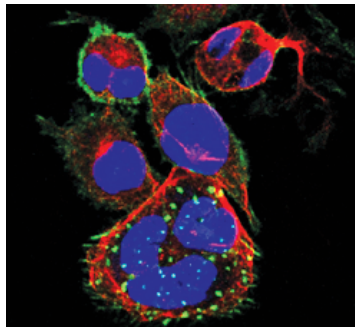
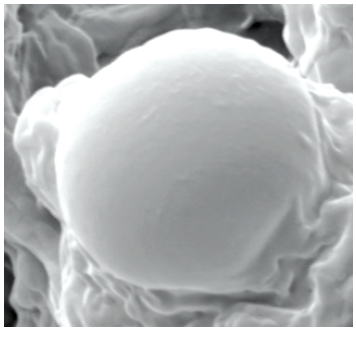
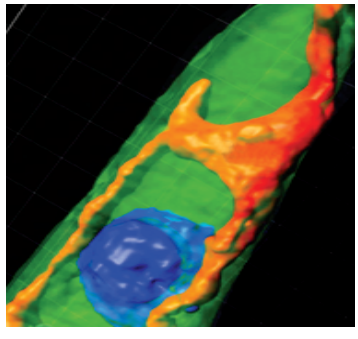
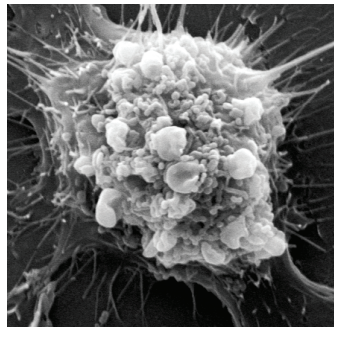
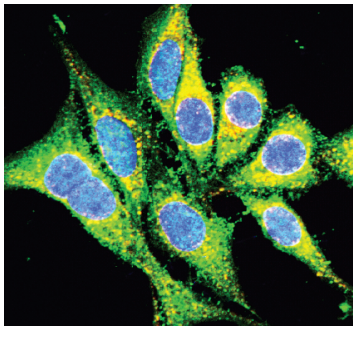
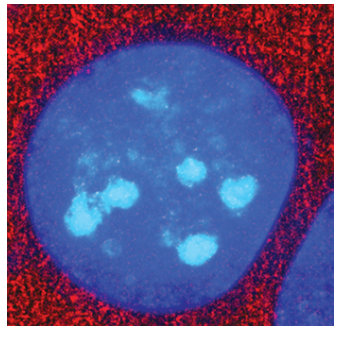
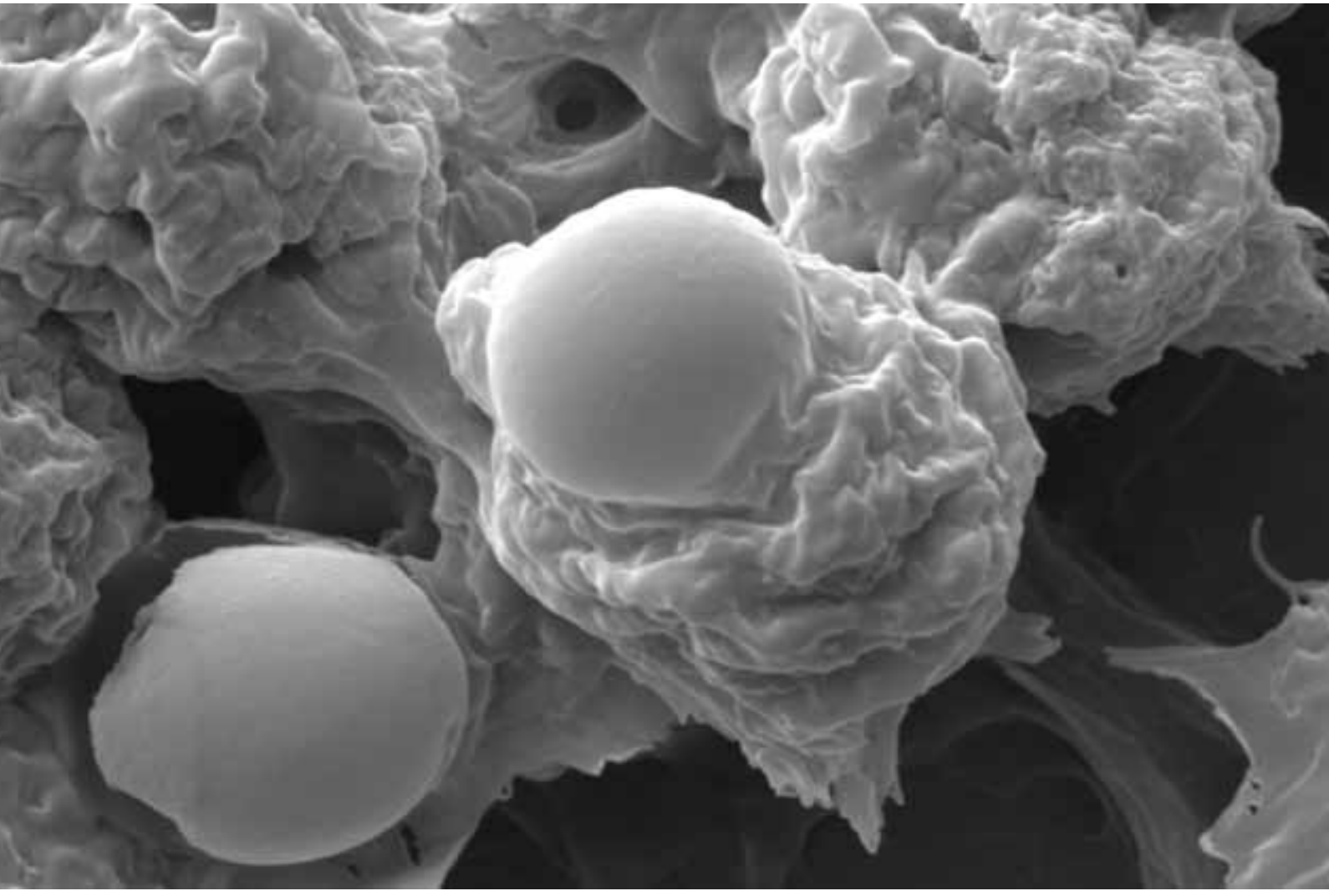
ANNUAL REPORT 2013-14

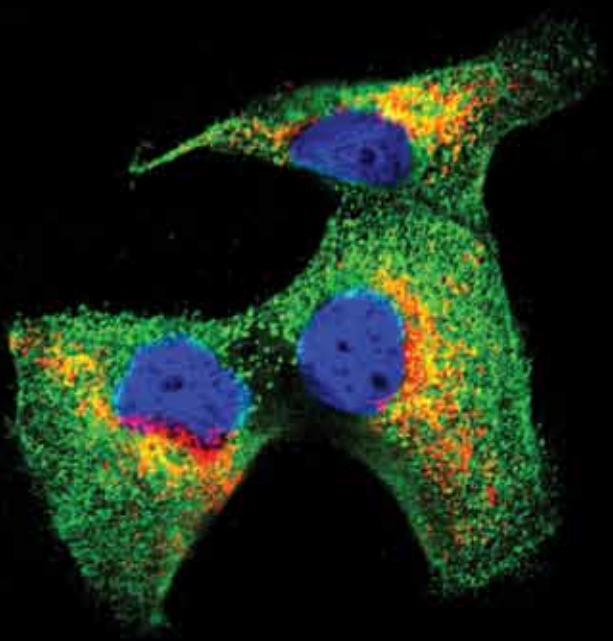







National Institute of Immunology



Macrophages interacting with polymer particles

CONTENTS



SPAG9 co-localization with endoplasmic reticulum in triple negative breast cancer cells

MANDATE OF THE INSTITUTE

- To undertake, aid, promote, guide and co-ordinate research of high caliber in basic and applied immunology.
- To carry out research for development of new vaccines and immunological reagents for communicable diseases.
- To develop immunological approaches for regulation of male and female fertility.
- To interact with industry for manufacture of vaccines and immunological reagents.
- To organise postgraduate courses, workshops, seminars, symposia and training programmes of a specialized nature in the field of immunology, vaccine development and related areas.
- To organise training programmes for technicians in immunological methods and related techniques.
- To establish affiliation with recognised universities and institutions of higher learning for the purpose of enabling research scholars to register for postgraduate degrees.
- To serve as a national reference centre for immunology and to provide consultancy services to medical and veterinary institutions, public health agencies and industries in the country.
- To provide and promote effective linkages on a continuing basis between various scientific and research agencies/laboratories and other organisations working in the country in the field of immunology, vaccine development and related areas.
- To collaborate with foreign research institutions, laboratories and other international organisations in fields relevant to the objectives mentioned above.

Amaltas in full bloom at NII campus

FOREWORD

I present the accomplishments of the National Institute of Immunology during the period 2013-14 with great pleasure.

As like all other years, the Institute witnessed significant achievements in various scientific frontiers. A sizeable number of faculty members received academic recognitions for their excellent contributions from a variety of sources including the scientific academies of the country showing appreciation within our peers in the academic arena. The scientists had a productive year with publications in many journals of repute; some of the important publications are summarized in later part of the foreword. The Institute continued its sensitivity towards societal needs by its endeavour to convert research leads to potential applications through generation of intellectual property and transfer of technology to the industry. During the current reporting year, one technology was transferred to the industry, nine patents were filed and five were granted. The Institute's efforts have been recognized by the Thomson Reuters India Innovation Award-2013 in the category of Pharma Academic and Government, an award that the institute is receiving for the second time. A total of eight bilateral and multilateral agreements for collaborative research have been executed between NII scientists and scientists at other academic organizations as well as the industry. The Institute joined the WIPO Re:Search Consortium that in partnership with the BIO Ventures for Global Health (BVGH) has facilitated collaboration with GlaxoSmithKline (GSK) to screen kinase inhibitors for drug development against malaria and tuberculosis.

The Institute continues to foster its doctoral, post-doctoral and training programmes in advanced biological sciences. The total number of Ph. D. students were 152 and 39 joined during the year. To inculcate highest level of aptitude and nuances in various aspects of research, for the first time, the Institute encouraged participation of the doctoral and post-doctoral students in the Research Area Panel meetings held during the reporting year. The students, have also been imparted training in various aspects of science communication through the art of writing papers and grants proposals, as a part of human resource development programme. They have participated in competitive poster presentations organized by the institute, aimed to create collegial interactions and the spirit of discussions in science. The Institute has embarked on a novel frontier to encourage training of foreign students from developing countries. Students from Nigeria, Tunisia, Egypt and Cameroon have either visited or completed their papers under the auspices of NAM S&T Centre, Organization for Women in Science for the Developing World and C V Raman Fellowship of the Department of Science and Technology. Summer trainees form a group of students who are trained in a variety of ways to look at science and a sizable number of summer students received training at the institute along with other long term associates and fellows. The Institute initiated multiple programmes to draw high quality trained post-docs that would adequately supplement ongoing programmes, e.g. the Institute has implemented NII-Young Investigator Award Scheme for fostering young and innovative scientific minds with outstanding track record to maintain the level of productivity and boost scientific creativity.

While the Institute continues to present regular seminars from faculty of various scientific organizations, two major workshops were organized. To encourage innovative thinking and develop aspirations to transfer technologies, a sensitization workshop on innovation was held at the Institute in collaboration with the Foundation for Innovation and Technology Transfer at FIIT and BioRx venture advisors. The second one, Indo-German Workshop on 'Chemical Biology of Infectious Diseases' in collaboration with the Max-Plank Institute, Germany, opened dialogues between scientists of two countries. Prominent personalities like, Ryoji Nyori, Nobel laureate and President of Riken, Japan and Professor C.N.R. Rao, National Research Professor and Honorary President and Linus Pauling Research Professor at the Jawaharlal Nehru Centre for Advanced Scientific Research delivered enlightening and inspiring lectures at the institute. The foundation day lecture delivered by Professor K.N. Ganesh, Director IISER, Pune was equally stimulating.

Other noteworthy achievements during the reporting period have been the establishment of a new upgraded Transmission Electron Microscopy facility for cutting edge scientific endeavours, modernisation of animal house and upgradation of facilities for biochemical research like establishment of modern cold room facilities.

A few of the important scientific achievements are summarized below.

Leishmaniasis or kala-azar is endemic in certain parts of India and fall in the category of neglected tropical diseases. Current treatments are costly with high toxicity, there are no available vaccines and drug-resistant parasites are common. Scientists at NII used haemoglobin receptor (HbR) of *Leishmania* as a vaccine candidate to test if it can serve to generate neutralizing antibodies. HbR is conserved across various strains of *Leishmania* and antibodies to HbR can be detected in infected patients' sera. A HbR-DNA vaccine was developed at NII where significant protection was recorded against *Leishmania donovani* challenge in both mice and hamsters. The vaccine elicited both T helper type 1 cytokines and multifunctional T cells. This forms a promising lead for development of a vaccine against the *Leishmania* parasite. In the field of tuberculosis research, IGPD (also known as HisB) is one of the most extensively studied enzymes of the histidine biosynthesis pathway and is a recognized target for herbicide discovery as mammals do not possess an IGPD. IGPD from *M. tuberculosis* (*Mtb*) was analysed through structural and biochemical studies of the native Mtb IGPD and its complexes with IGP and the inhibitor 3-amino-1,2,4-triazole. Information obtained from this study has the potential to help in the design of new compounds that can be used against *Mtb* with enhanced affinity and increased selectivity for IGPD.

The longevity of humoral protection relies on two factors, the generation of a pool of memory cells that can respond to subsequent infection with enhanced kinetics and vigor, and the lifespan of the plasma cells (PCs) that make antibodies, as the half-life of secreted antibodies is very short. Scientists at NII have identified inducible nitric oxide synthase (iNOS) as a major intermediate that supports the survival of PCs. They show that inducible nitric oxide synthase to be a component of signalling pathways that promote survival of PCs. Deficiency of iNOS is responsible for shorter lifespan of PCs while activation and terminal differentiation of B cells remain unaffected. PCs deficient in iNOS (Nos2–/– PCs) undergo enhanced death *in vitro*, after transfer into congenic adoptive hosts and in chimeras made with wild-type and Nos2–/– bone marrow. The iNOS-mediated protection involves activation of protein kinase G, modulation of endoplasmic reticulum stress components and diminished activation of caspases. Aging research at the institute has contributed some interesting observations on *C. elegans* during the last reporting year. In *Caenorhabditis elegans*, dietary restriction is achieved either by using the *eat-2* mutants with diminished pharyngeal pumping resulting in lower food intake or by feeding diluted bacterial food to the worms. Scientists at the institute demonstrate that when MEKK3-like kinase gene is knocked down, it initiates a process similar to dietary restriction without actually compromising food intake. They show that MEKK-3 possibly function as a central nutrient sensor and signalling component within the organism that controls metabolism.

