partnership Centre (PSPC) is a collaboration between Translational Health Science and Technology Institute (THSTI) and the Centre for Health Research and Development, Society for Applied Studies (CHRD-SAS), New Delhi. The two institutes have complementary skills and expertise; THSTI has the laboratory infrastructure and CHRD-SAS has huge field research experience.

The centre's mission is to utilize strengths and resources of the two institutes to create a potentially cost saving, scientific productivity and health impact enhancing collaboration. The aim is to purse collaborative research and innovation in population-based science, focused on development, evaluation and diffusion of affordable technologies and solutions for improved health and nutrition including conduct of clinical trials; emphasize on solutions and technologies of public health importance to India and to the poorest; promote utilization of under-used existing technologies through appropriate modification and evaluation.

The overall vision is to incorporate hypothesis-driven scientific questions in population cohorts and clinical trials related to diseases of major public health importance. The knowledge gained will provide insights for prevention, diagnosis and treatment of fetal, neonatal and childhood disorders that contribute most to the disease burden in the country.

A phase III randomized, double blind placebo controlled trial to evaluate the non-interference in the immune response of three doses of ORV 116E to antigens contained in childhood vaccines and to assess the clinical lot consistency of three production lots

Investigators
Temsunaro Rongsen-Chandola
Sudhanshu Vrati
Nidhi Goyal

Rotavirus infections are the leading cause of diarrhea-associated mortality in developing countries. Rotavirus infections are estimated to cause ~527,000 deaths annually, predominantly in developing countries. In India, by age 5, nearly every child will have an episode of rotavirus gastroenteritis. The Indian rotavirus vaccine based on a neonatal rotavirus strain 116E developed as a Public-Private Partnership (PPP), under the Indo- US Vaccine Action Program and coordinated by the Government of India (Department of Biotechnology) has recently completed a multicentre phase III clinical trial in India. The licensure has been obtained by Bharat Biotech International Limited in January 2014.

This study is a phase III, randomized, double blind, placebo-controlled trial to assess non-interference of ORV 116E to the childhood vaccines and clinical consistency in the immune responses to the three production lots of ORV 116E. The study is being conducted in the urban neighborhoods of Govindpuri-Sangam Vihar-Tigri-Dakshinpuri and Tuglakabad in South Delhi, India.

Ethical clearances were obtained from THSTI-IEC and Western Institutional Review Board. The study was approved by the office of the Drugs Controller General (India) and is being conducted as per the protocol, Schedule Y and Good Clinical Practices.

Prior to study initiation, the study clinic was set up. The clinic is manned by paediatricians and physicians, open 24x7, equipped to handle all emergencies and there are systems in place to escort subjects to the hospital, if required. All categories of staff were trained on the protocol, filling case report forms, standard operating procedures and good clinical practice.

Subjects were enrolled into the study at 6 weeks of age after obtaining consent under audio visual recording and subsequent screening at the study clinic. Subjects were given 3 doses of the ORV 116E/Placebo along with childhood vaccines at 6, 10 and 14 weeks of age. A baseline blood specimen of ~1.5 mL was collected from all subjects for rotavirus IgA assays. Around 6mL of post immune blood specimens was collected 28 days after the third dose of the Test Article/Placebo for assessing immunogenicity to the childhood vaccines.

The study team made daily contacts for 14 days after each of the three doses till four weeks after the third dose to ascertain serious adverse events and signs and symptoms of intussusception. Additionally, after four weeks of the third dose, the subjects were contacted weekly till the age of one year for signs and symptoms of intussusception and events of death, if any

All serious adverse events were reported to the regulatory authorities and THSTI-IEC within 24 hours of coming to know of the event. The study was initiated in May 2014. Enrolment of 1356 subjects was completed in September 2014. Enrolled subjects have received three doses of the vaccine. All subjects are being followed up till the age of one year. As of March 2015, 27 subjects have been censored for completion of follow up.

The last follow up of the youngest subject will be in August 2015. Data analysis and dissemination of the results is planned by Q4, 2015-Q12016.

Phase III, multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of live attenuated bovine-human rotavirus Reassortant pentavalent vaccine (BRV-PV) against severe rotavirus gastroenteritis In healthy Indian infants

Investigators
Nidhi Goyal
Sudhanshu Vrati
Vikash Kedia

According to the WHO-coordinated Global Rotavirus Surveillance Network, 37% of deaths are attributable to diarrhea and 5% of all deaths in children under 5 years. Five countries accounted for more than half of all deaths attributable to rotavirus infection; India alone accounted for 22% of deaths (98,621 deaths) in this study. Although commercial rotavirus vaccines are currently available and have been demonstrated to be safe and effective in low-income, high-burden populations, they are not affordable in developing countries. Serum Institute of India is developing a live attenuated bovine-human (UK) reassortant pentavalent rotavirus vaccine for oral vaccination against human rotavirus gastroenteritis in healthy infants and plans to establish proof of vaccine efficacy in the proposed efficacy trial, which will enroll infants in six sites in India representative of different demographic, climatic and socio-cultural factors. In Delhi, it is being conducted in the urban slums of south Delhi.

Prior to study initiation, the study clinic was set up. The clinic is manned by paediatricians and physicians, open 24x7, equipped to handle all emergencies and there are systems in place to escort subjects to the hospital, if required. All categories of staff were trained on the protocol, filling case report forms, standard operating procedures and good clinical practice. Ethical clearances were obtained from THSTI-IEC and Western Institutional Review Board. The study was approved by the office of the Drugs Controller General (India), ethics committees of each of the participating sites Government of India's Health Ministry Screening Committee and the State Governments.

Subjects were enrolled into the study at 6 weeks of age after obtaining informed consent under audio visual recording and subsequent screening by a physician at the study clinic. Subjects are given 3 doses of the BRV-PV/ Placebo at 6, 10 and 14 weeks of age along with OPV and Pentavalent vaccine (containing DPT, HepB and HiB) primary doses and Measles, MMR and booster vaccines according to the national immunization schedule. Active surveillance for Gastroenteritis, Intussusception and other illnesses is done by weekly contacts with the participating infants starting from the time of the first vaccination until infants reach two years of age. Once an episode of gastroenteritis is identified, study personnel maintain close contact with the parent until the child's illness is resolved.

Diarrhea Diary Cards are being used by the parents during gastroenteritis episodes to record number and duration of looser than normal stools, axillary temperature, number and duration of vomiting episodes, any treatment given and duration of hospital stay, if any. Stool samples are collected from all subjects having gastroenteritis. All Serious Adverse Event are reported to the regulatory authorities, Ethics committee and Sponsors, as per the applicable guidelines, throughout the study period.

The study was initiated in August 2014 and completed enrolment 2100 subject in April 2015. All subjects have received dose 1, ~1900 subjects have received

dose 2 and ~1800 subjects have received dose 3 of BRV-PV/Placebo. Subjects will be followed up till the age of two years. The last follow up of the youngest subject will be in Q1, 2017.

The effects of human intestinal microbiota on immune responses

Investigators
GB Nair
Nita Bhandari

There is recent evidence indicating the influence of different environments and diets on microbial ecology of the human gut. This study assumes that the composition of gut microbiota varies between people living in Japanese and Indian communities, and hypothesize that resistance to intestinal infection in Indian populations is regulated by their unique intestinal microbiota.

In this study, fecal samples of Indian and Japanese subjects were collected, and the intestinal microbiota were analyzed by molecular techniques. Fecal samples were collected from 50 healthy Indian adults residing in low socioeconomic dwellings in urban and rural areas of Delhi and Haryana states. These areas were typical urban resettlement neighborhoods and rural areas in South Delhi and Urban Faridabad and Rural Haryana, in India. The study population from Japan were comprised of healthy adults residing in high or average socioeconomic dwelling in Osaka prefecture. This area was typical urban area with good hygienic environment. The study was conducted in two phases. The first phase included collection, processing and transportation of fecal specimen from healthy adults in India and Japan to the testing laboratories in Japan. The specimens underwent specified analysis of the intestinal microbiota in the laboratory. In the second phase the analyzed fecal specimen will be fed to germ-free mice and the development of immune responses in these mice were tested.

DNA was extracted from 60 fecal samples and subjected to the Ion PGM sequencer to analyze the intestinal microbiota. Fecal suspensions were also subjected to high liquid performance chromatography (HPLC) to measure the concentration of short chain fatty acids (SCFAs). The principal component analysis showed that the plots of Japanese samples were clearly separated from those of Indian ones, but there were no distinct characteristics within the different areas. Hierarchical clustering showed that 60 subjects were broadly divided to 2 clusters: cluster I and II. All Japanese samples were grouped in the cluster I, which was characterized by the high abundance of *Bacteroides* genus, whereas Indian samples were in the cluster II, characterized by high numbers of *Prevotella* genus. The Indian samples were further subdivided to 2 clusters, IIa and IIb, mainly based on the differential proportion of *Prevotella*. Some Indian samples showed unique bacterial profilings. For examples, the sample from IND-47was dominated by *Klebsiella*, which is known to be the enteric pathogen. HPLC analysis showed that concentrations of total SCFAs and propionic acid in Indian samples were significantly higher than those in Japanese samples. In addition, concentrations of total SCFAs and propionic acid in cluster IIa were significantly higher than those in cluster IIb

The specimens from the Indian volunteers were collected in July 2013. Specimens have been collected from additional Japanese volunteers and analysis of the specimens is ongoing.

Gut inflammation markers as determinants of response to treatment and recovery in children with uncomplicated severe acute malnutrition undergoing community based rehabilitation

Investigators
Sunita Taneja
Sanjana Brahmawar Mohan
Nita Bhandari
Sarmila Mazumder
G. Balakrish Nair
Bhabatosh Das

Malnutrition is a global health problem affecting more than 300 million pre-school children worldwide. It is one of the major health concerns in India since around 50% of children below the age of two suffer from various forms of malnutrition. The gut microbiome plays an important role in nutrient pre-processing, assimilation and energy harvest from food. The human gut microbiome, collective genomes of all the microbes residing in the gastrointestinal tracts, provides several metabolic functions that are not encoded in our own genome. These functions facilitate the pre-processing of dietary nutrients and efficient harvest of dietary energy for the host. Consequently, dysbiosis of the gut microbiota has been implicated in malnutrition. Malnourished children may have underlying inflammation of the gut as a part of an enteropathy. This leads to malabsorption of processed dietary compounds. In addition, inflammatory molecules could affect gut homeostasis and there can be critical nutrient diversion.

The Society for Applied Studies coordinated a multicentre study to evaluate the impact of novel therapeutic foods embedded in various potentially usable feeding regimens for management of uncomplicated severe acute malnourished children (SAM) in children aged 6-59 months. SAM contributes to 25% of under 5 deaths. Preliminary results showed that nearly half the children do not respond to treatment with ready to use therapeutic foods (RUTF). We hypothesize that exposure to microbes and dietary deficiencies cause chronic gut inflammation and immune activation that contribute to poor response. A substudy was designed to ascertain presence of gut inflammation through measurement of specified biomarkers and examine the relationship between the gut microbiome, inflammatory markers and the response to treatment.

The study was conducted in three sites - urban slums and resettlement colonies in the national capital region, rural (predominantly tribal) Rajasthan, rural and urban Tamil Nadu - where the prevalence of SAM is above the national average of 6.4%. Study participants were children aged 6 to 59 months with weight for height less than -3 SD of WHO standard or oedema of both feet or both. Children with complicated SAM were excluded and referred to a hospital. All enrolled children were randomized to one of the three groups centrally produced RUTF, locally produce RUTF and augmented home prepared foods. These children were followed up weekly upto 16 weeks or recovery whichever was earlier and for 16 weeks after completion of the treatment phase. Faecal samples were collected at enrolment and at 8 weeks or recovery whichever was earlier. Blood sample was also collected at enrollment for analysis of high sensitivity CRP, Tissue Trans Glutaminase Antibody, Serum IgA, Serum Albumin, Platelet count and urine for Microalbuminuria. Total bacterial DNA sample was extracted in the laboratory from fecal sample, isolated DNA sample used for targeted metagenomic analysis using 454 GS FLX plus Pyrosequence. Inflammatory markers Calprotectin, Neopterin and Myeloperoxidas were analysed by competitive or sandwiched Elisa.

The follow up of the children is complete and analysis of specimen is ongoing.

Clinical Development Services Agency

An Overview

Sudhakar Bangera

The Clinical Development Services Agency (CDSA) was launched in September 2009 as an extramural unit of THSTI. It was created to facilitate development of affordable healthcare products for public health diseases. Registered in September 2010 as an autonomous, not-for profit research society by the Registrar of societies, Delhi, under the Societies Registration Act XXI of 1860, it aims to develop an eco-system for training and learning and work with public sector institutions, and small and medium enterprises (SME) to translate innovative technologies into medical products for public good.

CDSA has a simple governance structure. Led by a high-powered Governing Body, it has 12 members including the Program Director who acts as Member Secretary. The operational oversight of CDSA is provided by an Executive Management Committee (EMC) that comprises of experts in training, product development, and organizational development among others.

The main objectives of CDSA and the work done so far have been:

- As a training academy, CDSA aims to build capacity and capability in the area of clinical development and translational research. We conduct training programs for young clinical researchers, ethics committee members and other personnel in becoming an efficient clinical research professional

- Monitor public health studies for compliance to Schedule Y regulations, CDSCO-GCP guidelines, Study Protocol and other requirements.

- Support Investigators and SMEs by providing Clinical Study Support Services like regulatory consultation, project management, medical monitoring, audit, data management and biostatistics

- CDSA with 5 Centers of Excellence (CoEs) has formed a collegium of Centers of Clinical Research Excellence for collaboration in education, training and capacity building and Collaboration in Research, Innovation, and Clinical Development Support Services. The institutions are: KEM Hospital, Pune; Society of Applied Sciences (SAS), New Delhi; Center for Chronic Disease Control (CCDC), New Delhi; JSS University, Mysore and CMC Vellore.

Departmental Activities

TRAINING Department

CDSA has a national mandate of capacity and capability building in the area of clinical and translational research in India.

Training Programs

In 2014-15 and till date, CDSA has completed 17 trainings covering approximately 1329 participants with 181 resource personnel involving 155 attendees from institutions across India. Till date, 3003+ participants from 423 Institutions have attended 49 trainings that involved 494 resource personnel.

No.	Date	Venue	Workshop/ Courses	Resource Personnel	Participants
1	18 Jun 2014	IIC, New Delhi	BIRAC CDSA Regulatory Workshop: Emerging Needs & Regulations on Phytopharmaceuticals	08	62
2	25 Aug 2014	THSTI, Gurgaon	Good Clinical Practice (GCP) Awareness Programme for THSTI	06	39
3	18-19 Sep 2014	TMC, Kolkata	Good Clinical Practice (GCP) Awareness Programme for Tata Medical Center	09	53
4	11 Sep - 16 Nov 2014	Online Program	Bioethics Course with Manipal University	32	09
5	7-8 Oct 2014	Osmania Medical College, Hyderabad	Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness Program)	07	87
6	16 Oct 2014	IHC, New Delhi	BIRAC CDSA Regulatory Workshop: Current Regulation on Medical Devices and in vitro Diagnostics (IVD) Kits	11	58
7	18-19 Nov 2014	Smt. NHL Municipal Medical College, Ahmedabad	Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness Program)	07	135
8	21-22 Nov 2014	THSTI, Gurgaon	Workshop on Animal and Human Ethics for PhD students of THSTI	02	22
9	24-28 Nov 2014	ICGEB, New Delhi	Basics of Statistics and Fundamentals of SAS in Clinical Research	07	27
10	17 Dec 2014	ICGEB, New Delhi	IATA Requirements on Packing & shipping of infectious Biological Substances /Diagnostic Specimen and Cold Chain Management - Awareness Program	01	53
11	18-19 Dec 2014	ICGEB, New Delhi	Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness Program)	08	80

No.	Date	Venue	Workshop/ Courses	Resource Personnel	Participants
12	16-17 Jan 2015	SMS Hospital, Jaipur	Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness Program)	15	97
13	27-28 Jan 2015	Govt. Medical College, Trivandrum	Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness Program)	13	111
14	11-12 Feb 2015	NCCS, Pune	Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness Program)	12	108
15	26-27 Feb 2015	B M Birla Heart Research Centre, Kolkata	Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness Program)	16	130
16	11-13 Mar 2015	NIPGR, New Delhi	Statistical Concepts and Data Analysis used in Research	05	76
17	25-26 March 2015	Assam Medical College, Dibrugarh	Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness Program)	16	94
Total				181	1329

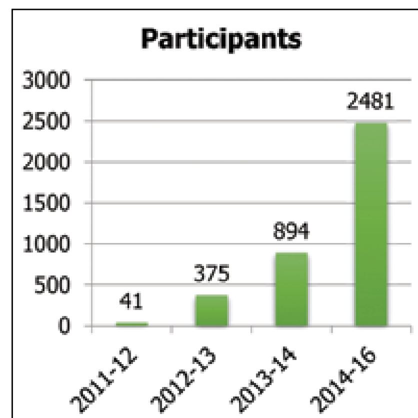
Outreach

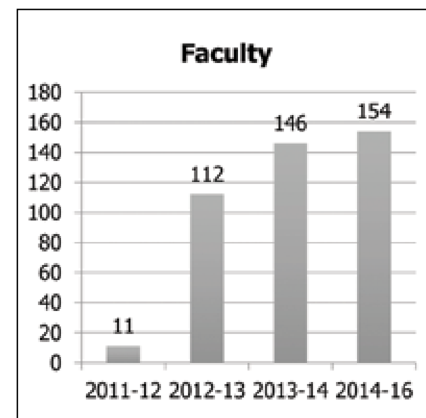
CDSA has mapped the cities from where the participants attend various training programs of CDSA along with the cities where programs are held or planned to oversee national outreach.

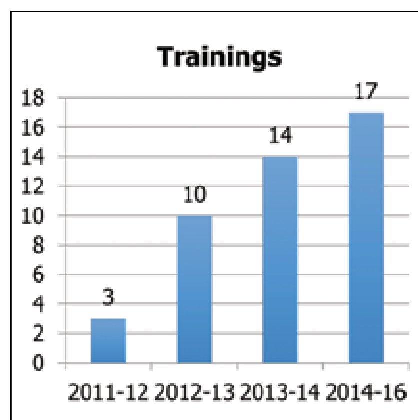
Figure 1: Cities from where the participants attended CDSA Programs.

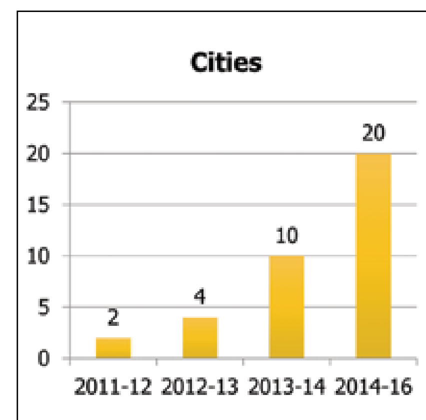
Figure 2: Cities where CDSA programs are conducted or to be conducted.

Trend Observed









Indian Clinical Researcher Development Program (InCReD)

InCReD is a prestigious program offered by Collegium of Clinical Research Excellence to attract and nurture established world-class clinician investigators to undertake cutting edge multi-disciplinary translational and clinical research in India. This non-accredited hybrid (online-offline) certificate program is designed for clinicians who want to be competent to conduct clinically oriented research. The program is intended to enable participants to acquire the requisite skills and experience to position themselves as independent investigator and help improve the quality of research.

Online Program with Manipal University

For the second year, CDSA in partnership with Manipal University is supporting online educational program "Bioethics Certificate Course".

Dedicated Portal

CDSA has developed a website portal totally dedicated for training activities (www.cidp.in). This website has all audio-video recordings, presentations, case studies, resource materials, faculty and participant details, etc. It has 'online registration' facility for all training programs.

Database

CDSA is working continuously towards development of database comprising of experts in various areas of clinical and translational research. In addition to this, a database comprising of following personal is being compiled to facilitate various surveys and reach out programs to a larger public for any upcoming training programmes.

No.	Details	Numbers
1	Ethics Committee Members	1350
2	Investigators	1122
3	Medical Colleges	276
4	Medical Device Organizations	26
5	Scientists	254
6	Sponsor/Contract Research Organizations	86
Total		3114

Clinical Study Support Services

Clinical research is becoming an essential activity for the rational development of new pharmacological treatments, diagnostic tests and devices. Clinical research includes both interventional and non-interventional studies which lacks public awareness as a research. The Supreme Court of India opined that no clinical research should be allowed till a mechanism is put in place to monitor and protect the lives of study participants. For interventional studies which also referred as clinical trials, there is a technical and regulatory system in place laid by concerned authorities which is still evolving in consideration to the safety of the study participants.

At the same time, there are non-interventional studies which provide an effective way of gathering real-world evidence. These evidences are becoming central to decision making when used in conjunction with clinical trials or used as a supplement in reforming policy and advocacy. Such studies generally take place in clinical or community set-up. Even though these studies have the ability to heighten disease awareness and to support of research and scientific inquiry, there is no proper mechanism to ensure the integrity of the collected data.

Department of Clinical Portfolio Management

The department (CPM) has been established to undertake and implement cost effective clinical research services in product development for government, semi-government, government-funded academic and non-academic institutions, non-profit, and small and medium sized enterprises. The main objective of Clinical Portfolio Management department is to direct, supervise and coordinate a network of clinical studies in India. Specific objectives include:

- Collate evidences from research projects for review to aid formulation of a national policy

- To provide monitoring support to the funding agencies for the clinical and community based studies as directed
- To facilitate allied activities as and when required:
  - Study start up activities
  - Site Assessment(s) and gap analysis
  - Project planning and Budgeting
  - Grant application
  - DSMB constitution and coordination
  - Developing SOPs
  - Convene expert review meetings.

CPM has been supporting investigators, academic institutes for clinical study planning, management and ensuring quality and integrity of data generated in compliance with project requirements and applicable guidance. Services also envisaged protecting the safety, confidentiality and well-being of the participants. Department also serve as a primary resource and point of communication for the investigators/sponsors/funding agency and project team.

Through active participation for policy and advocacy we engage in health programs to improve or protect health policies. For example, CDSA as per the directions from inter-ministerial committee for severe acute malnutrition (SAM) alliance program has convened national consultative meeting for projects implemented and completed under SAM program.

Department provides advisory support and assists researchers /academicians for the project planning and effective implementation of the research projects. This includes writing grants as co-applicant and /or reviewing the project proposal with special emphasis on operational aspect, project planning, tracking for milestones, risk identification and management for efficient implementation of research proposal.

Based on the experience gained while working as independent monitoring agency we plan to establish operational guidelines, educational module and study management tools customized to the need of the research in public health domain. These will be a value addition for the public health research for benchmarking the quality standards and CDSA will be extending advisory support for these as requested by the stakeholders.

We will establish a network of experts to support and mentor the department for improvisation of systems and processes, algorithm for the project selection and operational support mechanism for the research in public health domain.

Ongoing Clinical Projects/Programs

CDSA is providing clinical study support services to the following projects and programs listed in the table

Project Title	Principal Investigator/ Sites	Funding Agency	CDSA Role
Severe Acute Malnutrition (SAM) Programs			
To evaluate the impact of three feeding regimens on the recovery of children from uncomplicated severe acute malnutrition in India and to use the evidence to inform national policy	Dr. Nita Bhandari (03 sites: Delhi Vellore, Udaipur)	BMGF	• Co-monitoring with WHO • DSMB constitution & coordination with WHO
Undiluted animal milk with added sugar and micronutrients versus WHO feeding protocol for management of severe acute malnutrition in non-breastfed infants aged 2-6 months of age: a randomized controlled trial.	Dr. Satinder Aneja (03 sites: Delhi)	DBT	• Site start-up support • Clinical Monitoring • DSMB constitution and coordination • Data • Management
Validation of Mid Upper Arm Circumference (MUAC) for Detecting Severe Acute Malnutrition against Weight for Height Below -3 SD Using as Gold Standard in Children Aged 6-59 Months	Dr. Umesh Kapil (single site: Meerut)	ICMR	• Study start-up support • Clinical monitoring • Coordinate monitoring by domain experts
Other Projects/Programs			
Inter-Institutional Program for Maternal, Neonatal and Infant Sciences: A translational approach to studying preterm birth	Multi-Institutional Program – THSTI, RCB, NII, NIBMG, AIIMS & SJH (single site: General Hospital Gurgaon)	DBT	• Study Start Up Support • Clinical monitoring • Quality Management • Lab Monitoring
A Phase IV, Interventional, Open label, multicentric, Single arm clinical trial to assess the Safety, Tolerability & Immunogenicity of Bivalent Oral Polio Vaccine (bOPV) in healthy Indian infants.	8 sites across India	BIBCOL/ DBT	• Medical Writing • Regulatory advisory • Project management • Clinical monitoring • Data management • Statistical services
Evaluating the Efficacy and Safety of Indigenous Goat Lung Surfactant Extract (GLSE) in a pilot sample of preterm infants with Respiratory Distress Syndrome (Phase II study)	Dr. Ramesh Agarwal (AIIMS) (12 sites across India)	WT	• Project Management • Clinical Monitoring • Data Management • Safety Monitoring
Determination of efficacy and safety of reflexology therapy for the Patients with intractable epilepsy: a multicenter randomized clinical trial	Dr. Krishna Dalal (AIIMS) (02 sites: Dibrugarh, Imphal)	DBT	• Site Start Up Support • Clinical Monitoring • Operational Training
Inhibition of host-induced mycobacterial efflux pumps as a novel strategy to counter drug tolerance and virulence of pulmonary tuberculosis	Dr. Soumya Swaminathan (NIRT). Clinical site: LRS Delhi	DBT	• Regulatory Advisory • Clinical Monitoring