The temporal regulation of p53-mediated transcription is not very well understood despite being one of the most well-studied transcription factors. Scientists at the institute showed that histone deacetylase 5 (HDAC5) binds to p53 and abrogates K120 acetylation resulting in preferential recruitment of p53 to proarrest antioxidant targets at early phases of stress. However, upon prolonged genotoxic stress, HDAC5 undergoes nuclear export. Concomitantly, p53 is acetylated at the K120 residue and selectively transactivates proapoptotic target genes, leading to onset of apoptosis. Upon genotoxic stress in mice where HDAC5 expression is downregulated, the onset of apoptosis is accelerated in the highly vulnerable tissues. Findings suggest that HDAC5 is a key determinant of p53-mediated cell fate decisions in response to genotoxic stress. Another important report has been published by NII scientists on tumor microenvironment where the microenvironment is a major contributing factor towards the progression of a tumor. They have shown that the interaction between tumor-infiltrating hematopoietic cells and epithelial cancer cells results in their fusion in a variety of cancers. They were the first to demonstrate the presence of a hemato-epithelial cancer compartment contributing to stem cell markers and CXCR4 in epithelial carcinoma. This finding is important because it has impact on CXCR4-based therapeutics and opens up new avenues for discovering unique molecular targets against fusion and metastasis. Another interesting report in the area of cancer research was the demonstration of an important biochemical link between the ubiquitin/SUMO-dependent DNA damage response (UbS-DDR) and BLM-dependent pathways which are involved in maintaining genome stability. A translational success of a prior finding of NII has been reported by Cadilla pharmaceuticals. Based on earlier reports from this institute, on the activation of anti-tumor immune response and reduction of regulatory T cells with *Mycobacterium indicus pranii* (MIP) therapy in tumor bearing mice, Cadilla pharmaceuticals have carried out randomized controlled clinical trial in 221 patients diagnosed with advanced non-small cell lung cancer. There was an improvement of 17.48% in survival rates over a period of one year.

Development of small molecules that could alter glycan patterns in an antigen-selective and cell type selective manner might provide avenues for understanding biological functions of glycans. Mucin-type O-glycans form one of the most abundant and complex post-translational modifications on cell surface proteins that govern adhesion, migration and trafficking of hematopoietic cells. Scientists from NII demonstrated a novel observation that depending on the nature of the installed chemical moiety, non-natural GalNAc analogues drastically affect mucin-type O-glycosylation. These small molecules act as a first step in manipulating mucin-type O glycans and may further serve as valuable probes in understanding the relationship between structure and function of mucins in biological processes. Considering that cell surface glycans, particularly CD43, govern leukocyte trafficking in both immunity and autoimmune diseases, ability to modulate glycan patterns in a selective set of immune cells using carbohydrate-based small molecules may open up new avenues for understanding biological functions of glycans in immunity, autoimmune diseases and cancer.

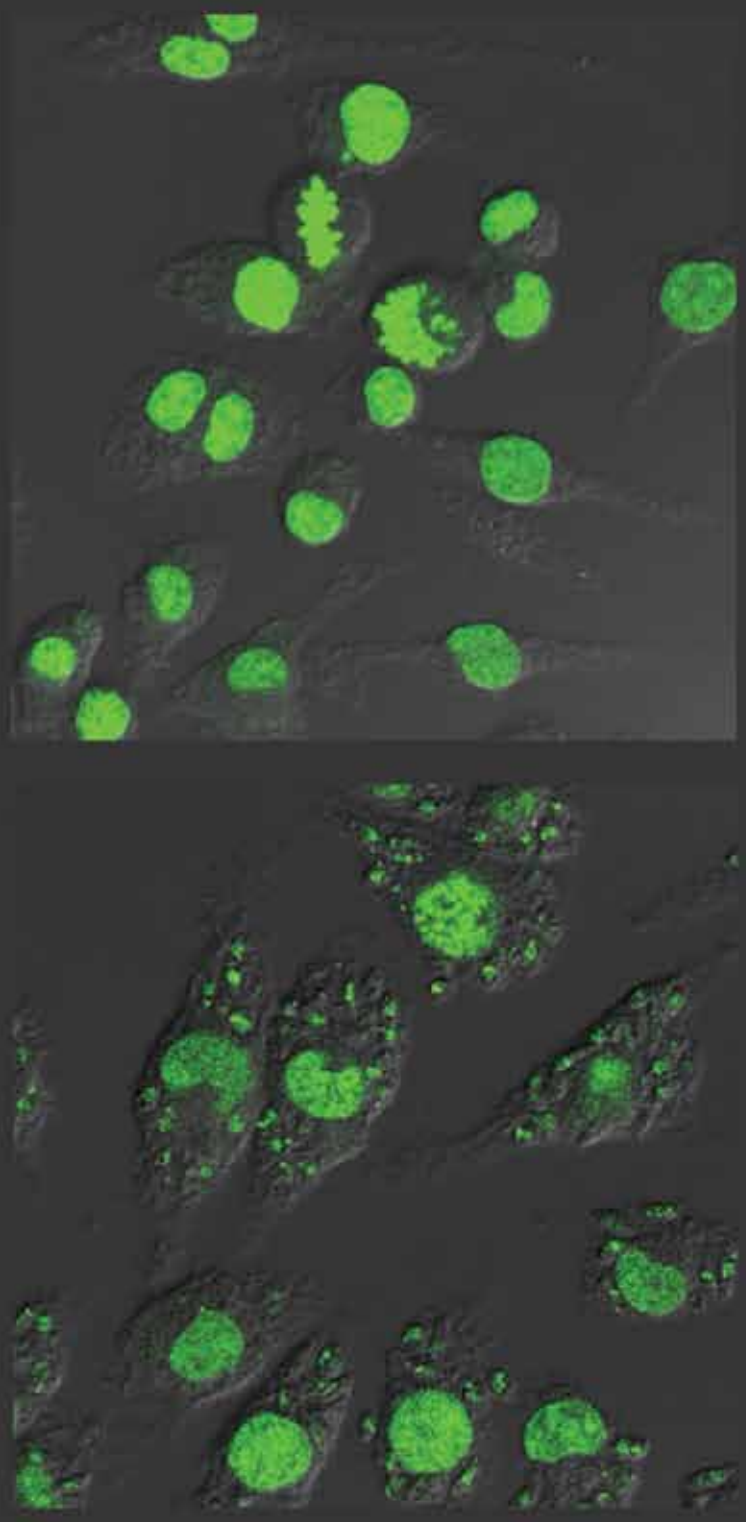
I would like to congratulate the entire scientific and allied staff of the institute, the doctoral students, the research associates, research assistants, junior research fellows and senior research fellows for their enthusiastic and stimulating participation in functioning of the institute. NII will endeavour to meet the challenges of the coming years and I am sure that all the members of the institute will help propel the institute in its growth towards a higher path to meet the growing needs of human health.

All the functioning of the institute would not have been possible without the constant support from the Department of Biotechnology.

Chandrima Shaha
Director

Date : May 28, 2014

RESEARCH REPORTS



***Leishmania* parasite uninfected and infected macrophages**

IMMUNITY AND INFECTION

Satyajit Rath

Analysis of antigen processing and presentation

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The aim of ongoing programmes in this group is to examine the generation and activation of T, B and antigen-presenting myeloid cells using multiple interlinked experimental systems. Data being reported here address the role of ligand density in controlling outcome of CD8 T cell activation.

Naïve CD8 T cells identify specific antigenic peptide-MHC class I (pMHCI) complexes on APC surfaces. TCR-pMHCI interactions trigger signaling cascades resulting in activation, proliferation and differentiation of CD8 T cells into effector cytotoxic T cells. The magnitude of CD8 T cell responses increases with ligand dose as well as with ligand affinity for the TCR. However, it is unclear how response-capable CD8 T cell populations convert detection of graded ligand densities into decisions controlling the corresponding response magnitude. Do more and more response-capable CD8 T cells actually get recruited, and/or do recruited individual CD8 T cells respond to a greater extent, perhaps by greater proliferation?

These questions are complicated by the fact that CD8 T cell responses are enormously specific and exquisitely sensitive, and models of T cell activation posit serial TCR triggering by a single ligand pMHCI as a signal amplifying mechanism to provide specificity and sensitivity. Also, in natural polyclonal T cell populations, the subset of cells that can respond to a given pMHC complex is heterogeneous in its affinity for the ligand. Even in a TCR-transgenic ‘monoclonal’ T cell population, strength of TCR signaling is affected by, not simply the ligand density and affinity, but also the duration of ligand availability, which could potentially allow multiple interactions to occur between responding T cells and ligand-bearing APCs, especially *in vivo*.

Complicating this further, responding T cells build quasi-stable interfaces, or immunological synapses, with ligand-bearing APCs, and TCRs are internalized from these interfaces, although the precise location, kinetics and functional significance of TCR internalization are still unclear. There is also evidence that responding T cells may remove ligand from APC surfaces, although again, the relationship of this phenomenon with TCR internalization and its significance for successful T cell activation is unclear.

Further, naturally generated pMHCI complexes, loaded with proteasomally derived peptides, are not uniformly distributed on the APC surface but as clusters. Also, some non-cognate self-pMHCI complexes on the same APC may also affect cognate ligand recognition outcome. The relationship between ligand density and ligand affinity is also complex; at certain ligand densities, very high ligand affinity is counter-productive for successful CD8 T cell activation perhaps due to failure of serial TCR triggering, while at some ligand densities, T cell activation increases as a function of ligand affinity.

On this background, using brief (3 h) exposure of TCR-transgenic CD8 T cells *in vitro* to varying densities and/or affinities of cognate peptide-MHC ligand followed by ligand-free culture in IL-2, we found that ligand density or affinity determined the frequencies of responding cells but not the expression levels of the early activation marker molecule, CD69. Cells with low glucose uptake capacity were less ligand-sensitive, implicating metabolic competence in the heterogeneity of CD8 T cell populations for signal receptivity. All responding cells also proliferated, but priming ligand density or affinity was associated with delayed entry into proliferation. Ligand density or affinity was also associated with the extent of cell-surface TCR downmodulation. Finally, TCR internalisation was associated, regardless of the ligand density, with robust IL-2Ralpha expression, faster loss of the cell cycle inhibitor p27kip1 and faster cell cycle entry. Together, our findings indicate that heterogeneity among responding CD8 T cell populations in signal receptivity for activation and for TCR internalization mediates detection of ligand density or affinity, contributing to graded response magnitudes.

Publications

Original peer-reviewed articles

1. Upadhyay M, Priya GK, Ramesh P, Madhavi MB, Rath S, Bal V, George A, Vaidya T*. (2014) CD40 signaling drives B lymphocytes into an intermediate memory-like state poised between naïve and plasma cells. *J. Cell. Physiol.* doi:10.1002/jcp.24572.
2. Sinha A, Gulati A, Saini S, Blanc C, Gupta A, Gurjar BS, Saini H, Kotresh ST, Ali U, Bhatia D, Ohri A, Kumar M, Agarwal I, Gulati S, Anand K, Vijayakumar M, Sinha R, Sethi S, Salmona M, George A, Bal V, Singh G, Dinda AK, Hari P, Rath S, Dragon-Durey MA, Bagga A*. (2013) Prompt plasma exchanges and immunosuppressive treatment improves the outcomes of anti-factor H autoantibody-associated hemolytic uremic syndrome in children. *Kidney Int.* doi:10.1038/ki2013.373.
3. Saini AS, Shenoy GN, Rath S*, Bal V*, George A*. (2014) Inducible nitric oxide synthase is a major intermediate in signaling pathways for the survival of plasma cells. *Nat. Immunol.* **15**: 275-282.

Reviews

1. Salam N*, Rane S, Das R, Faulkner M, Gund R, Kandpal U, Lewis V, Mattoo H, Prabhu S, Ranganathan V, Durdik J, George A, Rath S, Bal V. (2013) T cell ageing: Effects of age on development, survival and function. Indian *J. Med. Res.* **138**: 595-608.
2. Prabhu SB*, Khalsa JK, Banerjee H, Das A, Srivastava S, Mattoo HR, Thyagarajan K, Tanwar S, Das DS, Majumdar SS, George A, Bal V, Durdik JM, Rath S. (2013) Role of apoptosis-inducing factor (Aif) in the T cell lineage. *Indian J. Med. Res.* **138**: 577-590.

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Prafullakumar Tailor

Understanding the role of interferon regulatory factors in dendritic cell development and innate immunity

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Dendritic cells (DCs) are composed of multiple subsets that collectively provide early innate immunity, leading to subsequent adaptive immunity. Plasmacytoid dendritic cells (pDC), $CD4^+$ DC, $CD8\alpha^+$ DC and $CD4^-CD8^-$ DC are four major subtypes in the murine spleen. These subtypes of DCs express different sets of genes and assume distinct functions. We are interested in understanding the mechanisms of development of DC subsets and their functions. Members of Interferon regulatory factors (IRFs) play important role in DC subset development and their respective functions. Main area of research of the laboratory is to understand the significance of signaling pathways and contribution of IRFs and other critical transcription factors in DC subset development and functions.

We developed and characterized a mouse DC progenitor-like cell line, designated DC9, from *Irf8*$^{-/-}$ bone marrow cells as a model for DC development and functions. Expression of *Irf8* in DC9 cells led to pDC and $CD8\alpha^+$ DC like cells. *Irf8* expression in DC9 led to increase in *Id2* and *Batf3* transcript levels, transcription factors shown to be important for the development of $CD8\alpha^+$ DCs. We showed that *Id2* and *Batf3* expression without *Irf8* was not sufficient for directing classical $CD8\alpha^+$ DC development. *Batf3* and *Id2* when co-expressed with *Irf8*, resulted in a synergistic effect on classical $CD8\alpha^+$ DC development.