Medical Affairs and Medical Writing

CDSA supports the investigators in medical writing and medical affairs and contribute to the development and evaluation of new products. We support investigators/ innovators through their studies, and the decisions need to make between studies, to reveal opportunities for their program, along product development journey. The department is providing services as mentioned below:

Medical support of projects

- Training on BIBCOL Protocol has been imparted to Clinical operations team and all protocol related queries from the sites
- Defense for NIRTD study at SEC meeting, CDSCO office, New Delhi
- Health education on Polio
- Training session on MedDRA for CDSA team

Medical Writing Support

- Reviewed and revised Protocol, PIS, ICF, CRF, AE and SAE forms, attending Subject Expert Committee meetings for Surfactant study (AIIMS), Verapamil dose finding Study (NIRTD), Bivalent oral polio vaccine study (BIBCOL)
- A Repository of study related documents for the benefit of scientific fraternity. We plan to establish operational guidelines, educational module and study management tools customized to the need of the research in public health domain
    - Guidance document for Ethics committee
    - SOP templates for functioning of Ethics Committee
    - Medical Glossary in Layman Language (English)
    - Guide & Tool for calculating Compensation
- Faculty for the CDSA workshops in medical affairs and medical writing domain
- Collaboration with MVDP for Phase 1 malaria vaccine project
- Quarterly Newsletter (1 issues of Newsletters has been released)
- Journal Club
- An awareness program on Breast Carcinoma & Self Examination held for CDSA & THSTI staff

Data Management and Bio-Statistics

This division aims to provide data management and bio statistical support to various clinical projects. CDSA has the following softwares and program required for effective analysis of the data.

- Statistical Analysis System® (SAS) Version 9.3 as statistical analysis tool,
- Promasys® as a solution for clinical data management. Promasys has also been mapped with MedDRA dictionary to enable medical coding.

Also, a dedicated IT-infrastructure (data servers, systems, access-controls, etc.) has been deployed to run the activities as per the standards of data security

and safety. Currently it is helping ongoing projects for their data management and statistical requirements.

CDSA provides the following services through its data management & biostatistics group:

- Statistical inputs to study design and clinical trial protocol
- Sample Size Calculation
- Randomization
- Data Management Plan
- Case Record Form (CRF) Development
- Statistical Analysis Plan
- Data Cleaning and Coding
- Statistical Analysis of Data
- Reporting of Statistical Analysis (e.g. Figures and Tables)
- Training and Education
- Preparation of Technical Documents (Reports & Publications)

Delivery of Statistical Services

No	Project Title	Month	Funding Agency	PI
1.	Untargeted metabolic profiling of urinary sample to find urinary biomarker for diabetic nephropathy	Aug – Sept 14	DST	Dr. Sangeeta Kumari, Inspire Faculty, DDRC, THSTI
2.	Comparative study of HR Practices and their impact on Biotechnology Industry of India (PhD Thesis)	Sept – Oct 14	Old study	Mr. Sundeep Sarin Joint Director, Department of Biotechnology Ministry of Science & Technology
3.	A Phase III Randomized Double Blind Placebo Controlled Trial to Evaluate the Protective Efficacy of Three Doses of Oral Rotavirus Vaccine (ORV) 116E, Against Severe Rotavirus Gastroenteritis in Infants. Clinical Development of 116E Rotavirus Vaccine under Indo-US vaccine development program	Oct – Nov 14	**Old study** DBT, PATH, Bharat Biotech International Ltd.	Dr. Sudhanshu Vrati, Principal Research Scientist & Head, Virology Laboratory Vaccine and Infectious Disease Research Center, THSTI
4.	Exploring membrane vesicles of *Mycobacterium tuberculosis* as potential vaccine vehicle	Nov –Dec 14	DBT	Dr. Krishnamohan Atmakuri, Assistant Professor, Ramalingaswami Fellow THSTI
5.	Muscle quality assessment pre and post intervention in pre-diabetic Indians	April – 15	Welcome Trust	Dr. S. Sucharita, Additional Professor and Head Clinical Physiology Unit, Department of Physiology, St John's Medical College, Bangalore
6.	Assessment of effects of dexmedetomidine as an adjunct to local anesthetics for supraclavicular brachial plexus block on the onset and duration of block and the postoperative analgesia in patients undergoing upper limb surgeries	May 15	Old study	Dr. Neerja Bharti, Additional Professor Anaesthesia, PGIMER, Chandigarh
7.	Role of Vitamin D in Metabolic Disorders	Jun 15	Old study	Dr. Sanjay K Banerjee, Scientist E, DDRC, THSTI

Dissemination of awareness of Good Statistical Practice

No.	Training Title	Number of Days	Date	Venue	No. of Participants
1.	Knowledge Sharing Session	Three	August 2014	THSTI Seminar Room	25
2.	Basic Statistics and Fundamentals of SAS® in Clinical Research (Hands-on training program)	Five	Nov 24 – 28, 2014	ICGEB, New Delhi	27
3.	Statistical Concepts and Data Analysis used in Research	Three	Mar 11 – 13, 2015	NIPGR, New Delhi	85

Regulatory Affairs

CDSA provides regulatory advisory services for development and registration of new drugs, medical devices, diagnostics and biopharmaceuticals/biosimilars including vaccines to SMEs and public funded pre-clinical and clinical stage research projects. The advisory cell provides:

- Advice on regulatory processes encompassing product development & registration
- Consultation on regulatory dossier preparation, egg. IRB; IND; CTD etc.
- Regulatory input to ongoing clinical trials in CDSA
- Regulatory input in planning and compliance certification of GLP- bio analytical lab & Phase 1 facility.
- Advice on Registration of Ethics Committees as per new notification.
- Advice and review proposals coming from BIRAC (especially clinical trial protocols)
- Technical input (program design, faculty identification & preparation of resource materials).

Academia

Ph.D. Program

Translational Health Science and Technology Institute (THSTI) is a recognized R&D institute of the Jawaharlal Nehru University (JNU), New Delhi to offer doctoral programs in biomedical and clinical research tracks for candidates with medical, life sciences (including biomedical, health, pharmaceutical, nutritional science, public health, and nursing), veterinary science, engineering, or mathematics background.

The broad domains of ongoing research at THSTI are:

- Biology of infectious diseases such as dengue, Japanese encephalitis, hepatitis E and tuberculosis, vaccine and anti-viral development
- Physiology of nutrition and the developing immune system, immune responses in pregnancy and childhood
- Clinical research and epidemiology focused on maternal and child health
- Auto-immune diseases, infection and inflammation
- Understanding disease through the human microbiome
- Diagnostics and therapeutics
- Medical devices and implants
- Mathematical modelling to understand biological problems

Students selected for the program are required to undergo a pre-PhD course work followed by the submission of a research thesis. The PhD program at THSTI is governed by the JNU rules.

The students admitted to THSTI-JNU PhD program will be required to undertake courses to earn 14 credits to be eligible to undertake thesis research work. THS-1, THS-2, and THS-3 are core courses of 8 credits and compulsory for all students. All other courses are optional and the student has the choice to earn 6 credits by choosing any combination. The courses offered over the two semesters are:

Semester-I

- Biomedical Research : Concepts and Methods
- Clinical Research Methodology
- Research Internship

Semester-II

- Health Policy and Decision Analysis in Health
- Infectious Disease Biology
- Infectious Disease Epidemiology
- Immunology and Immunotechnology
- Special Topics in Epidemiology
- Essentials of Clinical Trials
- Essentials of Regulatory Trials
- Introduction to Biodesign

Biomedical research : concepts and methods

This course has been designed to introduce the students to the practical world of life science research. The curriculum covers fundamental concepts of basic and translational research as well as educates them in identifying and executing innovative research ideas. The course also

allows students to gain theoretical and practical understanding of techniques relevant to modern day life science research.

Clinical research methods

Students across life science disciplines require a sound knowledge of the fundamentals of research methods to understand, design, analyze and communicate their research. Students will be exposed to the basics of the components of a good research proposal and the essential epidemiological and statistical concepts that form the framework of sound research. Students will learn to use simple designs and statistical methods to formulate questions and analyze data. In addition to lectures and seminars the course will involve multi-faculty workshops, exercises in biostatistics with STATA statistical software and group tasks. Students across diverse fields are expected to understand the basics of clinical research methods and the language of clinical research by the end of the course.

Research internship

Students are required to work in the lab/clinic of the assigned supervisor in the afternoon and undergo training in various research methodologies and participate in laboratory/clinical discussions. At the end of the semester, students are required to write a report (8-10 pages) on various tasks assigned and make a presentation on their accomplishments to the assessment committee. The supervisor will provide his/her assessment of the student's performance during the internship, participation in laboratory/clinical activities and discussions, and the quality of report. Students are also expected to fine tune their analytical and scientific communication skills by presenting a seminar on a relevant topic (in consultation with their supervisor), detailing the existing knowledge and their opinion about future perspectives on that topic.

Health policy and decision analysis in health

Application of solutions for public health in the real world requires robust decision-making and analysis of risks. Economic analyses of policy decisions become even more critical in contexts of constrained resources. Public health professionals will be trained in techniques of decision and risk analyses. Many of these techniques can be used at the bedside as well for population & community health decisions. These methods can also be adapted to decision making for research priorities, outcomes and funding. Bayesian probability, evaluation of diagnostic tests, decision trees, QALYs, disease burden quantification, utilities and cost effectives analysis are some of the topics that will be covered. The course will involve lecture sessions with active class participation, in-class and take home exercises.

Infectious disease biology

This course aims at educating students about important human infectious diseases prevalent in India and worldwide. Introductory lectures by clinical experts on few important infectious diseases will provide a practical overview of those diseases from a doctor's point of view. Further emphasis will be given to understanding the molecular basis of various bacterial and viral infections. This course will also cover classical as well as modern approaches to developing prophylactic and therapeutic strategies against those pathogens.

Infectious disease biology

This course aims at educating students about important human infectious diseases prevalent in India and worldwide. Introductory lectures by clinical experts on few important infectious diseases will provide a practical overview of those diseases from a doctor's point of view. Further emphasis will be given to understanding the molecular basis of various bacterial and viral infections. This course will also cover classical as well as modern approaches to developing prophylactic and therapeutic strategies against those pathogens.

Immunology and immunotechnology

This course covers both theory and techniques relevant for immunological research. The first part of the course aims at explaining the fundamental concepts of immune system and its components and illustrate the importance of these under various conditions such as infections, cancer or transplantation therapy. The course also covers the importance of human microbiome in mediating immune functions. The second part of the course will cover theoretical and practical aspects of techniques important for immunological research in the laboratory as well as discuss the concepts of important diagnostic techniques.

Special topics in epidemiology

This course covers advanced topics in epidemiology providing detailed understanding of the design and implementation of cohorts and case control studies as well as other epidemiologic studies. The course will consist of lectures, seminars, and reading material. In addition, a number of in-class and take home exercises will form a strong component of the course. Students will also spend substantial time in group-activity across disciplines to learn to work collaboratively to formulate translational research questions and to design studies to answer them. Students are expected to participate in discussions in classes.

Essentials of clinical trials

This course addresses critical methodological aspects of clinical trials. At the end of this course the student will be able to demonstrate understanding of the principles of clinical trials, assess and select relevant research designs for clinical trials, conduct random allocation, blinding and sample size estimations, critically interpret published results from clinical trials.

Regulatory trials

This course is designed to create awareness about the regulations & guidelines related to clinical research in India as well as globally. The students will understand how to manage a clinical study effectively by good documentation and clinical data management practices. The course prepares the participants to face audit and regulatory inspections from regulatory agencies.

Post-doctoral Program

THSTI provides post-doctoral training under the mentorship of its senior faculty. Young scientists who have recently completed their doctoral training are encouraged to correspond with faculty members whose research area may be of interest to them. THSTI will provide sponsorship for DBT post-doctoral fellowship to deserving candidates based on the recommendation of a faculty member. THSTI offers post-doctoral training to young researchers through specific options for post-doctoral program at various niche centers of THSTI. These schemes usually span for a period of five years and are widely advertised in national newspapers and on THSTI website.

The various post-doctoral options are:

- 'Vaccine Research Innovation (VRI) Award Scheme' in the Vaccine and Infectious Disease Research Center.
- 'Innovation Award' scheme in Biodesign in the Centre for Biodesign and Diagnostics, focusing on diagnostics, implants and medical devices.
- 'Microbiome Innovation Award' scheme in the Centre for Human Microbial Ecology.

Also there is a sandwiched post-doctoral program, 'Indo-Finnish Post-doctoral fellowship in Diagnostics' funded by DBT and administered by THSTI. This program is for young researchers who have inclination for research in areas in diagnostic product development and platform technology development. The fellows get training at THSTI, India and University of Turku, Finnland.

Administration

THSTI Administration

M V Santo

The THSTI Administration performs relentlessly to provide unstinted support for the smooth scientific functioning of the institute. The personnel in administration comply with the Government of India Rules and related financial norms in their functioning. The THSTI Administration comprises of several functional sections. They are General Administration, Human Resource (HR) Management, Finance & Accounts, Stores & Purchase, Information Technology (IT), and Engineering & Estate Management. Information on some of the important activities performed by these various sections is provided below.

General Administration

THSTI started functioning from its new buildings in the campus of the NCR Biotech Science Cluster in Faridabad from January 2015. The new campus has excellent infrastructure which includes laboratories, offices, classrooms, library, seminar rooms, auditorium, cafeteria etc. The construction of faculty housing, hostel and the guest house are in progress. The administration's biggest challenge in the FY 2014-15 was to make the new campus operational. Some of the important activities included identifying competent vendors for shifting of furniture, lab equipment etc. to the new campus, finalizing the contractor for providing electro-mechanical and other engineering services, security services, housekeeping services and to provide transport services for the employees to reach the campus. Most of these services are now in place and the THSTI operations from the new campus are fully in place.

In order to provide support to the Executive Director in decision making and to fulfil statutory requirements, various internal committees have been constituted. The composition of various committees is given in the later part of this report.

On the communication front, it has been the endeavor of THSTI to maintain high degree of transparency with regard to the entire official processes through the website especially about recruitments and tenders. Strict compliance to the requirement of Right to Information (RTI) Act 2005 is practiced at the Institute with regard to suo-motu disclosures. During the period 2014-15, only 7 applications were received under the RTI act. Among these applications, only 4 were with respect to THSTI related activities and the information was disseminated under the provisions of RTI Act. The rest were applications transferred from DBT seeking general information. The Parliament questions, references from DBT and other organizations were responded within the stipulated deadlines.

THSTI observed all the important occasions as directed by the Govt. of India and the foundation day of THSTI. A brief on these activities is provided below.

Hindi Saptah Samaroh: Hindi Week was celebrated from 22nd September 2014 to 26th September 2014. As a part of the celebration, various competitions were organized in Hindi such as poem recital, essay competition, extempore speech and quiz. Professor Govind Prasad, Head of the Hindi Department from Jawaharlal Nehru University was the chief guest for the valedictory session.

Sadbhavana Diwas: Sadbhavana Diwas was observed on 20th August 2014. Sadbhavana Pledge was administered by the Executive Director and all students, staff, officers and faculty/scientists of the Institute took the pledge.

Cleanliness Drive: THSTI conducted cleanliness drive on 2nd October, 2014 on the occasion of the national campaign by the Government of India to clean the streets, roads and infrastructure under Swatch Bharat Mission.

Vigilance Awareness Week: THSTI observed vigilance awareness week from 27th October 2014 to 1st November 2014. The week commenced with a pledge administered by Dr. G. R. Medigeshi, CVO. During the week, various competitions were organized on the topic of anti-corruption drive.

5th Foundation Day: The occasion was celebrated with enthusiasm on 15th July 2014 by the THSTI community along with officials from the Department of Biotechnology, collaborators and well-wishers. The chief guest was Prof. K. VijayRaghvan and the guest of honour was Dr. T. S. Balaganesh.

Human Resource Management

During this financial year, THSTI issued 36 recruitment notices resulting in 144 recruitments. The rolling advertisement in the case of JRF/SRF/RA positions, which was introduced during the year 2013-14, to cater to the frequent requirements of the projects, was continued with new vacancies being advertised and filled up successfully every month. For administrative positions like Clerical Assistant, Accounts Assistant, etc., where the number of applications received were high; the selection process included a written test followed by the interview. Among the new recruitments, around 30% were to fill up clinical positions, 31% were for technical positions, 30% for scientific positions and the remaining 9% were for administrative positions.

In order to enhance the capabilities, competencies and effectiveness of employees, THSTI encourages employees to attend workshops, seminars etc. to widen their scientific knowledge for better output. Accordingly, 57 requests from employees were approved for various trainings / workshops/ seminars/ meetings within and outside the country during this financial year.

The consolidated staff position of THSTI as on 31-03-2015 employed under various grants is as shown below.

Name of the Unit	Numbers
THSTI core	27
VIDRC	11
PBC	12
CBD	20
NBA	3
CHME	9
DDRC	25
PCBR	6
CDSA	18
Projects	149
Outsourced manpower, Security, Maintenance and Housekeeping	130
Total	**410**

Finance & Accounts

The Institute gets grant-in-aid from the Department of Biotechnology (DBT) and extramural funds from DBT and various other external funding agencies like ICMR, DST, WHO etc. for various research projects. Finance & Accounts section of the Institute monitors and controls the expenditure against the above funds and also attends to the day-to-day financial matters, payments to contractors/suppliers, payment of salaries to staff, payment of personal claims in respect of the employees of the Institute etc. Since the numbers of employees have increased, salary software was purchased during the year 2014-15 which has made the process of preparation of salary more efficient with better MIS outputs for grant-wise details and automatic dissemination of salary slips to employees through email. The Section is also responsible for preparing the annual statement of accounts. The audited annual statements of accounts for the FY 2014-15 as presented before the Finance Committee, Governing Body and the Society has been provided in the later part of this report.

Stores and purchase

The Stores and Purchase section is responsible for the purchase of scientific equipment, perishable and non-perishable chemicals and reagents, other consumables and services from overseas and local markets. In order to minimize the procurement cost, the section has started warehousing the plastic-ware and the requirements are taken as a whole for all the labs. All perishable and non-perishable shipments were being cleared promptly from the port in order to avoid demurrage. The total consumables and equipment purchases made during the financial year 2014-15 are Rs.13,61,95,935 and Rs.11,61,73,154 respectively. In consonance with the directives received from the Department of Biotechnology (DBT), THSTI has started publishing their tenders through e-Procurement module of CPP portal (Central Public Procurement Portal). E-procurement not only makes the system transparent and efficient, it also instils confidence among the vendors in the tendering system. The Section procured all the equipment and non-consumable materials costing more than Rs. 10 lakh through e-procurement.

Information Technology

The IT section takes care of the hardware, networking, website and software requirements for the institute. The biggest challenge before the IT section this financial year was to provide communication facility at the Faridabad campus where the terrain is so complicated that Optical Fiber Cable connectivity could not be provided by any of the Internet Service Provider in India. However, the IT Section successfully managed to provide internet facilities throughout the campus through Radio Frequency Bandwidth of 30 Mbps leased lines from BSNL. The telephone lines were also provided through BSNL. THSTI's website was redesigned with add-on features to incorporate all requisite scientific and administrative information. The website is now hosted on NIC server which provides free hardware infrastructure, 24x7 maintenance and backup. THSTI is connected with social media like Facebook, Twitter and Blog. The information about the Institute is updated on a daily basis over social media. The google mail service being used by the Institute has been upgraded for 2000 users.

Engineering and Estate Management

The Engineering section develops and maintains the Institute's physical infrastructure and various facilities. The primary responsibility of this section is to ensure that all the scientific equipment is kept functional. The section takes full responsibility for the electricity supply system, HVAC system and water supply system. The section has started in-house repair of the equipment for minor repairs due to which the dependence on expensive maintenance contracts has been considerably reduced.

The engineering section successfully faced the biggest challenge to move safely the lab equipment from its interim facility at Gurgaon to its permanent campus at Faridabad. The process of handing and taking over of the new buildings, electromagnetic equipment in the electrical substation, HVAC system, AHU units, chiller plants, lifts, fire-fighting system, STP plant, pump house etc. was carried out methodically and maintenance contracts for these implemented.

Balance Sheet as at 31st March, 2015

Amount (In Rs.)

Liabilities	Schedule	31.03.2015	31.03.2014
Corpus / Capital Fund	1	1,140,333,546	1,006,667,651
Reserves and Surplus	2	97,914,323	72,818,285
Earmarked/Endowment Funds	3	-	-
Secured Loans and Borrowings	4	-	-
Unsecured Loans and Borrowings	5	-	-
Deferred Credit Liabilities	6	-	-
Current Liabilities and Provisions	7	96,397,847	87,605,293
Total		**1,334,645,716**	**1,167,091,229**
ASSETS			
Fixed Assets	8	1,069,559,605	1,048,699,338
Investment From	9	-	-
Earmarked/Endowment Funds			
Investment - Others	10	-	-
Current Assets. Loans, Advances etc.	11	265,086,111	118,391,891
Miscellaneous Expenditure (to the extent not written off or adjusted)		-	-
Total		**1,334,645,716**	**1,167,091,229**
SIGNIFICANT ACCOUNTING POLICIES AND NOTES ON ACCOUNTS	24		
CONTINGENT LIABILITIES	-		

Schedules 1 to 24 form an integral parts of Accounts

As per our separate Report of even date attached

For S.M. Saini as Associates
Chartered Accountants

Sd/-
(C. B. YADAV)
Finance & Accounts Officer

Sd/-
(Dr. G.B. NAIR)
Executive Director

Sd/-
(LAXMIKANT SAINI)
Partner
M No.512056

Place : Faridabad
Date : 24th October, 2015

Income and Expenditure Account for the Year Ended 31st March, 2015

Amount (in Rs.)

Income	Schedule	31.03.2015	31.03.2014
Income from Sales/ Services	12	123,080	
Grants/Subsides	13	175,000,000	180,400,000
Fees/ Subscriptions	14		
Income from Investments	15		
Income from Royalty, Publication etc.	16		
Interest Earned	17	15,793,029	8,451,613
Other Income	18	2,653,954	2,104,771
Increase/ (Decrease) in stock of Finished goods and works in progress	19		
Deferred Income-Fixed Assets		53,297,156	47,318,480
Total (A)		**246,867,219**	**238,274,864**
EXPENDITURE			
Establishment Expenses	20	54,959,696	59,102,624
Other Administrative Expenses etc.	21	112,878,329	118,368,626
Expenditure on Grants , Subsidies etc.	22		
Interest	23		
Depreciation (Net Total at the year-end-corresponding to Schedule 8)		53,297,156	47,318,480
Prior period Adjustment A/c (ANN-A)			
Total (B)		**221,135,181**	**224,789,730**
Balance being excess of Income Over Expenditure (A-B)		**25,732,038**	**13,485,134**
Transfer to special Reserve(Specify each)			
Transfer to /from General Reserve		25,732,038	13,485,134
BALANCE BEING SURPLUS /DEFICIT CARRIED TO CORPUS/CAPITAL FUND		–	.
SIGNIFICANT ACCOUNTING POLICIES AND NOTES ON ACCOUNTS	24		
CONTINGENT LIABILITIES			

Schedules 1 to 24 form an integral parts of Accounts

As per our separate Report of
even date attached
For S.M.Saini & Associates
Chartered Accountants

Sd/-	Sd/-	Sd/-
(C. B. YADAV)	(Dr. G.B. NAIR)	(LAXMIKANT SAINI)
Finance & Accounts Officer	Executive Director	Partner

Place : Faridabad
Date : 24th October, 2015

Consolidated Receipts and Payments Account for THSTI, Projects & Fellowship for the Year Ended 31st March, 2015

Amount-in-Rupees

RECEIPTS				
Particulars		**31.03.2015**		**31.03.2014**
OPENING BALANCE:				
THSTI	12,808,651		496,633	
Projects	91,124,287		15,410,435	
Fellowship	(103,932,937)		12,446,387	
Grant-in Aid Received:				
THSTI	224,337,000		370,000,000	
Projects	341,736,518		267,426,927	
Fellowship	14,296,543		31,418,667	
Other Receipts -THSTI				
Miscellaneous Receipts	10,010		19,441	
Sales of Scrap	123,080		-	
Overhead THSTI	2,295,324		1,833,936	
RTI Receipt	20		24	
Guest House Receipt	66,300		44,950	
Admission Fees	74,500		-	
Recruitment Fee	66,300		131,420	
Tender Fee	141,500		75,000	
Security / Hostel Deposit Received	402,014		1,367,776	
Earnest Money Deposit	-		11,467,070	
Interest Received	8,429,095		8,051,613	
Accrued Interest Received	7,353,934		-	
Income Tax Refund Received	-		1,340,418	
Govt. Dues Payable	431,672		1,140,753	
Other Liabilities/Payable	441,436		373,584	
Decrease in advances	943,669		9,517,612	
Total		**601,148,915**		**732,562,646**