Recent reports have identified a Zinc finger transcription factor zDC (Zbtb46), which is expressed specifically in cDC population and expression of zDC differentiates classical DCs from pDCs and monocyte-macrophage lineage population. We observed that cDC specific gene zDC is efficiently induced by *Irf8* and co-expression with *Id2* or *Batf3* led to a synergistic increase in zDC levels. One of the most important characteristics of $CD8\alpha^+$ DC is their ability to cross present antigens. Antigen cross presentation studies using B3Z line suggested that only DC9 cells expressing *Irf8* could cross-present antigen thus demonstrating an efficient development of classical $CD8\alpha^+$ DC subset. We demonstrated that *Irf8* is upstream of *Batf3* and *Id2* in the classical $CD8\alpha^+$ DC developmental program and defined the hierarchical relationship of transcription factors important for classical $CD8\alpha^+$ DC development.

Transforming growth factor-β (TGF-β) signaling is shown to have important roles in DC development. In DCs, TGF-β signaling induces *Id2* expression. ID (ID1-4) proteins are members of helix-loop-helix (HLH) group of transcription factors and are termed as antagonists of another activator class of HLH members. *Id2* expression is essential for CD8α$^+$ DCs and Langerhans cells. A recent study identified *Irf8* as a direct target of TGF-β signaling in DCs; and as per our studies, *Irf8* expression in DC9 cell line (*Irf8*$^{-/-}$ DC line) led to induction of *Id2* gene, suggesting that *IRF8* may regulate CD8α$^+$ DC development by controlling *Id2* gene expression. In our experiment with chemical inhibitor of TGF-β signaling or expressing *Smad7* and dnTGFbRII in the ongoing BMDC cultures, didn't affect *Irf8* transcript levels or Protein levels. Hence to further confirm our observations, we procured the transgenic mice expressing dnTGFbRII. First, the BMDC developed from dnTGFbRII transgenic mice and control mice showed a comparable *Irf8* and *Irf4* transcripts levels. Further, detailed study of the BMDC and splenic DC population by flow cytometry studies using various pDC and CD8α$^+$ DC markers didn't show abnormality in different major DC subtypes. Analysis of the DC populations from TGFbRII transgenic mice confirmed our preliminary studies from *in vitro* DC cultures. Thus, we conclusively showed that TGF-β signaling is redundant in pDC or CD8α$^+$ DC development. Further, we are conducting in depth experiments to demonstrate the role of *Irf8* in regulating *Id2* gene expression and understanding the role of TGF-β signaling in directing the DC subtype specific gene transcription.

Publications

Original peer-reviewed articles

1. Jaiswal H, Kaushik M, Sougrat R, Gupta M, Dey A, Verma R, Ozato K, Tailor P* (2013) *Batf3* and *Id2* have a synergistic effect on *Irf8*-Directed classical CD8α$^+$ dendritic cell development. *J Immunol* **191**: 5993-6001.

#2. Goel P, Tailor P, Chande A, Basu A, Mukhopadhyaya R (2013) An infectious HHV-6B isolate from a healthy adult with chromosomally integrated virus and a reporter based relative viral titer assay. *Virus Res* **173**: 280-285.

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#In press last year, since published.

Vineeta Bal

Biology of T lymphocytes

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To study mechanisms associated with renal dysfunction and proteinuria.

In the process of deciphering molecular mechanisms involved in proteinuria, in addition to TLR4 ligand LPS, it was observed that TLR2 ligand Pam3CysK4 and TLR3 ligand poly(I:C) also lead to proteinuria in mice. Using mice functionally sufficient or deficient in TLR4 signaling (C3H/OuJ and C3H/HeJ respectively) it was demonstrated that signaling through TLR4 is essential for LPS-mediated proteinuria. To delineate pathway of signaling downstream of TLRs MyD88-null mice were used. It was seen that TLR4 mediated signaling needed MyD88 as a functional molecule to induce proteinuria whereas ligation of TLR3, which signals in MyD88-independent fashion, resulted in proteinuria. Role of podocytes TLR expression in proteinuria was the next critical component to examine. Radiation chimera approach was used whereby TLRs were either present only on radiation resistant podocytes but not on the bone-marrow (BM) derived cells or vice versa. Challenge with LPS after complete reconstitution of irradiated mice showed that presence of TLRs on BM cells is essential and podocyte TLRs do not contribute to proteinuria. These data together with our earlier data show that inflammation in BM derived cells leads to proteinuria only in presence of CD80 expressed on podocytes. Next we examined the role of inflammatory process in proteinuria since it is seen in cases of sepsis as well. Chitohexaose (Chx) is a TLR4 ligand with anti-inflammatory signaling outcome. When competition experiments were done with LPS and Chx, extent of proteinuria could be reduced and CD80-uria could be prevented indicating a positive correlation with pro-inflammatory condition *in vivo*. These data suggest Chx as a significant anti-inflammatory agent capable with potential for treatement in CD80 associated proteinuia.

To study the role of T cells in Japanese encephalitis (JE) infection in mouse model.

A good animal model for studying many aspects of JE pathogenesis is still not available and we report use of adult T cell receptor beta (TCRβ) chain deficient mice as a potential model. These

mice have a normally developed blood brain barrier (BBB) and on i.v. infection with JE >80% mice succumb to infection over 16-20 day period. This is associated with gradual weight loss and worsening of clinical score. TCRβ sufficient WT mice show ~10% mortality. TCRβ-deficient infected mice show very high levels of JE virus in the brain as early as d7 post-infection whereas WT mice do not. TNFα and IL1β mRNA levels also go hand in hand with viral load. A stark BBB breach can be documented in infected TCRβ-deficient mice by d12 indicating a role of L/β TCR bearing T cells in preventing breach in BBB and viral entry in WT mice. Adoptive transfer of T cells from different mouse strains inTCRβ-deficient mice prior to infection showed that CD4 T cells are less important in providing protection whereas CD8 cells are critical. Further, granule releasing potential i.e. efficient target cell lysis function of CD8 cells is necessary otherwise mortality is high even in presence of CD8 T cells.

To characterise the effects of *in vivo* aging on CD4 T cell function and phenotypic features.

New data on the role of micro-RNA181a and malfunctioning of dual specificity phosphatase (DUSP)-6 in the process of aging was generated. It was shown that naïve CD4 cells from aged mice and those expressing lower CD4 levels from young mice show lower levels of micro-RNA181a and inhibition of DUSP-6 partially reverses the aging phenotype observed in these cells.

Publications

Original peer reviewed articles

1. Sinha A, Gulati A, Saini S, Blanc C, Gupta A, Gurjar BS, Saini H, Kotresh ST, Ali U, Bhatia D, Ohri A, Kumar M, Agarwal I, Gulati S, Anand K, Vijaykumar M, Sinha R, Sethi S, Saloma M, George A, Bal V, Singh G, Dinda AK, Hari P, Rath S, Dragon-Durey M-A, Bagga A* (2013) Prompt plasma exchanges and immunosuppressive treatment improves the outcomes of anti-factor H autoantibody-associated hemolytic uremic syndrome in children. *Kidney Int.* doi:10.1038/ki.2013.373.
2. Saini S, Shenoy G, Rath S*, Bal V*, George A* (2014) Inducible nitric oxide synthase is a major intermediate in signaling pathways for the survival of plasma cells. *Nat Immunol* **15**:275-282.
3. Upadhyay M, Priya GK, Ramesh P, Madhavi MB, Rath S, Bal V, George A Vaidya T*. (2014) CD40 signaling drives B lymphocytes into an intermediate memory-like state, poised between naïve and plasma cells. *J. Cell. Physiol.* doi:10.1002/jcp.24572.

Reviews/proceedings

1. Salam N*, Rane S, Das R, Faulkner M, Gund R, Kandpal U, Lewis V, Mattoo H, Prabhu S, Ranganathan V, Durdik J, George A, Rath S, Bal V* (2013) T cell ageing: Effects of age on development, survival and function. *Indian J Med Res* **138**: 595-608.
2. Prabhu SB*, Khalsa JK, Banerjee H, Das A, Srivastava S, Mattoo HR, Thyagarajan K, Tanwar S, Das DS, Majumdar SS, George A, Bal V, Durdik JM and Rath S (2013) Role of apoptosis-inducing factor (Aif) in the T cell lineage. *Indian J Med Res* **138**: 577-590.

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Sudhanshu Vrati

Biology of Japanese Encephalitis virus

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Japanese Encephalitis virus (JEV) is a member of the *Flaviviridae* family of animal viruses that contains several other medically important viruses such as Dengue and Yellow fever. JEV is a major cause of human encephalitis and is responsible for considerable mortality and morbidity in India. Frequent epidemics of Japanese encephalitis (JE) are being reported from various parts of India and JEV has become endemic in several parts of the country. We are studying the biology of JEV replication with a view to develop novel anti-virals.

JEV genome is a plus-sense single-stranded RNA of ~11 kilo bases. A minus-sense RNA template is generated during virus replication, which is then copied to produce a large number of plus-sense genomic RNA molecules. Based on the amino acid sequence homologies with other replicases, NS3 and NS5 viral non-structural proteins have been speculated to be involved in replication of the JEV genome. However, we do not know if any of the cellular proteins also are needed for viral replication. We are, therefore, studying cellular proteins that interact with JEV genome sequences, which are likely to be involved in viral replication. Besides, viral non-structural proteins may interact with viral genome or host proteins during replicaion. We wish to study these interactions.

We have studied interaction of JEV non-structural proteins with host proteins. The proteins under study are: NS5 which has the RNA-dependent RNA polymerase activity, and NS2A whose function is yet unknown. Yeast-two-hybrid system was used to fish out potential interacting partners with these two proteins.

The Yeast-2-hybrid system idnerified Rbm12b, Aldolase A, Rogdi, Itm2C and Cccd91 as potential interacting partners of NS5 protein. Interaction of these proteins with NS5 was further verified using co-immunoprecipitation assays. These assays indicated that Aldolase A and Rbm12b proteins interacted with NS5 in cultured cells infected with JEV. This was further explored by studying co-localization of these two proteins within the virus-infected cells using confocal microscopy. While no co-localization was observed for NS5 and Alodase A during the early phase of infection (4-16 h post infection), these two proteins co-localized in the cytoplasm at the later phase of infection (24-36 h post infection). No significant interaction with NS5 was observed in cells transfected with *Myc*-Rbm12b followed by infection with JEV. Thus Aldolase A appears to interact with NS5 in a time-dependent manner during the later phase of JEV replication.

A number of putative interacting partners for NS2A protein have been identified. These are under various stages of validation using pull down assys and confocal microscopy.

Publications

Original peer-reviewed articles

1. Bhattacharya S, Sen U, Vrati S* (2013) The RIDD pathway is activated during Japanese encephalitis virus-induced unfolded protein response and benefits viral replication. *J Gen Virol* **95**: 71-79.
2. Appaiahgari MB, Glass R, Singh S, Taneja S, Rongsen-Chandola T, Bhandari N, Mishra S, Vrati S* (2014) Transplacental rotavirus IgG interferes with immune response to live oral rotavirus vaccine ORV-116E in Indian infants. *Vaccine* **32**: 651-656.

Review

1. Bharati K, Vrati S* (2013) DNA vaccines: Getting closer to becoming a reality. *Indian J Med Res* **137** : 1027-1028.

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Agam Prasad Singh

Plasmodium proteins involved in virulence and host modulation: Host-Parasite interactions in Plasmodium liver stages

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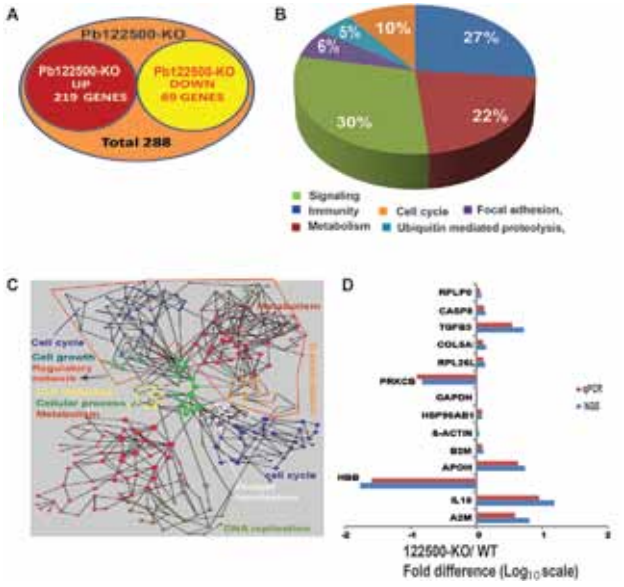
Plasmodium species introduce effector molecules into hepatocyte cytosol to manipulate host pathways for its own benefit. Those could prove good targets for drug development. Parasite kinases, phosphatases etc. targeted to hepatocytes are likely candidates. The host processes affected by them could also be target for intervention. Basic theme of this study is to identify, new parasite molecules that affect the host cellular processes, and possible intervention strategies. We use reverse genetics tool to characterize the parasite proteins with regard to its function. Currently, Primaquine is the only drug available for malaria liver stages (LS) treatment, but it can't be administered to pregnant women and people with G6PD deficiency as it causes toxicity. Alternative drugs are the need of hour.

This year we have characterized a liver-stage expressed and exported serine-threonine kinase with conserved FIKK domain. Kinase domain is present in the C-terminus of protein and active. Its activity could be inhibited with a known PKA inhibitor. We also determined the transcriptomic profile of FIKK kinase knockout infected hepatocyte in comparison to wild type infected parasite. We also performed transcriptomic analysis with another knockout parasite (tryptophan rich antigen) and found that this protein changed over 2000 host transcripts, of those ~ 10% were transcription factors. We noticed that this protein likely regulate immune metabolic switch through transcription factor MEF2C. We tested the anti-liverstage effectiveness of three inhibitors, curcumin, nano-curcumin and andrographolide in mouse challenge model. Andrographolide showed inhibition of liverstage parasite growth but curcumin or its nano form failed to show any activity at the highest dose tested.



(A) Venn diagram summarizing the significantly affected genes (>2 folds) in Pb122500-KO infection of host cell compared to WT infection.