Amount-in-Rupees

PAYMENTS		**31.03.2015**		**31.03.2014**
Particulars				
THSTI				
Fixed Assets	14,737,575		52,557,430	
Work -in- Process- Building	27,000,000		142,000,000	
Manpower	54,988,958		59,096,627	
Consumables	38,814,656		74,267,595	
Administrative Expenses	70,161,735		52,443,780	
Advances , Receivables & Liabilities	25,380,800		12,686,148	
Projects	226,115,471		194,198,905	
Fellowship	19,880,929		32,749,768	
Closing Cash & Bank Balance				
THSTI	**26,840,781**		**12,808,651**	
Projects	**206,745,334**		**88,638,457**	
Fellowship	**(109,517,323)**		**11,115,286**	
Total		**601,148,915**		**732,562,646**

As per our separate Report of
even date attached
For S.M.Saini & Associates
Chartered Accountants

Sd/-	Sd/-	Sd/-
(C. B. YADAV)	(Dr. G.B. NAIR)	(LAXMIKANT SAINI)
Finance & Accounts Officer	Executive Director	Partner

Place : Faridabad
Date : 24th October, 2015

S.M.SAINI &ASSOCIATES
Chartered Accountants

120, Mohyal Colony, B/H MMISchool,
Sector-40, Gurgaon-122001
Ph.09310832563, 09868275687, 0124-2381062
laxmikant_saini84@yahoo.co.in

AUDITORS' REPORT

To
Director
TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE
Faridabad

1. We report that we have audited the Balance Sheet of "TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE"as at 31 March 2015 and the relative Income &Expenditure Account& Receipt & Payment Accountfor the period ended on that date, annexed thereto. These financial statements are responsibilities of the society management. Our responsibility is to express an opinion on these financial statements based on our audit.
2. We conducted our audit in accordance with auditing standards generally accepted in India. These standards require that we plan and perform the audit to obtain reasonable assurance about whether the financial statements are free of material misstatement. An audit includes examining, on a test basis, evidence supporting the amounts and disclosures in the financial statements. An audit also includes assessing the accounting principles used and significant estimates made by management, as well as evaluating the overall financial statement presentation. We believe that our audit provides a reasonable basis for our opinion.

3. Further to our comments as follows , we report that:

   (a). We have obtained all the information and explanations, which, to the best of our knowledge and belief, were necessary for the purpose of our audit.
   (b). In our opinion, proper books of account have been kept as required by law so far, as appears from our examination of those books.
   (c). The Balance Sheet and Income & Expenditure Account and Receipt & Payment Account dealt with by this report are in agreement with the books of accounts.
   (d). In our opinion, the balance sheet and the Income & Expenditure account and Receipt & Payment Account dealt with by this report comply with the Accounting Standards issued by the Institute of Chartered Accountant of India, to the extent applicable.
   (e). In our opinion and best to the information and according to the explanation given to us, the said account a true and fair view in conformity with the accounting principles generally accepted in India

      i. In the case of the balance sheet, of the state of affairs of the Institute as at 31st March 2015; and

      ii. In the case of Receipt & Payment Account, of the receipt for the period ended on that date

      iii. In the case of Income and expenditure Account, excess of Income over income for the period ended on that date.

ForS.M.Saini & Associates
Chartered Accountants

Laxmikant Saini
Partner

Organizational Support System at THSTI

Achieving Operational Excellence

External Relations & Institutional Development (ERID)

Context & Conceptual Framework

A consolidated support system for research & innovation related activities, is of critical importance for the success of an organization like THSTI, especially in an era of fast scientific advancement and continuous information exchange. Regardless of organizational model, a support system with defined responsibilities of facilitating research and innovation is an asset to any ambitious organization, more so for THSTI, because of its intrinsic nature of a dynamic, futuristic and translational setting. This support system can establish an effective centralized mechanism to unite all upstream and downstream activities and connect it to the developmental process of the organization.

Goals/ Objectives

- To create a conducive system of innovation management
- To create a transparent machinery of proper implementation of biosafety, environmental safety, animal & human ethics.
- To create a support system for extramural fund generation and build effective investor relations.
- To create an effective communication to enhance the profile of the organization.

Processes at ERID

Innovation Management

ERID will develop and establish IP and technology transfer policy for THSTI. As a support system to the THSTI scientific community it will help in various IP management activities.

- Patent activities:
- Carry out IP portfolio analysis for THSTI's flagship programs
- IP landscaping and Freedom-to-Operate (FTO) analysis support
- Support in patent drafting, filing, follow up
- Licensing & tech transfer activities:
- Develop confidentiality agreements and maintaining records
- Support in finding industry partner for licensing
- Help in licensing and technology transfer activities
- Help in protecting IP and revenue sharing

Grants and Investor Relations

This function of ERID identifies new grant opportunities and keeps the faculty and scientists updated with various available options. The office aids in preparing grant applications to formats specified by funding agencies. It also supports the THSTI Faculty with registrations mandatory for grant

submissions. It will also develop mechanisms to make THSTI's identity more impactful to the investors.

- Grants management:
- Providing information on grants opportunities
- Supporting in grant application procedures
- Maintaining updates of grant application status
- Investor relations:
- Networking with investors, both from public and private space
- Arranging meetings and workshops to increase communication between investigators and investors

Statutory Committees

This function will facilitate faculty and scientists at THSTI to meet ethical and regulatory requirements to of their research. Guidelines will be developed for biosafety, chemical safety, animal ethics and human ethics in research. All research protocols will be cleared through the secretariat for review by the various statutory committees. The secretariat will organize committee meetings, will keep relevant records as per the regulatory requirements and update faculty on new regulations.

- Institutional Biosafety Committee:
- Guiding and supervising biosafety norms in institutional research activities
- Environmental safety committee
- Guiding and supervising chemical, electrical and other safety concerns
- Animal Ethics Committee:
- Guiding and maintaining ethical practice for animal studies in translational research
- Human Ethics Committee:
- Guiding and maintaining ethical practice in translational research involving human subjects

Communications

The Communication Unit will help achieve greater visibility and impact by means of various communication solutions. It will also develop mechanisms of effective internal communication among members of the research community at THSTI. Many of the Centre's publications, including the Annual Report, the quarterly newsletter, working papers, scientific reports, monographs, and special publications, will be developed, edited and produced by ERID.

- Internal Communications:
- Continuous communication with researchers and students at THSTI to engage them in organizational development
- Develop mechanisms for internal communications regarding events, publications, awards, etc.
- External Outreach Programs:
- Arrange outreach programs to raise the profile of THSTI at universities, public forums, etc.
- External Scientific Liaison:
- Build external scientific liaison of THSTI for all its major National and International Science and Technology partners

Develop and maintain an effective and dynamic relationship with the ministries, departments, councils, state bodies, NGOs, public and private

funding agencies, venture capital farms, private industries and policy makers

- Print & Electronic Media & Web Communications:
- Preparation of write-up/ features/ reports on THSTI activities and achievements for website and media coverage
- Design, development and release of special corporate briefings/brochures to help bridge the gap between academia & industry
- Preparation of Annual Report to aptly depict THSTI's unique vision and talent pool
- Improve THSTI website to enhance the brand value of THSTI to national and international scientific community

ERID Profiles

Ms.Vidhya Krishnamoorthy has a Post Graduate degree in Biotechnology followed by over a decade of academic research training in the field of Bacterial Pathogenesis from Madurai Kamaraj University, University of Texas at Houston and Harvard University. She is a non-voting member of the Ethics Secretariat at THSTI and by participating in ethics training conducted in the country she helps the investigators at THSTI to prepare appropriate regulatory documents to conduct research involving humans or human biological materials. She is responsible for organizing the Institutional Ethics Committee (Human Research) and animal ethics committee meetings, maintaining the records and updating the faculty on recent regulations in research involving humans and animals.

Dr. Susmita Chaudhuri has a PhD in Microbiology from National Institute of Cholera and Enteric Diseases, Kolkata. She did her M.Sc. in Zoology specializing in Microbiology from Calcutta University. She did her postdoctoral research in Medical Microbiology and Immunology from University of Alberta, Canada. She had a successful portfolio of bio-similar products as a Senior Research Scientist in the R&D of Panacea Biotec Ltd. She has a diploma in IPR and an MBA in Market Research. She specializes in technology due diligence and management. In the Innovation Management domain of ERID, she developed the Intellectual Property policy and Entrepreneurship policy of THSTI. She has initiated and developed a process of IP protection and utilization in THSTI and takes care of all the IP and technology transfer activities and IP awareness training for students and fellows.

THSTI Committees (2014-15)

S. No.	Committee	Members
01.	**THSTI Management Committee**	a. Dr. G.B. Nair b. Dr. Sudhanshu Vrati c. Dr. Shinjini Bhatnagar d. Dr. Kanury Rao e. Dr. Sudhakar Bangera f. Mr. M.V. Santo **Chairperson-Dr. G.B. Nair**
02.	**Academic Committee**	a. Dr. Sudhanshu Vrati b. Dr. Shinjini Bhatnagar c. Dr. Kanury Rao d. All convenors of the sub-committees formed by the Chairperson e. Mr. M.V. Santo f. Mr. J.N. Mishra **Chairperson - Dr. Sudhanshu Vrati**
03.	**Maintenance Committee**	a. Dr. Milan Surjit b. Dr. Bhabatosh Das c. Dr. Jonathan Pillai d. Dr. Rajkumar Haldar e. Dr. Sankar Bhattacharyya f. Dr. Savit B Prabhu g. Dr. Tripti Srivastava h. Mr. G R Agarwal i. Mr. Vishal Gupta j. Mr. Pitambar Behera k. Mr. Deepak Baghele **Chairperson – Dr. Milan Surjit / Dr. Bhabatosh Das**
04.	**Purchase Committee**	a. Dr. Ramandeep Singh b. Dr. Uma Chandra Mouli Natchu c. Dr. Samrat Chatterjee d. Dr. Ranjith Kumar C.T. e. Dr. Niraj Kumar f. Dr. Kaushik Bharati g. Mr. C.B. Yadav (In his absence Mr. Pitambar Behera) h. Mr. Mohd. Shahid **Chairperson- Dr. Ramandeep Singh / Dr. UC Mouli Natchu**

S. No.	Committee	Members
05.	**Faridabad Campus Development committee**	a. Dr. Shinjini Bhatnagar b. Dr. Guruprasad Medigeshi c. Mr. M.V. Santo d. Dr. Krishna Mohan Atmakuri e. Dr. Amit Awasthi f. Dr. Gaurav Batra g. Dr. Jonathan Pillai h. Dr. Shailaja Sopory i. Dr. Sushmita Chaudhuri j. Dr. Pallavi Kshetrapal k. Dr. Bratati Mukhopadhyay l. Mr. G.R. Agarwal m. Mr. C.B. Yadav n. Mr. Mohd. Shahid **Chairperson- Dr. Shinjini Bhatnagar/ Dr. Guruprasad Medigeshi**
06.	**IT & Communication Committee**	a. Dr. Bhabatosh Das b. Mr. M. V. Santo c. Dr Mona Duggal d. Dr. Deepak Sharma e. Dr. Sushmita Chaudhuri f. Dr. B. Debkumari g. Mr. Irudayaraj M h. Mr. Koushik Chatterjee **Chairperson – Dr. Bhabatosh Das/ Mr. M. V. Santo**
07.	**Human ethics committee**	a. Dr. Rakesh Lodha b. Dr. Madhulika Srivastava c. Mr. D. Raghunandan d. Mr. Rahul P. Dave e. Dr. Vineet Ahuja f. Dr. Ujjayini Ray g. Dr. Sivaram Mylavarapu h. Mr. M.V. Santo i. Dr. Nitya Wadhwa **Chairperson-Dr. Rakesh Lodha** **Ms Vidhya (Co-ordinator)**
08.	**Animal ethics committee**	a. Dr. Sudhanshu Vrati b. Dr. Amit Awasthi c. Dr. Amit Pandey d. Dr. Krishnamohan Atmakuri e. Dr. Niraj Kumar f. Dr. Natarajan j. Maj. Gen. Dhillon **Chairperson – Dr. Sudhanshu Vrati** **Ms Vidhya (Co-ordinator)**

S. No.	Committee	Members
09.	**Biosafety Committee**	a. Dr. Sudhanshu Vrati b. Dr Sushmita Chaudhuri c. Dr Nisheeth Agarwal d. Dr Shailaja Sopory e. Dr Vinay Kumar Nandicoori f. Dr Uma Chandra Mouli Natchu g. Dr. Anirban Basu **Chairperson – Dr. Sudhanshu Vrati**
10.	**RTI Act**	a. Dr Nisheeth Agarwal – PIO b. Dr Shinjini Bhatnagar – Appellate Authority c. Dr Sudhanshu Vrati – Transparency Officer d. Mr M.V. Santo – Nodal Officer e. Dr. G. B. Nair – Public Authority
11.	**Complaints Committee (to enquire into complaints of sexual harassment)**	a. Dr. Shinjini Bhatnagar b. Dr. Shobha Broor (external member) c. Dr Nita Bhandari d. Dr. Manjula Kalia e. Dr. Monika Bahl f. Mr. M. V. Santo **Chairperson – Dr. Shinjini Bhatnagar**
12.	**Cafeteria Committee:**	a. Dr. Amit Kumar Pandey b. Dr. Gaurav Batra c. Dr. M. B. Appaiahgari d. Dr. Saikat Boliar e. Mr. J. N. Mishra f. Mr. Prashant Bhujbal **Chairperson – Dr. Amit Kumar Pandey / Dr. Gaurav Batra**
13.	**Student Welfare and Hostel Committee**	a. Dr. Milan Surjit b. Dr. Nitya Wadhwa c. Dr. Ramandeep Singh d. Dr. Manjula Kalia e. Dr. Arup Banerjee f. Dr. Sucheta Kurundkar g. Mr. J. N. Mishra h. Two students' representatives **Chairperson – Dr. Milan Surjit / Dr. Nitya Wadhwa**
14.	**Tender Opening Committee**	a. Mr. P. Behera b. Mr. G.R. Agarwal c. Mr. Deepak Baghele
15.	**Vigilance Officer**	Dr. Guruprasad R. Medigeshi

People at THSTI as on 31st March 2015

S.No.	Name	Designation
Faculty and Scientists		
1	Dr. G.B. Nair	Executive Director
2	Dr. Sudhanshu Vrati	Dean (Academics) and Head-VIDRC
3	Dr. Shinjini Bhatnagar	Professor and Dean (Clinical Research) & Head - PBC
4	Dr. Kanury Venkata Subba Rao	Adjunct Faculty & Head - DDRC
5	Dr. Guruprasad R. Medigeshi	Associate Professor
6	Dr. Ramandeep Singh	Associate Professor
7	Dr. Nisheeth Agarwal	Assistant Professor
8	Dr. Amit Kumar Pandey	Assistant Professor
9	Dr. Krishnamohan Atmakuri	Assistant Professor
10	Dr. Milan Surjit	Assistant Professor
11	Dr. Amit Awasthi	Assistant Professor
12	Dr. Uma Chandra Mouli Natchu	Assistant Professor
13	Dr. Bhabatosh Das	Assistant Professor
14	Dr. Gaurav Batra	Assistant Professor
15	Dr. Samrat Chatterjee	Assistant Professor
16	Dr. Jonathan D. Pillai	Assistant Professor
17	Dr. Rajkumar Halder	Scientist E
18	Dr. Sanjay K. Banerjee	Scientist E
19	Dr. Manjula Kalia	Research Scientist D
20	Dr. Arup Banerjee	Research Scientist D
21	Dr. Mohan Babu Appaiahgari	Research Scientist D
22	Dr. Sankar Bhattacharyya	Research Scientist D
23	Dr. Nitya Wadhwa	Scientist D
24	Dr. Shailaja Sopory	Scientist D
25	Dr. Ashutosh Tiwari	Research Scientist C
26	Dr. Niraj Kumar	Research Scientist C
27	Dr. Susmita Chaudhuri	Research Scientist C
28	Dr. Amit Kumar Yadav	Scientist C
29	Dr. Savit B. Prabhu	Scientist C
30	Dr. Shailendra Asthana	Scientist C
31	Dr. Shilpa Jamwal	Scientist C
32	Dr. Ranjith Kumar C.T.	Ramalingaswami Fellow
33	Dr. Pallavi Kshetrapal	Ramalingaswami Fellow
34	Dr. Sangeeta Kumari	INSPIRE Faculty
35	Dr. Sagarika Haldar	INSPIRE Faculty
36	Dr. Sameena Khan	INSPIRE Faculty
37	Dr. Rana Pratap Singh	Scientist B (Medical)
38	Dr. Supratik Das	Senior Scientist
39	Dr. Huma Qureshi	Senior Scientist
40	Dr. Shubbir Ahmed	Senior Scientist
41	Dr. Saikat Boliar	Scientist

S.No.	Name	Designation
42	Dr. Tripti Shrivastava	Scientist
43	Dr. Sweety Samal	Scientist
44	Dr. Rajesh Kumar	Scientist
45	Dr. Bratati Mukhopadhyay	Sr. Program Officer
46	Dr. Sanjukta Sengupta	Sr. Program Officer
47	Dr. Gautam Kumar Saha	Program Officer
48	Ms. Vidhya Krishnamoorthy	Professional Expert (Grants & Ethics)
49	Dr. Pawan Mehrotra	Professional Expert

	Research Staff	**Designation**
1	Ms. Nisha Arora	Junior Analyst
2	Ms. Swati Verma	Junior Analyst
	Research Fellows	
1	Dr. Vikas Sood	VRI-Awardee
2	Dr. Atoshi Banerjee	VRI Awardee
3	Ms. Reena Kumari	Microbiome Innovation Award
4	Dr. Anurag Sankhyan	Innovation Awardee (Diagnostics)
5	Dr. Subham Banerjee	Innovation Awardee (Devices)
6	Dr. Tarun Kumar Sharma	Innovation Awardee
7	Dr. Chandresh Sharma	Innovation awardee
8	Dr. Prabhakar Tiwari	Research Associate
9	Dr. Rajat Anand	Research Associate
10	Dr. Mukul Kumar Midha	Research Associate
11	Dr. Varshneya Singh	Research Associate
12	Mr. Rajpal	Research Associate
13	Dr. Suchitra Devi Gopinath	Research Associate
14	Dr. Suprit Deshpande	Research Associate
15	Dr. Kamlesh Gidwani	Post-Doctoral Fellow
16	Dr. Ram Krashan Kasera	Post-Doctoral Fellow (Indo – finish)
17	Dr. Sheikh Mohd. Talha	Post-Doctoral Fellow (Indo – finish)
18	Dr. Parvez Syed	Post Doctoral Fellow (Indo – finish)
19	Dr. Muzamil Yaqub Want	Post Doctoral Fellow
20	Ms. Eira Chaudhary	Sr. Research Fellow
21	Ms. Garima Arora	Sr. Research Fellow
22	Ms. Deepa Nair	Sr. Research Fellow
23	Ms. Shruti Saxena	Sr. Research Fellow
24	Mr. Deepak Rohila	Sr. Research Fellow
25	Ms. Renu Khasa	Sr. Research Fellow
26	Mr. Nishant Joshi	Sr. Research Fellow
27	Mr. Rahul Sharma	Sr. Research Fellow
28	Ms. Pratishtha Jain	Sr. Research Fellow
29	Ms. Vidya P. Nair	Sr. Research Fellow
30	Mr. Ramu Adela	Sr. Research Fellow
31	Ms. Neha Kaushik	Sr. Research Fellow

S.No.	Name	Designation
32	Ms. Shilpi Gupta	Sr. Research Fellow
33	Ms. Ojasvi	Research Fellow
34	Mr. Pawan Kumar	Research Fellow
35	Ms. Nidhi Vishnoi	Jr. Research Fellow
36	Mr. Sankalp Srivastava	Jr. Research Fellow
37	Ms. Sheba Solomon	Jr. Research Fellow
38	Ms. Mayanka Dayal	Jr. Research Fellow
39	Ms. Abhilasha Madhvi	Jr. Research Fellow
40	Mr. Srikanth Sadhu	Jr. Research Fellow
41	Ms. Archana Pant	Jr. Research Fellow
42	Ms. Anica Dadwal	Jr. Research Fellow
43	Mr. Manitosh Pandey	Jr. Research Fellow
44	Ms. Poulami Dasgupta	Jr. Research Fellow
45	Ms. Saimah Raza	Jr. Research Fellow
46	Mr. Ramendrapati Pandey	Jr. Research Fellow
47	Mr. Parmeshwar Katare	Jr. Research Fellow
48	Mr. Arjun	Jr. Research Fellow
49	Ms. Sapna Jain	Jr. Research Fellow
50	Ms. Indu Bisht	Jr. Research Fellow
51	Ms. Ekta Dhamija	Jr. Research Fellow
52	Mr. Raubins Kumar	Jr. Research Fellow
53	Mr. Dikshat Gopal Gupta	Jr. Research Fellow
54	Ms. Apeksha Bhalla	Jr. Research Fellow
55	Mr. Ravi Jain	Jr. Research Fellow
56	Ms. Arti Kataria	Jr. Research Fellow
57	Ms. Juhi Sharma	Jr. Research Fellow
58	Mr. Ajay Akhade Suresh	Jr. Research Fellow
59	Mr. Prasanta Dey	Jr. Research Fellow
60	Mr. Prashanta Kumar Deb	Jr. Research Fellow
61	Ms. Manu Kandpal	Jr. Research Fellow
62	Mr. Chakit Arora	Jr. Research Fellow
63	Ms Diksha Awadhesh Varma	Jr. Research Fellow
64	Mr. Chirantan Debnath	Jr. Research Fellow
65	Ms. Arti Jatwani	Jr. Research Fellow
66	Mr. Bharath Kumar N.	Jr. Research Fellow
67	Mr. Savera Aggarwal	Jr. Research Fellow
68	Mr. Subrata Mondal	Jr. Research Fellow
69	Ms. Sheetal Kaul	Jr. Research Fellow
70	Ms. Akanksha Srivastava	Jr. Research Fellow
71	Mr. Sumit Kumar Sharma	SIIP Fellow
72	Ms. Lakshita	SIIP Fellow
73	Ms. Aparna Rani	SIIP Fellow
74	Ms. Shweta Roy Chowdhury	SIIP Fellow

S.No.	Name	Designation
	Research Students	
1	Ms. Preeti Thakur	Ph.D. Student
2	Ms. Minu Nain	Ph.D. Student
3	Mr. Manish Sharma	Ph.D. Student
4	Mr. Nishant Sharma	Ph.D. Student
5	Ms. Bhavya Khullar	Ph.D. Student
6	Ms. Rinki Kumar	Ph.D. Student
7	Mr. S. Chandru	Ph.D. Student
8	Ms. Praapti Jayaswal	Ph.D. Student
9	Ms. Tarang Sharma	Ph.D. Student
10	Ms. Sakshi Aggarwal	Ph.D. Student
11	Ms. Saumya Anang	Ph.D. Student
12	Ms. Nidhi Kaushik	Ph.D. Student
13	Ms. Bharti Kumari	Ph.D. Student
14	Ms. Anita Chaudhary	Ph.D. Student
15	Ms. Meenakshi Kar	Ph.D. Student
16	Ms. Sakshi Malik	Ph.D. Student
17	Ms. Sakshi Talwar	Ph.D. Student
18	Ms. Shilpi Sehgal	Ph.D. Student
19	Ms. Smita S. Hingane	Ph.D. Student
20	Mr. Ajitesh Harihar Lunge	Ph.D. Student
21	Ms. Deepika Chaudhary	Ph.D. Student
22	Ms. Hina Lateef Nizami	Ph.D. Student
23	Ms. Jyoti Singh	Ph.D. Student
24	Ms. Kiran Bala	Ph.D. Student
25	Mr. Naseem Ahmed Khan	Ph.D. Student
26	Ms. Niti Singh	Ph.D. Student
27	Mr. Ashok Kumar	Ph.D. Student

	Administration Personnel	
1	Dr. G.B. Nair	Executive Director
2	Mr. M.V. Santo	Head-Administration
3	Mr. C.B. Yadav	Administrative Officer (F&A)
4	Mr. J. N. Mishra	Administrative Officer (P&A)
5	Mr. Mohd. Shahid	Section Officer
6	Mr. Deepak Bhagirath Baghele	Section Officer (Store & Purchase)
7	Mr. Shiv Kumar	Management Assistant (P&A)
8	Ms. Rajni Verma	Management Assistant (P&A)
9	Mr. Alok Kumar Gupta	Management Assistant (F&A)
10	Mr. Manoj Kumar	Management Assistant (F&A)
11	Mr. Satish Kumar	Management Assistant (S & P)
12	Md. Arif Saifi	Management Assistant
13	Mr. Radhesh Notiyal	Executive Management Assistant
14	Mr. Maneesh Kumar Sharma	Program Manager
15	Mr. Hanumantha Rao S	Personal Assistant
16	Ms. Greeshma J.	Executive Secretary