(B) Pathways analysis of Pb122500-KO affected host genes compared to WT parasite infection by KEGG pathway. The host genes showing fold change of > ±2 were considered for pathways analysis. Pie chart showing pathways in which host genes were up regulated during Pb122500-KO infection compared to WT parasite.

(C) Functional cluster analysis of host genes affected by Pb122500-KO parasite. Nodes represented in one color are involved in same biological process.

(D) Validation of NGS data with quantitative real time PCR. Blue and red bar represent the fold change in log scale for host genes observed in NGS Data and real time PCR validation.

Akhil C. Banerjea

Genetic and functional analyses of host and HIV-1 genes that affect progression of HIV-1 and development of nucleic acid based antiviral approaches

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HIV-1 Vpr redirects host ubiquitination pathway

HIV-1 is known to rely heavily on modulation of host ubiquitin pathway particularly for counteraction of antiretroviral restriction factors, viral assembly and release. Reports till date have focused on molecular hijacking of ubiquitin machinery by HIV-1 at the level of E3 ligases. Interaction of a viral protein with an E3 alters its specificity to bring about selective protein ubiquitination. However, in case of infection, multiple viral proteins can interact with this multi-enzyme pathway at various levels making it much more complicated. Here, we have addressed manipulation of ubiquitination at the whole cell level post HIV-1 infection. Our results show that HIV-1 Vpr is necessary and sufficient to bring about redirection of host ubiquitin pathway towards HIV-1 specific outcomes. Our work, first of its kind, provides novel insight into regulation of ubiquitin system at whole cell level by HIV-1.

Role of MicroRNA34a in HIV-1 replication

HIV-1 alters the host cell microRNA expression patterns. MicroRNA-34a (miR34a) is one of the cellular microRNAs found to be elevated during HIV-1 infection. The functional consequences, however, remain unexplored. Our data suggests

that miR34a functions to promote HIV-1 replication in T cells and there exists a positive feedback loop in HIV-1 mediated activation of microRNA-34a (miR34a) expression and vice versa. We are greatly interested in studying its role in HIV-1 mediated apoptosis especially in human T-cells and neuronal cells.

Role of AKT in HIV-1 pathogenesis

A number of knockdown studies have reported survival pathways to be important for HIV-1. Protein kinase B/AKT is one of the crucial factors needed by cells for their survival. The exact role of this kinase which lies downstream of the PI3 kinase pathway has not been properly elucidated in HIV-1 though it is known to be important. The aim of this study is to elucidate the role of AKT and as well as its downstream effector molecules during HIV-1 infection. The main focus of this project is MDM2 (target substrate for AKT) and its regulation in presence of HIV-1.

Role of metabolism in HIV-1 pathogenesis

HIV-1depends on host cell for its biosynthetic demands due to small genome size using domain and motifs for protein-protein interaction. In this study we explore how HIV-1 alters the metabolic state of the T cell and if this can be used as a therapeutic target. We have checked the levels of metabolic sensors i.e. mTOR, AMPK and Akt during time course of infection. Because these sensors are master regulators of metabolic state of the cells hence, downstream targets of these sensors will give better insights into the exact alteration in the metabolic state of the cell by HIV-1.

Genetic and Functional analysis of Tat exon 1 variation and Tat-Vpr interaction from North Indian HIV-1 infected individuals

Tat and Vpr genes from HIV-1 infected individuals from North India were genetically analyzed and extensive phylogenetic analyses were carried out and some key functional activities related to gene expression were tested. Both of these genes are critically important for viral gene expression and viral fitness. We report novel Tat- B/C recombinant with a precise breakpoint in the middle of the ORF and their HIV-1 LTR promoter activating properties were studied in detail in comparison with prototype subtype B and C Tat Exon1. Functional implications of natural Tat-Vpr variants were studied for their ability to augment the HIV-1 promoter activity and apoptosis for the first time.

Publications

Original peer reviewed articles

1. Ponia SK, Arora S, Kumar B, Banerjea AC* (2013) Arginine rich short linear motif of HIV-1 regulatory proteins inhibits dicer dependent RNA interference. *Retrovirology* **10**:97.
2. Kakumani PK, Ponia SS, SRK, Sood V, Chinnappan M, Banerjea AC, Medigeshi GR, Malhotra P, Mukherjee SK, Bhatnagar RK* (2013) Role of RNAi in dengue viral replication and identification of NS4B as a RNAi suppressor. *J Virol.* **87**: 8870 -8883.
3. Ronsard L, Lata S, Singh J, Ramachandran VG, Das S, Banerjea AC* (2014) Molecular and genetic characterization of natural HIV-1 Tat Exon-1 variants from north India and their functional implications. *PLoS ONE* **9**: e85452.
4. Lata S, Ronsard L, Sood V, Dar SA, Ramachandran VG, Das S, Banerjea AC* (2013) Effect on HIV-1 gene expression, Tat-Vpr interaction and cell apoptosis by natural variants of HIV-1 Tat Exon 1 and Vpr from northern India. *PLoS ONE* **8**: e82128.
5. Gupta S, Neogi U, Srinivasa H, Banerjea AC, Shet A* (2014) HIV-1 coreceptor tropism in India: Increasing proportion of X4-tropism in subtype C strains over two decades. *J Acquir Immune Defic Syndr*. **65**:397-404.

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Anna George

Study of mucosal immune responses

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We have been looking at the relative importance of signal transduction downstream of antigen recongnition by the B cell receptor (BCR) versus B cell-mediated antigen presentation to T cells following internalization of the antigen-BCR complex into endosomes in shaping the outcome of B cell responses. For our analysis, we took advantage of the *beige* mice which carry the *Lyst* mutation that leads to impaired trafficking of endosomes to lysosomes where antigen degradation is most efficient. In this context, we have reported previously that B cells from *beige* mice show delayed antigen presentation to T cells on the one hand and to enhanced and more sustained BCR-mediated signaling on the other. Over the past year, we tracked the movement of the antigen-BCR complex by confocal microscopy and report that endocytosis of the antigen-BCR complex is similar in B cells from both strains. However, while the endocytosed BCR trafficks rapidly to LAMP-1+ve (lysosomal) compartments in wild-type (WT) B cells, a substantial fraction remained in LAMP-1-ve compartments in *beige* B cells even at 120 min, leading to continued BCR mediated signaling in *beige* B cells. We also found that unlike WT B cells, in which peptide-MHC complexes were efficiently generated in lysosomal compartments, they were poorly generated outside lysosomes in the mutant B cells. A consequence of higher and more sustained BCR signaling is the enhanced generation of memory cells and long-lived plasma cells in *beige* mice following immunization.

The longevity of antibody-mediated protection *in vivo* is known to depend on the lifespan of plasma cells (PCs) and we have been looking at factors that influence PC viability. In this context, we have shown previously that PCs from iNOS-deficient mice show relatively poor survival *in vitro* and *in vivo*. Over the past year we have continued our studies on the role of iNOS in PC biology. We have shown that iNOS promotes PC viability via a biochemical pathway that involves soluble guanylyl cyclase mediated activation of PKG leading to modulation of endoplasmic stress and caspase activation, and that pro-survival mediators such as IL-6 feed into this pathway by inducing iNOS. We confirmed that iNOS deficiency did not affect B cell proliferation

(by CFSE dilution experiments) or the initiation of terminal differentiation (by showing similar downmodulation of Pax5 and Bach2 and similar induction of IRF4 and Blimp-1) and generation of PCs (by flow cytometry and ASC assays) over a 72 h period in WT and iNOS-null mice. However, purified iNOS-null PCs die more rapidly *in vitro*, and protection is afforded by adding a nitric oxide donor. Conversely, WT PCs show enhanced death when cultured with a pharmacological inhibitor of iNOS. To directly score PC death *in vivo*, we tracked antigen-specific ASC numbers following immunization of the two strains with NP-Ficoll and found that the temporal drop in ASC numbers is sharper in the spleens and bone marrow of iNOS-null mice. Similar findings were seen mixed marrow chimeras (WT+iNOS-null) immunized with NP-OVA, with ASCs declining more rapidly in the iNOS-null compartment. We also tested whether iNOS inhibition could lead to inhibition of an established antibody response by treating mice with aminoguanidine from day 28 post immunization (after a primary response that included germinal center formation, IgG antibodies and localization of PCs to the bone marrow had occurred). While aminoguanidine treatment had no effect on the antibody response of iNOS-null mice, it led to a dramatic fall in titers in WT mice.

Publications

Original peer-reviewed articles

1. Sinha A, Gulati A, Saini S, Blanc C, Gupta A, Gurjar BS, Saini H, Kotresh ST, Ali U, Bhatia D, Ohri A, Kumar M, Agarwal I, Gulati S, Anand K, Vijayakumar M, Sinha R, Sethi S, Salmona M, George A, Bal V, Singh G, Dinda AK, Hari P, Rath S, Dragon-Durey MA, Bagga A* (2013) Prompt plasma exchanges and immunosuppressive treatment improves the outcomes of anti-factor H autoantibody-associated hemolytic uremic syndrome in children. *Kidney Int*. doi:10.1038/ki.2013.373.
2. Saini AS, Shenoy GN, Rath S, Bal V, George A (2014) Inducible nitric oxide synthase is a major intermediate in signaling pathways for plasma cell survival. *Nat. Immunol*.**15**: 275-282.
3. Upadhyay M, Priya GK, Ramesh P, Madhavi MB, Rath S*, Bal V, George A, Vaidya T (2014) CD40 signaling drives B lymphocytes into an intermediate memory-like state, poised between naïve and plasma cells. *J. Cell Physiol*. doi:10.1002/jcp.24572.

Reviews

1. Prabu SB*, Khalsa JK, Banerjee H, Das A, Srivastava S, Mattoo HR, Thyagarajan K, Tanwar S, Das DS, Majundar SS, George G, Bal V, Durdik JM, Rath S (2013) Role of apoptosis-inducing factor (Aif) in the T cell lineage. *Ind J Med Res* **138**:577-590.
2. Salam N*, Rane S, Das R, Faulkner M, Gund R, Kandpal U, Lewis V, Mattoo H, Prabhu S, Ranganathan V, Durdik J, George A, Rath S, Bal V (2013) T cell ageing: Effects of age on development, survival & function. *Ind J Med Res* **138**:595-608.

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Ayub Qadri

Analysis of *Salmonella Typhi*-host cell interaction

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Pathogenic *Salmonella* species produce different clinical manifestations depending upon *Salmonella* serovar and the type of host. In humans, these manifestations range from self limiting localized gastroenteritis caused by *Salmonella typhimurium* to systemic disease produced by *Salmonella typhi*. *Salmonella typhi* does not however establish infection in normal mice. On the other hand, *Salmonella typhimurium* infection in mice results in a systemic outcome that is analogous to human typhoid. The reasons for these different clinical outcomes and different host specificities are not understood. Further, the mechanisms by which pathogenic *Salmonella* manipulate host immune defenses in order to establish systemic infection are also not very clear. Work in our laboratory addresses these two aspects of *Salmonella*-host cell interaction.

Caspase-1 regulates expression of TLR/NLR ligand from pathogenic *Salmonella*

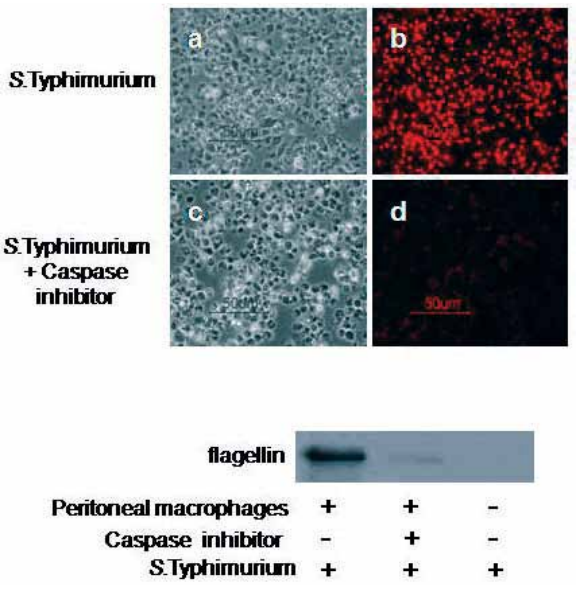
Previous work from our laboratory showed that sensing of lysophospholipids derived from intestinal epithelial cell (IEC) activates release of proinflammatory flagellin from *Salmonella*, thereby revealing a previously unappreciated mechansim by which inflammatory responses could be regulated during infection with this pathogen (*Naeha Subramanian and Ayub Qadri. Nat. Immunol. 7:583, 2006*). Post intestinal invasion, pathogenic *Salmonella* species infect and reside within mononuclear phagocytes which can recognize bacterial flagellin not only through membrane TLR-5 but also through the intracellular inflammasome, Nlrc4; the responses through these sensors play a vital role in innate immunity against *Salmonella* infection. We therefore thought it pertinent to investigate regulation of flagellin secretion during infection of macrophages with *Salmonella*. The results showed significant release of flagellin during interaction of *Salmonella* with macrophages. To investigate if this host stimulus-dependent regulation of flagellin was also operative during intracellular residence of *Salmonella*, we looked at expression of this TLR/NLR ligand (flagellin) in *Salmonella* obtained from WT or caspase-1 deficient bone marrow - derived macrophage cell lines infected with *S.typhimurium*. The data showed that while *Salmonella* obtained from WT cells continued to express flagellin, *Salmonella* obtained from caspase-1 deficient cells reduced expression of flagellin with the progression of infection. These results suggest that caspase-1 – derived stimulus modulates expression of flagellin during infection with *Salmonella*.

Host lipids amplify TLR – generated inflammatory response

We had previously reported that TLR-5 - induced CXCL8 secretion from human and mouse intestinal epithelial cells, and human monocytes is considerably increased in the presence of non-proteinaceous component(s) of serum. Further investigations revealed that this phenomenon was not restricted to TLR5. One of the lipids that could mimic the effect of serum in monocytes was serum-borne bioactive lipid, lysophosphatidylcholine (LPC). The enhancement in inflammatory responses was produced as a result of priming of immune cells by TLR-activation to respond to serum/LPC. The upregulation brought about by LPC was inhibited with antibody against G2A, one of the G-protein coupled receptors which regulate cellular responses to LPC. LPC - stimulation of TLR-sensitized cells led to activation of JNK MAP-kinase, which was sufficient to enhance inflammatory response. Our work presents a novel mechanism for the amplification of inflammatory responses through TLRs.

T cell receptor activation regulates TLR responses from human T cells

T cells are responsible for providing adaptive immunity, yet these cells express Toll-like receptors (TLRs) which are vital to innate immunity. To understand how activation of T cells through the T cell receptor (TCR) might influence the innate capability of T cells, we stimulated these cells through the TCR and tested their responses to TLR-2 (Pam3csk) and TLR-5 (flagellin) ligands. While freshly isolated T cells readily secreted neutrophil chemoattractant CXCL8 upon stimulation with flagellin or Pam3csk, TCR-activated cells secreted negligible amounts of CXCL8. TCR engagement did not however affect the ability of TLRs to activate MAP-kinases and NF-κB indicating that signals transduced through the TCR do not produce general unresponsiveness to TLRs. The reduction was seen at the level of mRNA as well as protein raising a possibility that in TCR-activated T cells, CXCL8 locus might be unresponsive to TLR - generated intracellular signals. These findings highlight the role of TCR in shaping innate immune activation of T cells.



Caspase – Dependent release of flagellin during infection of macrophages with *Salmonella*.
Peritoneal macrophages were infected with *S.typhimurium* in presence or absence of the pan-caspase inhibitor zVADfmk for 1 h. Flagellin released by *S.typhimurium* into the supernatant was analyzed by Western blotting with antibody to *S.typhimurium* flagellin. Cell death was determined by staining with propidium iodide (b & d); a & c show phase contrast pictures.

Publications

Original peer-reviewed article

1. Santhanam SK, Dutta D, Parween F Qadri A* (2014) The Virulence polysaccharide Vi released by *Salmonella typhi* targets membrane prohibitin to inhibit T-Cell activation. *J. Infect. Dis*. doi:10.1093/infdis/jiu64.

#2. Sharma N, Akhade AS, Qadri A* (2013) Sphingosine-1-phosphate suppresses TLR-induced CXCL8 secretion from human T cells. *J Leukoc Biol* **93**: 521-528.

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#In press last year, since published.

Devinder Sehgal

Molecular basis of B cell responses

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The theme of research is to decipher how *Streptococcus pneumoniae* (pneumococcus) causes disease and what interventions can be made to stop this from happening. The research is focused on the pneumococcal products and strategies that allow the pathogen to avoid being destroyed by the mammalian immune system, and the types of immune responses that can circumvent these strategies and products.

The main objectives are (a) identification and characterization of pneumococcal virulence factors that are or may be related to pathogenesis, (b) how these virulence factors interact with the immune system and host cell to alter its cellular and molecular processes, and (c) evaluating the vaccine potential of pneumococcal surface proteins.

Identification and functional characterization of secreted nuclease(s) from *S. pneumoniae*

During pneumococcal infection, neutrophils are among the first immune cell types that infiltrate the site of infection. In a recently recognized mechanism, interaction of the bacterial pathogen induces neutrophils to die and generate neutrophil extracellular traps (NETs). The bacteria trapped in this framework chromatin, histones and antimicrobial proteins are subsequently killed thereby restricting bacterial dissemination. Secreted nucleases can help bacteria to establish infection by evading the NETs. In this regard, we aim to identify potential nucleases in the pneumococcal secretome. Culture supernatant from the logarithmic phase lytA deficient pneumococci showed nuclease activity which was completely abrogated upon treatment with proteinase K, heat or EDTA suggesting that the nuclease activity is heat labile and required divalent cations as cofactors for its activity. In-gel assay indicated the presence of 3 bands with nuclease activity. Currently, efforts are underway to identify these nucleases by mass spectrometry. The role of the secreted nucleases in host-pneumococcal interaction is being evaluated.

Functional characterization of lipoproteins from *S. pneumoniae*

Alveolar macrophages on encountering pneumococci produce proinflammatory cytokines that regulate the function of other immune cells and microbicidal factor nitric oxide (NO). The objective of this study is to decipher the role of pneumococcal lipoproteins in immunomodulation of alveolar macrophage effector functions. It was observed that the adhesion of lipoprotein deficient pneumococci to model murine alveolar macrophage cell line MH-S is decreased three fold compared to wild type pneumococci. Live, heat inactivated lipoprotein deficient pneumococci and Triton X-114 extract from lipoprotein deficient pneumococci induced production of IL-6, IL-12 and NO was reduced compared to the corresponding preparation of wild type pneumococci. L-NAME, an inducible nitric oxide synthase (iNOS) inhibitor, suppressed wild type and lipoprotein deficient pneumococci induced production of NO from MH-S cells, and in the presence of Triton X-114 extract from wild type pneumococci suggesting iNOS is involved in lipoprotein mediated NO production. Stimulation of MH-S cells with extract from the wild type pneumococci activated MAP kinase and NF_κB pathways, and induced iNOS as observed by immunoblotting. Signaling pathway specific inhibitors helped in identifying JNK as the major pathway that leads to NO production. NO production induced in response to stimulation with extract from wild type pneumococci was abrogated by anti-TLR2 blocking antibody. Taken together, the data suggest that lipoproteins contribute to adhesion to alveolar macrophages and their interaction with TLR2 triggers signaling pathways that result in inflammatory responses.

Publication

Original peer-reviewed article

1. Anish C, Khan N, Upadhyay AK, Sehgal D, Panda AK* (2014) Delivery of polysaccharides using polymer particles: Implications on size-dependent immunogenicity, opsonophagocytosis and protective immunity. *Mol Pharm*. **11**:922-937.

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Amulya K. Panda

Studies on immune response from antigen loaded biodegradable polymer particles and protein refolding from inclusion bodies

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The main objective of the laboratory is the exploration of immunomodulation activities using biodegradable polymer particles. High-throughput refolding of inclusion body proteins into bioactive form is another objective of the research group. Researches in the following areas are conducted in the laboratory to achieve the objectives:

1. Analysis of immune response from antigen loaded polymer particles and evaluation of adjuvant properties associated with polymeric particle formulation. Evaluation of memory antibody response from polymer particle based immunization. Large porus polymer particle are also used to form scaffold for tissue engineering application.
2. Solubilization and refolding of inclusion body proteins from *Escherichia coli*. This involves analysis of inclusion body formation during protein expression and understanding of protein aggregation with an aim to recover higher amount of bioactive protein.

(A) Immune response from polymeric particles entrapping antigens

We have been reporting improved immunogenicity of antigens by entrapping them in polylactic acid (PLA) particles. It was observed that single dose immunization with polymeric particles entrapping antigens leads to development of immunological memory. This concept of developing memory

antibody response from single point immunization was extended to carbohydrate vaccine such as Pneumococcal capsular polysaccharides. Entrapment of capsular polysaccharides in polymer particles improved its immunogenicity and generated memory antibody titer from single point intramuscular immunization (Figure 1).

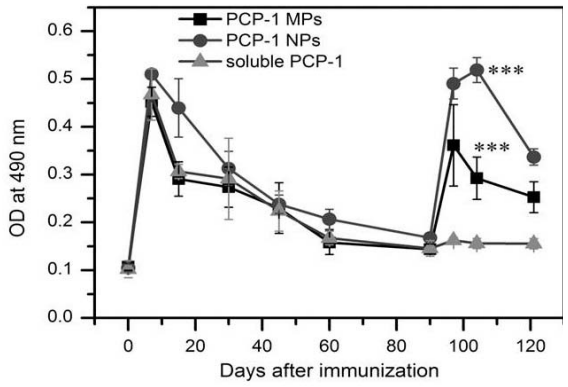


Figure 1. Comparison of anti-PCP-1 IgG responses elicited by immunizing animals with different PCP-1 formulations.

PLA nanoparticles entrapping capsular polysaccharide alone were also able to elicit protective antibody titer from a single immunization dose. Detailed studies involving *in vitro* release of protein antigens from polymer particles, cellular uptake by macrophages and DCs are being studied to understand the mechanism of antigen processing and presentation (Figure 2).

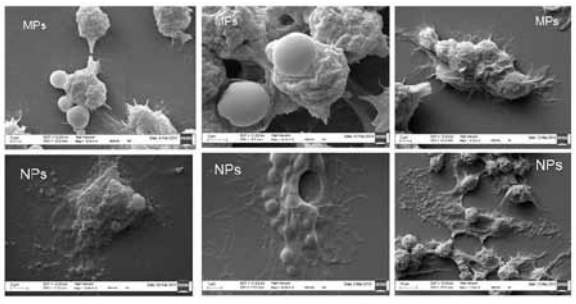


Figure 2. Interaction of MPs induce formation of active phagocytic cups where as Nanoparticles undergo change in morphology as a result of uptake of particles.

Polymeric particle formulation process was optimized using spray drying. The process engineering and formulation parameters were optimized to get desired sized particles in reproducible way. Spray drying was also used to produce dry powder alum for immunization. Antigens (DT, TT and Lysozyme) mixed with alum were spray dried to make dry powder vaccine formulations. The size of particle were around 2-5 µm. Alum particles were characterized in detail and compared with liquid alum preparation. In spite of the fact that the antigen was released quickly from spray-dried alum particles, antibody response in mice was found to be similar to that observed with traditional alum adsorbed antigen. This was true for both primary and secondary antibody responses. The vaccine formulation powder possessed good long-term stability. This opens up new possibilities of making dry powder, room temperature stable vaccine containing alum as an adjuvant.

(B) Solubilization and refolding of inclusion body proteins

Our focus on inclusion bodies (IBs) has been on two aspects: (1) to improve the recovery of bioactive protein and (2) to understand the nature of aggregation during inclusion body formation. Previously we have reported that different sized inclusion body aggregates are formed during expression of recombinant protein in *E. coli*. To obtain native protein from these aggregates, it is necessary to solubilize these aggregates followed by refolding of the solubilized protein by appropriate refolding method. A novel solubilization method using n-propanol in presence of low concentration of urea was developed. N-propanol in combination with 2 M urea was found to be sufficient for the efficient solubilization of human growth hormone (hGH) inclusion bodies. Aggregation during refolding was also studied and it was found that solubilization

with n-propanol based buffer resulted into bioactive hGH without aggregation in comparison to those obtained with urea and GdmCl based buffers. We are currently screening other organic solvents for their solubilization potential. We hypothesize that organic solvents with a high log $P_{octanol/water}$ value (partition coefficient) are efficient in inclusion body solubilization.

Presence of enzyme activity in inclusion bodies along with other structural studies indicates the presence of native-like secondary and tertiary structures. There are no detailed reports on the presence of enzyme activity in inclusion bodies of oligomeric proteins. One of such proteins is *E. coli* Asparaginase II which is active only in its tetrameric form where the active site is formed by intimate interaction of two subunits. Inclusion bodies of Asparaginase are found to be of non-classical type which display significant enzyme activity when solubilized. This observation strongly suggests the presence of native-like quaternary structures in inclusion bodies. Currently we are exploring the structural basis of enzyme activity in these IBs employing various biophysical and biochemical techniques.

Publications

Original peer-reviewed articles

#1. Gupta N, Shrestha A, Panda AK, Gupta SK* (2013). Production of tag-free recombinant fusion protein encompassing promiscuous T cell epitope of tetanus toxoid and dog zona pellucida glycoprotein-3 for contraceptive vaccine development. *Mol Biotechnol* **54**: 853-862.

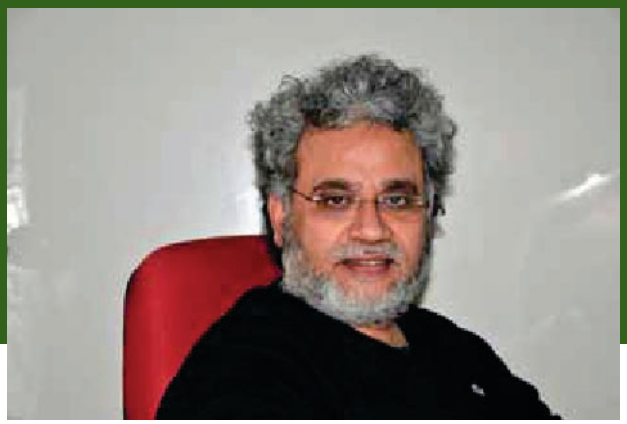
2. Sharma M, Sharma V, Panda AK, Majumdar DK* (2013). Development of enteric submicron particles formulation of α-amylase for oral delivery. *Pharm Dev Technol* **18**: 560-569.
3. Alam A, Mirza A, Talegaonkar S, Panda AK, Iqbal Z* (2013). Development of Celecoxib complex: characterization and cytotoxicity studies in MCF-7. *Pharmaceutics Anal Acta* **4**: 211
4. Haider S, Alam MS, Hamid H, Shafi S, Nargotra A, Mahajan P, Nazreen S, Kalle AM, Kharbanda C, Ali Y, Alam A, Panda AK (2013). Synthesis of novel 1,2,3-triazole based benzoxazolinones: Their TNF-α based molecular docking with *in-vivo* anti-inflammatory, antinociceptive activities and ulcerogenic risk evaluation. *Eur J Med Chem* **70**: 579-588.
5. Anish CK, Khan N, Upadhyay AK, Sehgal D, Panda AK* (2014). Delivery of polysaccharides using polymer particles: Implications on size dependent immunogenicity, opsonophagocytosis and protective immunity. *Mol Pharmaceutics* **11**: 922-937.

Patents

1. Panda AK, Gopimohan R and Anish CK (2014). Biodegradable polymer scaffold & process for preparation thereof. *(Patent no. AU 2008315318; Granted on February 06, 2014).*
2. Panda AK, Singh MS, Upadhyay AK (2013). Process for obtaining bioactive recombinant protein from inclusion bodies. *(Patent No. EP2147093; Granted on May 31, 2013).*
3. Panda AK, Anish CK and Goswami DG (2013). Spray dried formulation of antigen containing alum or antigen entrapped polymer particles with surface coated of alum. *(Application No. US 13/824864; Filed on May 2, 2013).*
4. Panda AK and Anish CK (2013). A vaccine composition capable of inducing memory antibody response from single point immunization. *(Application No. US 14/122,923; Filed on November 27, 2013).*

*Corresponding authors

#In press last year, since published.