S.No.	Name	Designation
17	Ms. Taruna Sharma	Programmer
18	Mr. Dharmendra Sharma	Programmer
19	Mr. Mukesh Juyal	Data Entry Operator
20	Ms. Shilpa Chopra	Data Entry Operator
21	Mr. Rahul Kumar Chauhan	Data Entry Operator
22	Mr. Ali Baksh	Data Entry Operator
23	Mr. Raj Kumar Tanwar	Data Entry Operator
24	Mr. Rakesh Kumar	Data Entry Operator
25	Ms. Aakriti Sinha	Executive Assistant
26	Ms. Upasana Sharma	Executive Assistant
27	Mr. Rahul	Clerical Assistant
28	Mr. Lalit Kumar	Clerical Assistant
29	Ms. Priyanka Kapoor	Clerical Assistant
30	Mr. Amit Kumar	Clerical Assistant
31	Mr. Pradeep Jakhar	Clerical Assistant
32	Mr. Ranjan Kohli	Clerical Assistant
33	Ms. Kriti Kohli	Clerical Assistant
34	Ms. Nidhi Yadav	Clerical Assistant
35	Mr. Sudhanshu Apat	Clerical Assistant
36	Ms. Sweety Jain	Accounts Assistant
37	Ms. Reena Pal	Accounts Assistant
38	Mr. Rinku Gurahiya	Accounts Assistant
39	Ms. Deepika Kanaujia	Front Office Executive

	Technical Personnel	
1	Mr. Gopal Raman Agarwal	Inst. /Elec. Engineer
2	Dr. Manpreet Kaur	Vaccine Technologist
3	Mr. Vishal Gupta	Sr. Technical Officer
4	Mr. Irudayaraj M.	Sr. Technical Officer (IT)
5	Mr. Pradeep Kumar	Technical Officer
6	Mr. Sharanabasava	Asstt. Vaccine Technologist
7	Ms. Taranjeet Kaur	Asstt. Vaccine Technologist
8	Dr. Madhu Pareek	Technical Officer I
9	Ms. Sonali Porey Karmakar	Technical Officer I
10	Mr. Saqib Kidwai	Technical Officer I
11	Mr. Uttam Kumar Saini	Technical Assistant
12	Mr. Gaurav Singh	Technical Assistant
13	Ms. Nidhi Sharma	Technical Assistant
14	Mr. Saurabh Vaishnav	Technical Assistant
15	Mr. Anbumani D.	Technical Assistant
16	Mr. Eklavya Srivastava	Technical Assistant
17	Mr. Abhishek Sharma	Technical Assistant
18	Mr. Babu Mathew P.	Technical Assistant
19	Mr. Suresh Kumar	Technical Assistant
20	Mr. Roshan Kumar	Technical Assistant (Lab)
21	Mr. Sitesh Jana	Technical Assistant (Lab)

S.No.	Name	Designation
22	Ms. Neeta Rai	Technology Management Specialist
23	Ms. Bipasa Saha	Technical Officer – II
24	Mr. Satyabrata Bag	Technical Officer II
25	Mr. Sandeep Singh	Lab Technician
26	Mr. Imran Khan	Lab. Technician
27	Mr. Ranjeet Rai	Lab. Technician
28	Mr. Chandra Prakash Bhaskar	Lab Technician
29	Mr. Subhash Chandra Tanwar	Lab Technician
30	Mr. Srikant Kumar	Lab Technician
31	Mr. Sandeep Goswami	Lab. Technician
32	Mr. Ashish Kumar Tyagi	Lab. Technician
33	Mr. Naresh Kumar	Lab Technician
34	Mr. Naveen Kumar	Lab Technician
35	Ms. Sangita Kumari Sinha	Lab Technician
36	Mr. Manas Ranjan Tripathy	Lab Technician
37	Mr. Manish Bansal	Lab Technician
38	Ms. Shilpa Shivanand Patil	Lab Technician
39	Ms. Deepika Kannan	Lab Technician
40	Mr. Ananta Kumar Saha	Lab Technician
41	Mr. Shailesh Kumar	Lab Attendant
42	Mr. Pooran Singh	Lab Attendant
43	Mr. Manoj Mahato	Technician - II
44	Mr. Shri Chand Pandey	Technician - II
45	Dr. Venkatasamy Manivel	Consultant (Mass Spectrometry)

	Clinical Personnel	
1	Dr. Poonam Yadav	Consultant (Radiologist)
2	Dr. Piyush Jain	Clinical Research Coordinator
3	Dr. Neha Mehta	Sr. Resident
4	Ms. Nisha Piplani	Sr. Resident
5	Dr. Mahadev Dash	Senior Research Officer
6	Dr. Kanika Sachdeva	Senior Research Officer
7	Dr. Sumit Misra	Research Officer
8	Dr. Shubhra Agarwal	Research Officer
9	Dr. Deepak K. Rathore	Research Officer
10	Dr. Chitrangada Mistry	Jr. Resident
11	Mr. Swapnil Rajvanshi	Jr. Resident
12	Dr. Ravinder	Jr. Resident
13	Ms. Sushmita Kumari	Study Nurse
14	Ms. Neha Thakur	Study Nurse
15	Ms. Rinki	Study Nurse
16	Ms. Kumari Meera	Study Nurse
17	Ms. Suman Rawat	Study Nurse
18	Ms. Renu Gehlawat	Study Nurse
19	Ms. Sangeeta Singh	Study Nurse

S.No.	Name	Designation
20	Ms. Jyoti Sharma	Study Nurse
21	Ms. Khushboo	Study Nurse
22	Ms. Dimple Kumari	Study Nurse
23	Ms. Sheetal	Study Nurse
24	Ms. Swati	Study Nurse
25	Ms. Sonia Rani	Study Nurse
26	Ms. Gurdeep Bhamra	Study Nurse
27	Ms. Manisha Sharma	Study Nurse
28	Ms. Harshlata Dayal	Study Nurse
29	Ms. Jyoti Rani	Study Nurse
30	Ms. Moumita Maity	Study Nurse
31	Mohd. Usman Khan	Technical Assistant
32	Mr. Ajay Kumar	Technical Assistant
33	Dr. Prashant Gupta	Technical Assistant (Field)
34	Mr. Ramgopal	Field Assistant
35	Mr. Srikant Dimri	Technician – II
36	Mr. Kapil Dev	Technician - II
37	Ms. Ritu Rani	Technician - II
38	Ms. Aasma Khan	Technician - II
39	Mr. Ashok Saini	Technician - II
40	Mr. Brij Mohan	Technician - II
41	Mr. Vijay	Technician - II
42	Mr. Parveen Kumar	Technician – II
43	Mr. Ankit Dogra	Technician – II
44	Mr. Rakesh Kumar	Technician - II
45	Mr. Devendra Kumar	Technician
46	Mr. Dinesh Kumar	Technician
47	Mr. Premveer Singh	Technician
48	Mr. Basant Kumar Nivoriya	Technician
49	Mr. Naresh Kumar	Technician
50	Ms. Vaishanvi Rai	Technician
51	Mr. Ravi Kaushik	Technician
52	Mr. Shahbaz Ahmad	Technician
53	Mr. Dev Kumar Sharma	Technician
54	Mr. Abdul Kalam Khan	Technician
55	Dr. Richa Mehra	Site Manager
56	Ms. Deepika Yadav	Supervisor
57	Mr. Amanpreet Singh	Project Manager
58	Ms. Bharati Khatwani	Data Manager
59	Mr. Gaurav Khurana	Project Assistant
60	Mr. Murari U.	Statistical Assistant

S.No.	Name	Designation
CDSA Personnel		
1	Sudhakar Bangera	Program Director
2	Monika Bahl	Director (Clinical) Posrtfolio Management
3	Sucheta Banerjee Kurundkar	Director, Training
4	Pawandeep Kaur	Associate Medical Director
5	Prashant Bhujbal	Finance Manager
6	Sanjeeva Kumar	Administrative Manager
7	Rita Francis	Secretary to PD and HR Associate
8	Gayatri Vishwakarma	Biostatistician
9	Neha Mishra	Training Coordinator
10	Mahender Singh	IT Administrator
11	Jasmine Luke	Medical Writer (Level - I)
12	Vineeta Baloni	(Clinical) Database Designer
13	Deepika Sharma	Accounts Associate
14	Karma lhamo	Study Monitor
15	Rajat Sharma	Study Monitor
16	Divya Pillai	Senior Research Officer
17	Renuka Gahlawat	Quality Manager
18	Ligi Anil	Program Officer

Our Augmented Strength

Chair & Honorary Faculty

Biotechnology Chair

Prof. John David Clemens
Executive Director
International Centre for
Diarrhoeal Disease Research Dhaka, Bangladesh

Visiting Professor of Eminence

Prof. N. K. Ganguly

Honorary International Visiting Faculty

Dr. Madhukar Pai, MD, PhD
Associate Professor, McGill University
Associate Director, McGill International TB Centre.

Adjunct Faculty / Honorary Visiting Professor

Dr. Satyajit Rath
Sr. Scientist, National Institute of Immunology

Dr. Vineeta Bal
Sr. Scientist, National Institute of Immunology

Prof. Anil K. Tyagi
Vice Chancellor, Indraprasth University

Dr. Navin Khanna
Group Leader, ICGEB

Dr. Kanury Venkata Subba Rao
Head-DDRC

Dr. Nita Bhandari
Jt. Director, CHRD-SAS

Dr. Amit Sharma
Group Leader, ICGEB

Dr. Jaya Sivaswami Tyagi
Professor, Department of Biotechnology
All India Institute of Medical Sciences

Extramural Grants

(Rs. In Lakhs)

Title of the Project		Funding Agency	Total Approved Budget	Grant Received in F.Y 2014-15
	Name of the P.I			
Pediatrics Biology Centre (PBC)	Dr. Shinjini Bhatnagar	Department of Biotechnology	817.66	116.30
Centre for Biodesign and in-vitro Diagnostics (CBD) Ph1 & 2		Department of Biotechnology	2,749.88	1,103.85
HIV Vaccine Translational Research		Department of Biotechnology	2,078.90	386.06
Vitamin D supplementation to improve immune responses to vaccines administrated in early infancy- The NutriVac -D Trial	Dr. Uma Chandra Mouli Natchu	Department of Biotechnology	160.64	0.44
Development of a rapid diagnostic test for diagnosis of celiac disease - Phase I-II	Dr. Shinjini Bhatnagar	Department of Biotechnology	66.03	15.97
Investigating the role of MazF toxins in pathogenesis and persistence of Mycobacterium tuberculosis	Dr. Ramandeep Singh	Department of Biotechnology	33.49	0.13
Molecular Mechanisms of Minimal Change Disease Nephritic Syndrome : Role of CD 80	Dr. Shailaja Sopory	Department of Biotechnology	47.09	7.68
Collaboration for translational and clinical research between Translational Health Science and Technology Institute ,National Brain Research Centre, Regional Centre for Biotechnology and Gurgaon Civil Hospital	Dr. Shinjini Bhatnagar	Department of Biotechnology	211.29	-
Deciphering Mycobacterium Tuberculosis Artillery	Dr. Krishnamohan Atmakuri	Department of Biotechnology	64.60	24.55
Understanding the role of Polyphosphate Kinases and Ployphosphatases in Physiology of Mycobacterium Tuberculosis	Dr. Ramandeep Singh	Department of Biotechnology	49.48	16.71
Role of microRNAs in establishment of Japanese Encephalitis Virus (JEV) infection and Disease Progression -	Dr. Arup Banerjee	Department of Biotechnology	55.60	16.53
Policy Centre for Biomedical Research (PCBR)		Department of Biotechnology	712.00	44.91
Innovative Young Biotechnologist Award -IL-27 dependent regulation of TH 17 and regulatory cells 2011 (IYBA 2011)	Dr. Amit Awasthi	Department of Biotechnology	38.81	7.62
Human Microbial Ecology (CHME)	DR. G.B. Nair	Department of Biotechnology	2,355.00	22.00
Centre for Drug Discovery Research Centre (DDRC)	Dr. Kanury Rao	Department of Biotechnology	5,695.00	701.95
Characterization of Hepatitis E Virus RNA dependent RNA polymerase and its associated proteins in the replicase complex -Dr. Ranjith Kumar CT	Dr. Ranjith Kumar C.T	Department of Biotechnology	77.06	19.57
Genetic requirement of mycobacterium tuberculosis growth in cholesterol under hypoxia using high density mutagenesis RGYI -	Dr. Amit Kumar Pandey	Department of Biotechnology	46.00	0.18
To identify novel therapeutic compounds that inhibit the interaction between Hepatitis E Visas ORF 3 protein and TSG 101 and to explore the molecular mechanisms controlling the release Hepatitis E Virions from infected cells RGYI-	Dr. Milan Surjit	Department of Biotechnology	32.05	10.56
MicroDiab Studies of interaction between the gut Microbiome and the human host biology to elucidate novel aspects of the pathophysiology and pathogenesis of type 2 Diabetes	Dr. G.B. Nair	Department of Biotechnology	374.95	-