Rahul Pal

Disorders of proliferation: Analysis of novel pathways and targets

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A. hCG and tumorigenesis

Tumor-associated human chorionic gonadotropin (hCG) has been linked with poor patient prognosis. Additionally, hCG reduced the loss of viability induced by 5-flourouracil, curcumin, cisplatin, tamoxifen and etoposide. Enhanced viability in the presence of hCG was attributable to a significant reduction in drug-induced apoptosis.

Tumor cells incubated with hCG demonstrated increases in the levels of cIAP-1, XIAP, survivin HIF-1α, HO-1, PON2 and Hsp27. siRNAs against HIF-1α, HO-1, Nrf-2 and survivin decreased transcription and expression of respective targets in tumor cells and decreased hCG-induced chemoresistance.

Some TLR ligands acted in synergy with hCG to provide enhanced resistance against chemotherapeutic agents. Cells incubated with hCG along with ligands for either TLR2 or TLR8 showed enhanced ERK phosphorylation. Interestingly, all three components (hCG, TLR ligand and drug) were required for maximal phosphorylation of signaling intermediates.

Mice implanted with syngeneic Lewis Lung cancer cells (LLC) were immunized with a βhCG-based vaccine formulation and also received either curcumin or tamoxifen. Co-administration of drugs and immunotherapy resulted in a further decrease in both tumor volume and incidence, and in animal survival. The data suggests that combining anti-hCG immunotherapy with standard chemotherapy may result in substantial benefit in individuals carrying gonadotropin-sensitive tumors.

Implantation of syngeneic tumor cells βhCG transgenic animals resulted in higher tumor incidences and larger tumor volumes. Markedly higher transcript levels of IL-6, IL-8, TNFα, VEGF, MMP-2, MMP-9, Bcl-2, Bcl-xl and survivin were observed. Serum derived from TG animals induced secretion of the tumor-associated moieties VEGF, KC, TGF-β and IL-6 from LLC cells.

B. Systemic autoimmunity

The immunobiology of hemoglobin (Hb)

Splenocytes derived from both autoimmune-prone and non-autoimmune-prone mice responded to ferric (but not ferrous) murine

Hb by the increased secretion of IL-6, IL-8 and TNF-α. Of interest was the fact that ferric (but not ferrous) murine Hb induced a significant increase in levels of maturation markers (CD80, CD86, CD83, CD40) on BMDCs from autoimmune-prone mice.

The influence of hCG on systemic autoimmune responses

hCG enhanced the maturation of bone marrow-derived dendritic cells driven by ssRNA (a TLR ligand implicated in lupus), as assessed by cell-surface phenotype and the ability to induce allogeneic proliferative responses. hCG preferentially enhanced the pro-proliferative effects of LPS in splenic B cells derived from autoimmune-prone mice and significantly increased mixed lymphocyte-induced proliferative responses. While synergistic increases in IL-6 and IL-10 upon anti-CD3 and hCG stimulation occurred in both autoimmune and non-autoimmune murine strains, enhancements in T cell receptor-induced proliferative effects and associated increases in the phosphorylation of ERK, p38 and AKT were preferentially observed in autoimmune mice, as was the heightened generation of autoantibodies to apoptotic blebs.

The influence of apoptotic blebs and apoptotic cell-specific antibodies on BMDC generation and maturation

Purified blebs potently inhibited the generation of bone marrow-derived dendritic cells (BMDCs), an effect more severe in autoimmune-prone animals. Blebs were also more effective than healthy cell lysate in the induction of BMDC maturation. BMDCs matured in the presence of blebs produced heightened levels of inflammatory cytokines and mediated higher allogeneic proliferative cytokine responses. Previous work had described some characteristics of monoclonal apoptotic cell-specific antibodies, including the ability to induce hyper-gammaglobulinemia and expansion of the autoantibody repertoire upon immunization into syngeneic, autoimmune-prone animals. Much like apoptotic blebs, such autoantibodies also induced both the maturation BMDCs as well as the secretion of inflammatory cytokines.

Publication

Original peer-reviewed article

1. Bose A, Huhtaniemi I, Singh O*, Pal R* (2013) Synergistic activation of innate and adaptive immune mechanisms in the treatment of gonadotropin-sensitive tumors. *PLoS ONE* **8**: e61288.

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Rajni Rani

Study of genetic and immune factors associated with autoimmune disorders: Type1 diabetes and vitiligo

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The project aims to decipher the immunogenetic and autoimmune factors involved in the destruction of pancreatic beta cells and melanocytes in Type 1 diabetes (T1D) and vitiligo respectively.

Type 1 diabetes

Genetic basis of Type 1 diabetes

Three SNPs in LMP2 and LMP7 genes were studied in a large number of cases and controls and the results revealed that the SNP in LMP7 exon 2 that results in an amino acid change from Glutamine to Lycine (Q49K) at position 49 showed a significant increase which was independent of the predisposing MHC alleles suggesting a role of antigen processing genes in T1D manifestations.

Mesenchymal stem cell treatment of non-obese diabetic mice

Since the expression of immunosuppressive genes indoleamine 2,3 dioxygenase (IDO) and inducible nitric oxide synthase (iNOS) were enhanced in TNF-α and IFN-γ treated mesenchymal stem cells (MSCs), we treated non-obese diabetic (NOD) mice with these MSCs and followed them up for 32 weeks. While 40% of the mice in the control group remained non-diabetic, 100% of the NOD mice in the treated group remained non-diabetic for 32 weeks (Figure 1).

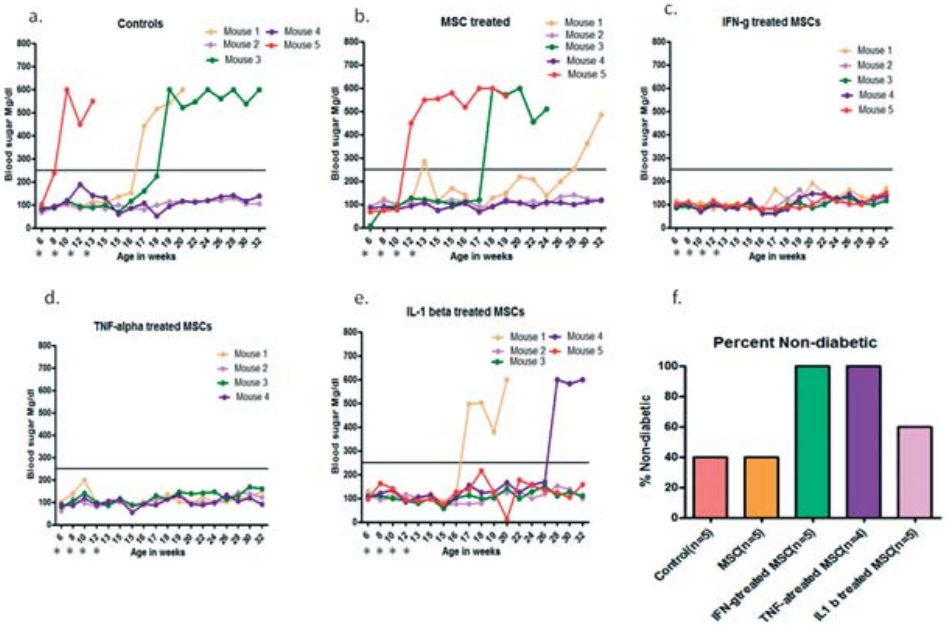

Figure 1. Blood glucose levels of control NOD mice (a), NOD mice treated with MSCs (b), NOD mice injected with IFN-γ treated MSCs (c) NOD mice injected with TNF-α treated MSCs (d), NOD mice injected with IL-1 β treated MSCs (e) and Percent non-diabetic mice (f)

Vitiligo

Micro-array analysis results obtained from keratinocytes treated with cytokines increased in vitiligo have been validated using real time PCR that confirmed that cell proliferation genes, cyclin and CDK genes were down-regulated and CDK inhibitors CDKN1A, CDKN1B, CDKN2A, CDKN2B were up-regulated with combined treatment of IFN-γ and IL-17A. Propidium iodide staining confirmed cycle arrest both at the G1/S transition, and at the G2/M transition. With respect to keratinocyte differentiation, basal keratinocyte markers KRT5 and KRT14 were down-regulated, while abnormal keratinocyte differentiation marker KRT16 and ITGA2 and cornification marker IVL were up-regulated (Figure 2).

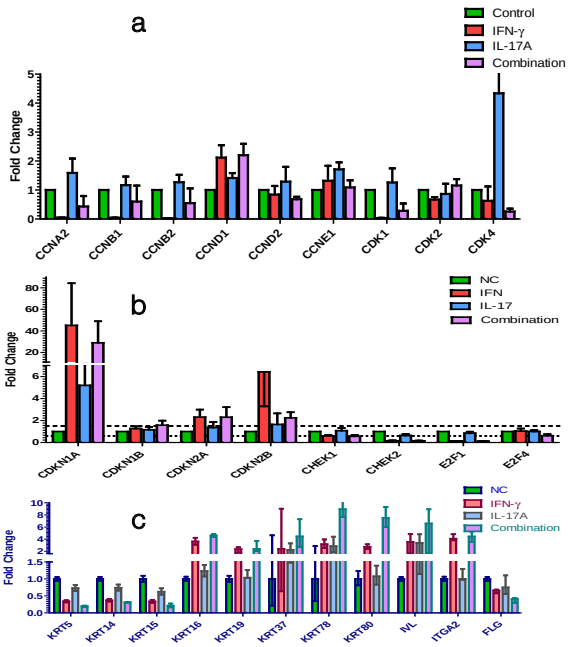

Figure 2. a. Real time PCR for cyclin and CDK genes showing downregulation of cyclins CCNA2, CCNB1, CCNB2 CCNE and their catalytic partners CDK1 and CDK2 and CDK4 with combined treatment of IFN-γ and IL-17A. (N=3). **b**. IFN-γ and combination treatment of IFN-γ and IL-17A upon keratinocytes resulted in the up-regulation of CDK inhibitors CDKN1A, CDKN1B, CDKN2A, CDKN2B, while cell cycle check point genes CHEK1, and CHEK2 were down-regulated, and E2F1 S-phase gene was down-regulated as well. **c**. Alteration in keratins and keratinocyte differentiation markers by IFN-γ and IL-17A: Real time PCR for keratin and keratinocyte differentiation markers after NHK treatment with IFN-γ and IL-17A for 48 hrs. Basal keratinocyte markers KRT5 and KRT14 are down-regulated, while abnormal keratinocyte differentiation marker KRT16 and ITGA2 and cornification marker IVL are up-regulated.

At translational level, vitiligo patients were transplanted with autologous epidermal cells and pure melanocyte cultures bilaterally in 30 patients and the results show significantly better pigmentation in the sides treated with pure melanocyte cultures. This work was done in collaboration with Dr. Kar from RMLH.

Publications

Original peer-reviewed articles

1. Natarajan VT, Ganju P, Singh A, Vijayan V, Kirty K, Yadav S, Puntambekar S, Bajaj S, Dani P, Kar HK, Gadgil CJ, Natarajan K, Rani R, Gokhale RS. (2014) IFN-**γ** signaling maintains skin pigmentation homeostasis through regulation of melanosome maturation. *Proc Natl Acad Sci U S A.* **111**:2301-2306

#2. Saini C, Prasad HK, Rani R, Murtaza A, Misra N, Shankar Narayan NP, Nath I. (2013). Lsr 2 of Mycobacterium leprae and its synthetic peptides elicit restitution of *in vitro* T cell responses in erythema nodosum leprosum and reversal reactions in lepromatous leprosy patients. *Clin Vaccine Immunol* **20**:673-682.

Review/Proceeding/Chapter

1. Rani R*, Singh A.(2014) Functional implications of MHC associations in autoimmune diseases with special reference to Type1 diabetes, Vitiligo and Hypoparathyroidism. Accepted for publication in the book, "HLA & Associated Human Diseases", Book edited by Yongzhi Xi. ISBN 980-953-307-1107-5. InTech Open Access Publishers.
2. Rani R (2013) MHC Applications. In W. Dubitzky, O. Wolkenhauer, K. Cho & H. Yokota (eds.), Encyclopedia of Systems Biology, Springer New York. Vol. 3 , 2013: 1158-1162.

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Sangeeta Bhaskar

Study of immunotherapeutic potential of *Mycobacterium indicus pranii* (MIP) and the underlying mechanisms in animal models of tuberculosis and tumor model

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The present study aims to investigate the protective efficacy of MIP immunisation in live or killed form, through parenteral route as well as by aerosol immunization, against subsequent infection with *M.tuberculosis* in animal models. Study of immune response to *M.tb* in animals immunised with MIP as compared to those immunized with BCG. Evaluation of immunotherapeutic efficacy of MIP along with chemotherapy in animal infection models.

Another objective is to evaluate immunoprophylactic and immunotherapeutic activity of MIP in mouse syngeneic tumor model. Study of MIP as an adjunct to chemotherapy in combination with commercial anti cancer drugs in tumor bearing mice and simultaneous study of mechanism of MIP mediated host immune activation.

A. Immunotherapeutic potential of MIP and the underlying mechanisms in mouse tumor model

Ex-vivo studies with macrophages and dendritic cells showed that TLRs has major role in MIP mediated activation of these cells. Tumors were implanted in MyD88 knockout and wild type mice and MIP treatment was given by peritumor injections. While in wild type mice treated with MIP about 40-50% mice had no visible tumor or very small tumor, MIP treated MyD88 knockout mice had tumors comparable to PBS treated mice. These results provide evidence of major role of TLRs in MIP mediated protection against tumor. Further studies with TLR knockout mice strains showed that TLR2 has important role in MIP mediated protection. TLR2 knockout mice treated with MIP showed no reduction in tumor volume whereas MIP treated TLR4 knockout mice had tumor volumes comparable to wild-type MIP treated mice.

B. Protective efficacy of MIP against tuberculosis and mechanistic insights as compared to BCG.

Evaluation of immunostimulatory activity of different fractions of MIP

Differential immunostimulatory activity of cellular fractions of MIP was investigated. Cell wall fraction showed highest immune stimulating activity. It was further fractionated into aqueous soluble and lipid soluble parts and determined their immune stimulatory activity in terms of macrophage and T cell activation. Aqueous extract of MIP cell wall was found to be very potent immune stimulator. Mycobacterial cell walls have potent TLR agonistic activity hence, to analyse the TLR agonistic activity of MIP cell wall fraction, immune response in the presence of specific pharmacological inhibitors of TLRs was studied. About 20% reduction in secretion of major proinflammatory cytokine was observed with TLR4 inhibitor, which further increased to almost 50% when a combined TLR2 and TLR4 inhibitor was added to MIP cell wall treated macrophages. As chemical inhibitors are not 100% efficient for blocking the signal transduction through a particular receptor, we repeated the experiments with macrophages isolated from TLR2 and TLR4 knock out mice. About 75% reduction in secretion of major proinflammatory cytokine was observed in TLR2 knock out mice as compared to the wild type. Reduction in cytokine secretion was about 30% in TLR4 knock out mice. This data provide evidence of strong TLR2 agonistic activity and moderate TLR4 stimulating property of MIP cell wall fraction.

C. Efficacy of MIP as a booster to BCG: Immunogenicity, protection and safety study when given by aerosol route in animal models.

BCG is effective against severe form of childhood tuberculosis however, effective protection from TB in adults is still a challenge which indicates that there is a need to boost the protective immune response against *M.tb.* However, paradoxically, multiple doses of BCG have resulted in reduced protection and poor survival in the susceptible animal model. Vaccination with MIP gives protection against TB in both BCG responder & non-responder strains of mice. MIP has shown higher protective efficacy as compared to BCG in animal models. Hence, it was proposed that a booster with MIP may enhance the protective immunity in animals primed with BCG. The intranasal route is proposed to induce both local and systemic immunity and provides a non-invasive delivery system intended to target lung. Hence, it is aimed to evaluate the efficacy of MIP administered through aerosol as booster to BCG vaccine in animal model. Initial results have shown higher protection in terms of lung and spleen bacterial load in guinea pigs given MIP booster, two months after BCG priming as compared to only BCG vaccinated animals. Further experiments to confirm the findings are underway. We are also analyzing the lung immune response in the above groups of animals.

Publication

Original peer-reviewed article

#1. Roy A, Singh M, Upadhyay P*, Bhaskar S* (2013). Nanoparticle mediated co-delivery of Paclitaxel and a TLR-4 agonist leads to tumor regression and enhanced immune response in the tumor microenvironment of mice. *Int J Pharm* **445**:171-180.

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#In press last year, since published.

Soumen Basak

Developmental LTβR reinforces TLR4 triggered inflammatory RelA/NF-κB responses to ameliorate the pathogen clearance in the gut

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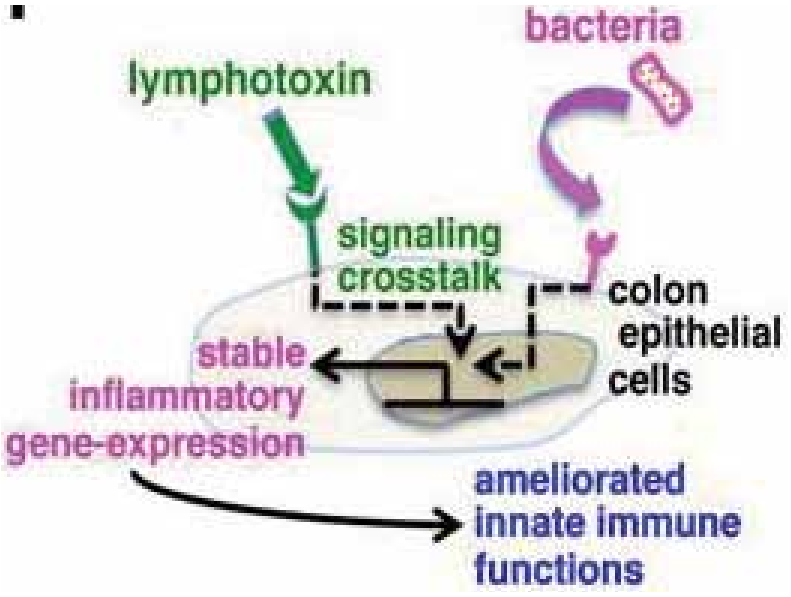
Tissue microenvironment functions as an important determinant of the inflammatory response elicited by the resident cells. Yet, the underlying molecular mechanisms remain obscure. Our systems-level analyses identified a duration code that instructs stimulus specific crosstalk between TLR4 activated canonical NF-κB pathway and lymphotoxin-κ receptor (LTβR) induced non-canonical NF-κB signaling. Indeed, LTβR costimulation synergistically enhanced the late RelA/NF-κB response to TLR4 prolonging NF-κB target gene-expressions. Concomitant LTβR signal targeted TLR4-induced newly synthesized p100, encoded by *Nfkb2*, for processing into p52 that not only neutralized p100 mediated inhibitions, but potently generated RelA : p52/NF-κB activity in a positive feedback loop. Finally, *Nfkb2* connected lymphotoxin signal within the intestinal niche in reinforcing epithelial innate inflammatory RelA/NF-κB response to Citrobacter rodentium infection, while *Nfkb2*$^{-/-}$ mice succumbed to gut infections owing to stromal defects. In sum, our results suggest that signal integration via the pleiotropic NF-κB system enables tissue microenvironment derived cues in calibrating physiological responses.



The proposed model depicting the regulatory role of *Nfkb2* in integrating lymphotoxin signals within the intestinal niche to prolong innate inflammatory NF-κB responses to gut pathogens.

Amitabha Mukhopadhyay

Hemoglobin receptor is a potential vaccine candidate against visceral *leishmaniasis*

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Previously, we have shown that *Leishmania* acquires heme for their survival from the degradation of endocytosed hemoglobin mediated through a specific receptor (HbR) present on the surface of the parasite. In the reporting period, we have shown that immunization with HbR-DNA induces complete protection against virulent *Leishmania donovani* infection in both BALB/c mice and hamster. We have found that HbR-DNA immunization stimulates the protective Th1 (IFN-γ, IL-12, and TNF-α) with concomitant suppression of disease promoting Th2 response (IL-10 and IL-4). In addition, HbR-DNA immunized animals also produces anti-HbR antibody and thereby blocks hemoglobin endocytosis in parasites. Thus, HbR-DNA immunization triggers all arms of protective immune response and renders sterile protection against virulent *Leishmania donovani* challenge in animals. These findings demonstrate that HbR is a potential vaccine candidate against visceral *leishmaniasis*.

It is now well evident that several intracellular pathogens modulate host cellular machinery for their survival in the host cells through some of their own proteins, collectively known as effectors.

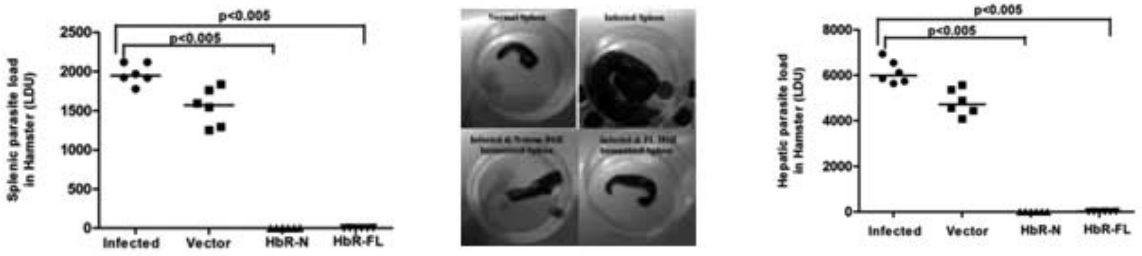

Fig.1. HbR-DNA vaccination renders sterile protection against virulent Leishmania donovani challenge in hamsters

Earlier, we have identified and determined the role two such effectors namely SopE and SipC from *Salmonella* which modulate host cell trafficking pathways by recruiting Rab5 and Syntaxin6, respectively. In the reporting period, we have identified another effector protein of *Salmonella* which specifically binds with host syntaxin 8. Bioinformatic analysis of SipA has shown that this effector protein consists of a N-terminal domain ($SipA^{1-435}$) which is subdivided into Chaperone binding domain (1-267aa), SNARE domain (180-242aa), putative transmembrane domain (250-265aa) and a caspase 3 cleavage site DEVD (432-435aa) and a C-terminal domain (436-685aa). Accordingly, we have made several truncated mutants of SipA with N-terminal His6; SipA1-169, $SipA^{1-242}$, $SipA^{1-277}$, $SipA4^{8-277}$, $SipA^{436-685}$. Our results have shown that SipA1-277 which contains putative transmembrane domain, binds with GST-Syntaxin8. However, $SipA^{1-169}$ which lacks SNARE domain, unable to bind with GST-Syntaxin8 indicating possibly SipA acts as a cognate SNARE of Syntaxin8. Currently, we are trying to determine the role of such interaction in the trafficking of *Salmonella* in the host cells.

Publications

Original peer-reviewed articles

1. Guha R, Gupta D, Rastogi R, Vikram R, Krishnamurthy G, Bimal S, Roy S, Mukhopadhyay A* (2013) Vaccination with *Leishmania* hemoglobin-receptor-encoding-DNA protects against visceral Leishmaniasis. *Science Transl. Med.* **5**: 202ra121.
2. Ezougou CN, Ben-Rached F, Mos DK, Lin J, Black S, Knuepfer E, Green JL, Khan SM, Mukhopadhyay A, Janse CJ, Coppens I, Yera H, Holder AA, Langsley G* (2014) Plasmodium falciparum Rab5B is an N-terminally myristoylated Rab GTPase that is targeted to the parasite's plasma and food vacuole membranes. *Plos One* **3**: e87695.

Patent

1. Mukhopadhyay A, Roy S, Gupta D, Guha R and Rastogi R (2013) Hemoglobin receptor as novel vaccine for leishmaniasis. *(Application No. 1449/DEL/2013; Indian Patent Filed on May 15, 2013)*.

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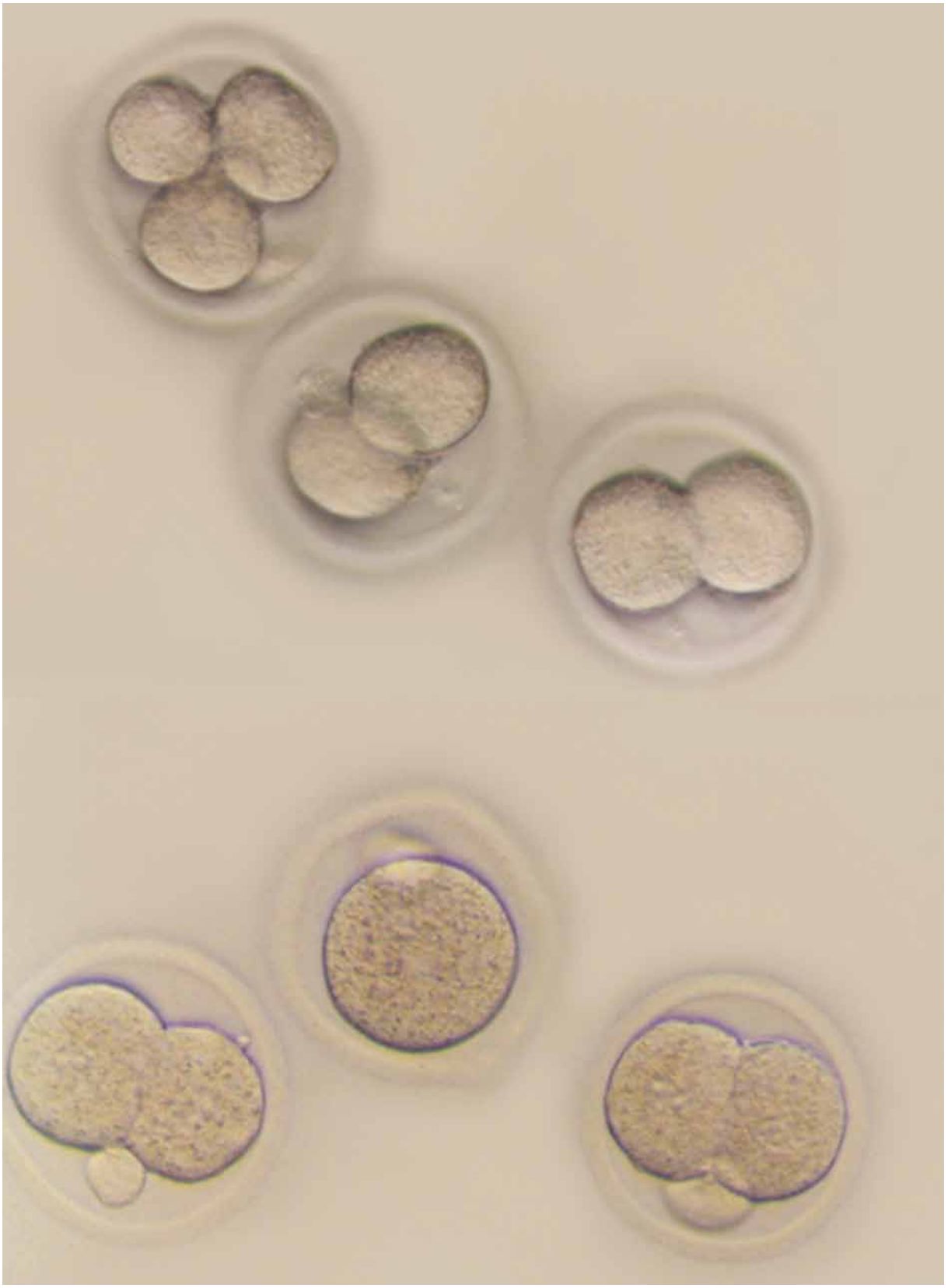

Mouse embryos

REPRODUCTION AND DEVELOPMENT

Subeer S. Majumdar

Studies of Sertoli cells and spermatogonial stem cells of the testis and other endocrinology related research

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Samir Bhattacharya, Vishwabharti, Shantiniketan

Objectives

1. To exploit spermatogonial stem cells of testis for insertion and propagation of transgene through several generations in an attempt to over express or knock down specific genes.
2. To undertake gene expression studies of rat, mice and monkey Sertoli cells to identify factors important for induction of spermatogonial stem cell division and differentiation in the testis.
3. To study biology of spermatogonial stem cells and to use germ cell transplantation technique for restoration of fertility following chemotherapy.
4. To study paracrine and endocrine modulation of signal transduction in target cells of the endocrine system.