(Rs. In Lakhs)

Title of the Project		Funding Agency	Total Approved Budget	Grant Received in F.Y 2014-15
Technology Platform for Simple and efficient production of recombinant antibodies (Tech Sepra)	Dr. Gaurav Batra	Department of Biotechnology	63.76	18.23
Vitamin A is the "Microenvironmental cue" for triggering disease activity in patients with Inflammatory Bowel disease (Ulcerative colitis,Crohn's Disease)	Dr. Amit Awasthi	Department of Biotechnology	29.31	7.78
Neonatal immune profiles infections and toxicants	Dr. Satyajit Rath & Dr. Nitya Wadhwa	Department of Biotechnology	88.08	0.55
Inter Institutional program for Maternal Neonatal and infant sciences-A translational approach to studying preterm bity (PTB)	Dr. Shinjini Bhatnagar	Department of Biotechnology	2,922.71	301.22
Animal Facility for Research on Infectious Disease	Dr. Sudhanshu Vrati	Department of Biotechnology	1,828.19	145.18
HLA-G 5" URR genotyping in small for gestational age neonates compared to appropriate for gestational age neonates"" under IYBA 2013 grants - Dr. Pallavi Kshetrapal	Dr. Pallavi Kshetrapal	Department of Biotechnology	19.50	20.01
Large scale proteomics analysis of post-translational modifications and their crosstalks in cardiovascular dieases" under IYBA-2013 grants-	Dr. Amit Kumar Yadav	Department of Biotechnology	38.81	19.84
Laboratory assays for the phase-III trial for the non-interference of ORV116E	Dr. Sudhanshu Vrati	Department of Biotechnology	58.75	58.75
A system approach to analyze changes in global phophorylation status of protien in macrophages infected with Mycobacterium tuberculosis complex bacteria and their repercussions on mycobacterial virulence	Dr. Nisheeth Aggarwal	Department of Biotechnology	23.38	23.38
Human Gastrointestinal Immunology Translational Program	Dr. Amit Awasthi	Department of Biotechnology	46.10	15.32
Transcriptome analysis for identification of novel bimarker for disease progression in dengue patients	Dr. Arup Banerjee	Department of Biotechnology	56.00	57.51
Effect of viral infections on zinc homeostasis: focus on modulation of zinc transporters	Dr. Guruprasad Medigeshi	Department of Biotechnology	29.80	29.80
Understanding the distinct developmental and functional properties of the neonatal immune system and their clinical consequences in the neonatal period (HDDB Grant)	Dr. Shailaja Sopory	Department of Biotechnology	55.00	22.43
Identification of neutralizing antibody epitopes on Indian and South African HIV-1 subtype C Viruses for HIV vaccine design	Dr.Jayanta Bhattacharaya	Department of Science and Technology (DST)	13.42	13.42
Characterization of HIV -1 primary envelopes obtained from broadly cross neutralizing plasma for their degree of cleavage, sensitivity to neutralizing and non- neutralizing antibodies and immunogenicity	Dr.Jayanta Bhattacharaya	Department of Science and Technology (DST)	5.00	5.00
Cardio Protective effect of garlic: Role of nitric oxide and mitochondrial biogenesis	Dr. Sanjay Banerjee	Department of Science and Technology (DST)	32.00	7.22
Mathmatical approaches to understand host-pathogen cross-talk in mycobacterial pathogenesis	Dr. Samrat Chatterjee	Department of Science and Technology (DST)	13.00	4.70
Metabolism in mycobacteria: role of PPK-1 and PPK-2 in stationary phase survival and virulence of M. Tuberculosis	Dr. Ramandeep Singh	Department of Biotechnology	59.60	15.10
Immune function in infancy – special focus on low birth weight and small for date infants and the role of Vitamin D deficiency	Dr. Uma Chandra Mouli Natchu	Department of Biotechnology	72.85	-

(Rs. In Lakhs)

Title of the Project		Funding Agency	Total Approved Budget	Grant Received in F.Y 2014-15
Understanding the biology of Hepatitis E Virus and development of vaccine and drugs against it.	Dr. Milan Surjit	Department of Biotechnology	85.60	16.10
Regulation of Cholesterol Metabolism in Mtb	Dr. Amit Kumar Pandey	Department of Biotechnology	74.50	-
Mycobacterial outer membrane-derived vesicles: Role in pathogenesis and exploration as noval submit vaccine vehicles against tuberculosis.	Dr. Krishanmohan Atmakuri	Department of Biotechnology	74.50	16.10
The role of Notch synergies in pediatric T-Alls	Dr. Pallavi Kshetrapal	Department of Biotechnology	82.00	18.72
Modulation of innate immune response and characterization of viral polymerases for the development of potent vaccines.	Dr. Ranjith Kumar	Department of Biotechnology	85.60	16.08
DBT Fellowship to PhD students		Department of Biotechnology	5.54	5.54
Indo- Finland Post Doct Fellowship		Department of Biotechnology	223.68	19.09
INSPIRE Fellowship	Dr. Sangeeta Kumari	Department of Science and Technology (DST)	35.00	-
INSPIRE Fellowship	Dr. Sagarika Haldar	Department of Science and Technology (DST)	35.00	19.00
INSPIRE Fellowship	Dr. Sameena Khan	Department of Science and Technology (DST)	35.00	19.00
Welcome Trust Fellowship	Dr Amit Awasthi	Wellcome Trust	269.13	-
Welcome Trust Fellowship	Dr Guruprasad Medigeshi	Wellcome Trust	363.00	73.12
Establishment of a mammalian cell culture based hepatitis E Virus (HEV) expression system to study the viral life cycle and application of the secreted virion as a candidate vaccine-	Dr. Milan Surjit	Science and Engineering Research Board(SERB)	25.00	7.00
Integration and excision mechanism of integrative mobile genetic elements essential for Vibrio cholera pathogenicity (IMGE)	Dr. Bhabatosh Das	Science and Engineering Research Board(SERB)	17.00	2.10
Biotechnology Industry Partnership Programme (BIPP)	Dr. Sudhanshu Vrati	Biotechnology Industry Partnership Programme (BIPP)	1.96	1.96
Social Innovation Immersion Program (SIIP)	Dr. Uma Chandra Mouli Natchu	BIRAC	66.90	20.80
The HIB Initiative –Hospital based Sentinel Surveillance for Meningitis in Children	Dr. Shinjini Bhatnagar	The INCLEN Trust	10.39	3.28
IGSTC workshop on " Diagnostics and translational genome sequencing in clinical and public health microbiology		Indo-German Science & Technology Centre	1.07	1.07
World Health Organisation (WHO) -APW"- Dr. Shinjini Bhatnagar	Dr. Shinjini Bhatnagar	World Health Organization (WHO)	5.22	5.22
Novel Sample processing for the simple and rapid diagnosis of TB,MDR-TB and XDR-TB"(Phase-I)under SBIRI-	Dr. Sagarka Haldar	BIRAC	34.00	10.00
Host-Virus Interactions and Antibody Therapy for Japanese Encephalitis	Dr. Manjula Kalia	Indo French Centre	68.00	22.51

Seminars

Date	Topic	Speaker
19-12-2014	Host-directed therapies for controlling chronic Mycobacterium tuberculosis infection	Dr. Amit Singhal, Project Leader & Senior Research Scientist Tuberculosis Program, Singapore Immunology Network (SIgN)
25-11-2014	Modeling disease spread and control in farms.	Prof. Ezio Venturino, Dipartimento di Matematica Giuseppe Peano, Universita di Torino, Italy
27-11-2014	Abcam technical talk - Introduction to ChIP, Optimization of western blot, Optimization of IHC/ICC	Dr. Katarzyna Dudek, Senior Scientific Support Specialist, Abcam
20-08-2014	Effect of Type I Interferon on West Nile Virus specific CD8 T cells: An intricate phenomenon	Siddhartha Kumar Bhaumik, Ph.D., Research Associate, Dept. of Pediatrics (Infectious Diseases), School of Medicine, Emory University
19-08-2014	Xenogene silencing, stress response and chromosome architecture in E.	Dr. Aswin Sai Narain Seshasayee, Young Investigator, Faculty of Biochemistry, Biophysics and Bioinformatics, National Centre for Biological Sciences, Banglore
14-08-2014	Health Benefits and Microbiology of Some Ethnic Fermented Foods	Prof. Jyoti Prakash Tamang (Dean), School of Life Sciences, Department of Microbiology, Sikkim University
05-08-2014	West Nile Virus Pathogenesis: An Interplay Between Virus and Innate Immune Signaling Pathways	Dr. Saguna Verma, Assistant Professor, Dept. of Tropical Medicine, Medical Microbiology and Pharmacology , John A. Burns School of Medicine, University of Hawaii
10-07-2014	Intervention of bacterial and viral infection with probiotics	Dr. Palok Aich, Associate Professor, School of Biological Sciences, National Institute of Science Education and Research, Bhubaneswar
12-06-2014	Rapid Bionanomaterials Assembly and Bioassay Readout on a Chip	Dr. Shalini Gupta, Assistant Professor, Department of Chemical Engineering, Indian Institute of Technology Delhi
05-06-2014	Small molecules to control cell fate	Rajkumar Halder, Drug Discovery Research Center, THSTI
01-05-2014	Rapid lateral Flow based POC test for TB diagnosis	Dr. Suman Laal, Associate Professor, Department of Pathology, Research Career Scientist, VA Medical Center, New York
10-03-2014	BioMedical Image Analysis	Prof Alison Noble Technikos Professor or BioMedical Engineering Oxford University, UK
04-03-2014	Demo & Presentation of mst	Dr. Moran Jerabek, NanoTemper Technologies GmbH, Munich, Germany
31-01-2014	Understanding metabolic cooperation between tumor and stromal cells	Dr. Debu Banerjee Rutgers Cancer Institute of New Jersey Graduate School of Biomedical Sciences, Rutgers Biomedical Health Sciences The State University of New Jersey

Major International Collaborations

Indo-Finnish Collaboration

The goal of the Indo-Finnish Diagnostic Research Centre (IFDRC) is to complement and enhance the research capabilities of its Indian and Finnish scientific networks from academia and industry in the area of diagnostics. Through the IFDRC, the intention is to stimulate the exchange of both junior and advanced students and researchers between the countries around common collaborative endeavors and research topics. To create a sizable pool of researchers who specialize in development of diagnostics in India, the Department of Biotechnology has instituted five postdoctoral fellowships in the field of diagnostics for training in various Finnish Institutes for a period of two years.

Indo-Japanese Collaboration

Translational Health Science and Technology Institute and Osaka University mutual Ph.D. program in Biomedical Science at the Graduate School of Medicine, Osaka University, Japan

Translational health science and technology institute (THSTI) and Osaka University, Japan are jointly conducting interview to select bright Indian students for Ph.D. program at Osaka University, Japan under interdisciplinary program for biomedical sciences (IPBS). The program was initiated in the academic year 2013. In last two years four students were selected for the Ph.D. program. Details of the program, selection procedure, and schedule are published in THSTI official website as well as national newspapers in each year. Since its inception in 2013, the program is being coordinated by Dr. Bhabatosh Das, Assistant Professor, THSTI.

Indo-Norwagian Collaboration

There is a long standing partnership between scientists from Centre for International Health (CHN-CIH) University of Bergen and the investigators at PBC, THSTI. They are now a part of a consortium called 'Centre for Intervention Science in Maternal and Child Health' (CISMAC), which is being funded by the Research Council of Norway. It represents a unique opportunity for interaction between the Centre for International Health (CIH) at the University of Bergen (UiB), the Norwegian Institute of Public Health (NIPH), and the Christian Michelsen Institute (CMI), and the Society for Applied Studies (SAS) in India.

Events at THSTI

5th Foundation Day

Launch of Celiac Disease Kit

Hindi Saptah at THSTI

Swachh Bharat Abhiyan

ट्रांसलेशनल स्वास्थ्य विज्ञान
एवं प्रौद्योगिकी संस्थान

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