Functional genomic studies of genes selected from studies of differential genomics by DNA microarray using mRNA from Rat Sertoli cells.

We cultured rat Sertoli cells (Sc) from 5 days and 12 days old rat testes to compare genes expressed during spermatogenically inactive and active phase of the testis, respectively. Earlier, we have undertaken such studies using Monkey Sc and after using tools of bioinformatics, selected genes and made transgenic mice (different species). Now, we used rat Sc as a source of information and generated transgenic rats to study functional genomics.

In rats, in spite of sufficient level of circulating hormone, since birth, the division and differentiation of spermatogonia do not occur until, 11-12 days of age. In our study, we evaluated the differential gene expression of Sc cultured from infant and pubertal rat. In view of this hypothesis, we choose microarray technique to study the differential transcriptome related to robust onset of spermatogenesis. Sc were cultured from infant (5 day old) as well as pubertal (12 day old) rats and RNA from such Sc was further used for DNA microarray experiment

utilizing the Agilent platform. Three sets of hormone treated infant and pubertal Sc were hybridized independently to account for culture to culture variation. DNA microarray reads were normalised and processed by Genespring Gx software to generate comparative transcriptome profile of hormone treated infant and pubertal Sc. Three such data sets, representing independent microarray analysis of hormone treated infant and pubertal Sc, were combined to generate a general transcriptomic profile wherein genes which were over expressed in Sc during infancy as well as genes which were over expressed in pubertal Sc were adequately represented. Specific signalling pathways and biological processes that were differentially expressed in infant and pubertal rat Sc were identified by *in silico* analysis of the combined microarray analysis. Differential gene expression was observed in various biological processes. However, our study was aimed at evaluating few genes which may have relevance to Sc mediated process of initiation of Gc division and differentiation. To this end, we analyzed differentially expressed pathways which are known to influence cell division and differentiation. We found 2003 genes as up regulated in 12 day old rat Sc as compared to 5 day old rat Sc and 2205 genes as down regulated in 12 day old rat Sc as compared to 5 day old rat Sc.

However, to be certain that selected genes were truly up or down regulated at the transcript level, RT PCR of Sc mRNA was performed. We found that certain genes like EII Associated Factor (eaf2), Ninjurin 2 (ninj2), Neuramedin (nmu), Nuclear receptor 4A3 (Nr4a3) were up regulated in pubertal rat Sc as compared to infant rat Sc. Sostdc1, Angiotensin type 2 receptor (Agtr 2), Extracellular growth factor ligand 3 (Egfl3), Tetraspanin 8 (Tspn8) were found to be down regulated in pubertal rat Sc as compared to infant rat Sc. Eaf2 is positive regulator of RNA Pol II, Ninj2 is a cell- cell adhesion molecule, Nmu is involved in energy homeostasis, Nr4a3 is a orphan nuclear receptor, Sostdc1 is a Wnt and BMP inhibitor, Agtr 2 is a receptor for apoptosis, Tspn 8 is an inhibitor of differentiation whereas Egfl3 is a ligand for EGF pathway.

To undertake functional genomics study of the differentially expressed genes obtained from rat microarray, transgenic rat models are being generated. We wish to over express specific genes at an age when they have limited or no expression, naturally. We have made constructs using murine PEM proximal promoter (a kind gift from M. Wilkinson) because its expression occurs in Sc of the pubertal testis only, but Pem expression is low or absent in the infant testis. Agtr 2, Tspn8 and Sostdc1 naturally having low expression in Sc during puberty were cloned with a flag tag at their c- terminal region downstream of PEM promoter to make transgenic rats for evaluating regulation of spermatogenesis by these genes.

Endocrine signaling

Sertoli cell: We have demonstrated that unlike pubertal testicular Sertoli cells (Sc), infant Sc fail to produce substantial amount of cAMP upon FSH treatment. In an effort to understand why cAMP production is restricted in the infant Sc, we attempted to divulge molecular basis of this deficiency in the signal transduction. In this study, we have compared the FSH-R mediated signaling events in Sc of infant and pubertal rhesus monkeys. We found that expression of GαS and its activator, Ric8b are very low in infant Sc. This may be responsible for suboptimal cAMP generation and insufficient expression of spermatogenically relevant genes by infant Sc, in spite of sufficient expression of FSHR, circulating levels of FSH and their binding to Sc. We also found that levels of Gαi increased upon FSH treatment in infant Sc unlike pubertal Sc. Our experimental observations

suggested that infant Sc are as competent as pubertal Sc for transcriptional events related to spermatogenesis, when sufficient amount of cAMP is generated inside these cells by treatment with pharmacological agents like forskolin and 8Bromo-cAMP.

This observation in primates, generates a remarkable scope for generating advanced Gc, *in vitro*, using a fraction of seminiferous tubules from infertile individuals displaying FSH resistance. Intracellular cAMP in Sc of such tubules may be augmented by treatment with forskolin or supplementation with 8Bromo-cAMP which may lead to robust transcription of genes conducive to generation of advanced Gc which may be used for assisted reproduction. This may become a new approach to enable an infertile individual to father a child from his own genome, when hormonal therapy fails. A set of new information generated about some signal transduction mediator molecules of primate Sc by us may provide basis for diagnosis and treatment of certain forms of idiopathic male infertility.

Adipose tissue: We have shown earlier that Fetuin A is necessary for Free fatty acids (FFAs) mediated augmentation of adipose tissue inflammation through the TLR4 pathway which causes insulin resistance. Macrophage infiltration into adipose tissue during obesity and their phenotypic conversion from anti-inflammatory M2 to proinflammatory M1 subtype significantly contributes to develop a link between inflammation and insulin resistance; signaling molecule(s) for these events, however, remains poorly understood. Studies in Prof Bhattacharya's lab with our collaboration demonstrated that excess lipid in adipose tissue ambience may trigger one such signal. Adipose tissue from obese diabetic *db/db* mice, high fat diet (HFD) fed mice and obese diabetic patients showed significantly elevated fetuin-A (FetA) levels compared to their controls; partially hepatectomized HFD mice did not show noticeable alteration indicating adipose tissue to be its source. In adipocytes, fatty acid (FA) induces FetA gene and protein expressions resulting in its copious release. We found that FetA could act as chemoattractant for macrophages. To simulate lipid induced inflammatory condition when proinflammatory adipose tissue and macrophages create a niche of altered microenvironment, we set up transculture system of macrophages and adipocytes; addition of FA to adipocytes released FetA into the medium which polarized M2 macrophages to M1. Taken together, lipid induced FetA from adipocytes is an efficient chemokine for macrophage migration and polarization. These findings opened a new dimension for understanding obesity induced inflammation.

Germ Cell transplantation

We have generated a new technique for evacuating testicular germ cell niche without compromising bone marrow, for studies related to germ cell transplantation for restoration of fertility, post chemotherapy. Using this model, we have started doing homologous germ cell transplantation post *in vitro* expansion of spermatogonial stem cells.

Publications

Original peer-reviewed articles

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Patent

1. Majumdar SS, Ganguli N and Usmani A (2013). Isolation, Cloning, Sequencing and Functional analysis of ß-casein promoter along with the regions of exon1, intron1 and exon2 using mammary gland derived cell line of Buffalo. (Bubulus bubalis) *(Application No. US 13/898137; Filed on May 20, 2013).*

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Cellular and molecular biology of human cancer

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Over the last three decades, knowledge on molecular biology of human cancers has vastly expanded. A host of genes and proteins involved in cancer development and progression have been identified and many mechanisms at the molecular, cellular and even tissue level have been, at least partly, elucidated. In fact, cancer research has now reached a critical stage, in which the accumulated knowledge on molecular mechanisms ought to be translated into improved prevention, diagnosis, and treatment.

Breast cancer is the most common cause of cancer-related deaths among women worldwide, with highest mortality incidence in developing countries. Recent studies have documented that breast cancer disease is a resultant of accumulation of genomic and epigenomic alterations resulting in reduced apoptosis, unchecked proliferation, increased motility and invasion abilities and metastasis in various other distant sites. Recently, we reported an association of sperm-associated antigen 9 (SPAG9) expression, a new member of CT antigen family, in early stages of breast cancer

patients. Collectively, our data suggested that SPAG9 could be playing a potential role in various malignant properties of breast tumorigenesis.

Metastasis is a complex process involving multiple steps including epithelial mesenchymal transition (EMT) and mesenchymal epithelial transition (MET) resulting in migration, invasion, colony forming abilities and subsequently tumor growth at distant sites. In this context, it is important to investigate genes and gene products involved in early spread, tumor progression and metastasis Majority of the breast cancer patients express proteins such as estrogen receptor (ER) and progesterone receptor (PR) for which targeted hormone therapy is available with better clinical outcome. In addition, around 15-20% patients express human epidermal growth factor receptor 2 (HER2) protein, for which effective trastuzumab therapy is available with good prognosis. In contrast, around 15% of diagnosed breast cancers are designated as triple-negative and are characterized as ER negative (ER-), PR negative (PR-) and HER2 negative (HER2-). Triple-negative breast cancers patients represent an important clinical challenge because these patients do not respond to endocrine therapy or any other available targeted agents.

In the present study, we investigated the SPAG9 expression in four breast cancer cell lines of various subtypes, harbouring different hormone receptors, such as MCF-7 (luminal-A, ER+ PR+ Her2-), BT-474 (luminal-B, ER+ PR+ Her2+), SK-BR-3 (HER2 overexpressing, ER- PR- Her2+) and MDA-MB-231 (highly metastatic basal, triple-negative ERPR-Her2-). Our analysis revealed that SPAG9 expression was found in all breast cancer cell line models used in the present study [MCF-7 (ER+/PR+/Her2- luminal-A subtype), SKBR- 3 (ER-/PR-/Her2+ ERBB2 associated subtype), BT- 474 (ER+/PR+/Her2+ triple-positive luminal-B subtype) and MDA-MB-231 (ER-/PR-/Her2- triple-negative basal subtype)]. Further, involvement of SPAG9 was investigated both *in vitro* and *in vivo* on various malignant properties [cellular proliferation, colony forming ability, migration and invasion] in triple-negative MDA-MB-231 cells, employing plasmid-based small interfering RNA (siRNA) approach. Small interfering RNA mediated gene silencing approach was used to selectively knock down SPAG9 to study its role in various malignant properties. Highly aggressive triple-negative basal subtype MDA-MB-231 cells were used for *in vitro* and *in vivo* gene silencing studies.

In conclusion, to the best of our knowledge, this is the first report where we have put forth an evidence of potential role of SPAG9 in cellular growth, migration, invasion and colony forming ability in highly aggressive triple-negative MDA-MB-231 breast cancer cells. In addition, *in vivo* xenograft studies further strengthen the role of SPAG9 in breast cancer. Our study provides an association between SPAG9 expression and its potential role in breast cancer, and thus lays a foundation for developing a promising therapeutic target for triple-negative breast cancer.

Publications

Original peer reviewed articles

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3. Saini S, Agarwal S, Sinha A, Verma A, Parashar D, Gupta N, Ansari AS, Lohiya NK, Jagadish N, Suri A* (2013) Gene silencing of A-kinase anchor protein 4 inhibits cervical cancer growth *in vitro* and *in vivo*. *Cancer Gene Therapy* **20**: 413-420.

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#5. Agarwal S, Saini S, Parashar D, Verma A, Jagadish N, Batra A, Suri S, Bhatnagar A, Gupta A, Ansari AS, Lohiya NK, Suri A* (2013) Expression and humoral response of a-kinase anchor protein 4 in cervical cancer. *Int J Gynecol Cancer* **23**:650-658.

Patent

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Asok Mukhopadhyay

Study on expansion and plasticity of bone marrow stem cells

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Stem cells niche controls self-renewal and lineage commitment of the progenitor cells. It has been show by us and others that the fate of hematopoietic or mesenchymal stem cells can be altered if exposed to different microenvironmental conditions. Therefore, it is utmost important to critically analyze how a niche control its own stem cells or the stem cells of the other adult tissues for fate change. We intend to dissect hematopoietic stem cell niche to understand its control over the self-renewal of stem cells, and how bone marrow-derived stem cells respond to different pathological conditions of the solid organs. Thus, overall goals of the laboratory are:

1. Study of molecular control in self-renewal and differentiation of HSCs,
2. Study of mechanism for the involvement of marrow stem cells in regeneration of liver under various pathological conditions,
3. Role of BM cells in the progression of ovarian cancer, and
4. Molecular analysis for transdifferentiation of MSCs into dopaminergic neurons

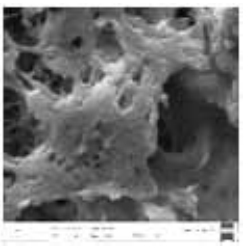
A. Hematopoietic stem cells niche

We are working on elucidating the role of amphiregulin (*Areg*) in maintaining of HSCs in murine bone marrow. Knock-down experiments suggest that *Areg*-mediated activation of ErbB pathway is crucial for Stat5 expression for the gain of anti-apoptotic properties in HSCs. A

known inhibitor of ErbB pathway, pimozide, has also shown similar results. Down-stream gene expression analysis has validated anti-apoptotic role of Areg. All together, these results implicate an active role of ErbB pathway for conferring protection to HSCs from apoptosis before they undertake self-renewal or differentiation.

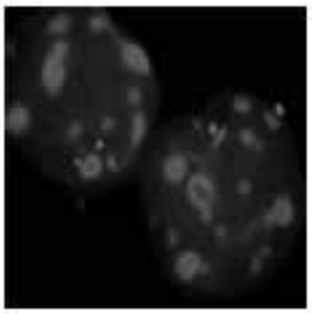
B. Plasticity of BM stem cells

In past couple of years, our group has been engaged in liver regeneration studies by BM-derived stem cells in different diseased models in mouse, like hemophilia A, Alpha1-antitrypsis deficiency and chemical-induced liver fibrosis. Interestingly, in all such diseased mice, a significant recovery has been observed, as marked by decline of collagen deposition, normal liver function, and phenotypic correction as per the test models.



SEM of fibrotic liver tissue after transplanting bone marrow-derived cells (magnification 10K)

The donor cells are found to be integrated in the liver tissue, primarily as hepatic cells. A part of them also contribute as liver sinusoidal endothelial and Kupffer cells in the recipient organ. XY chromosome FISH analysis reveale that the engrafted donor-marked hepatic cells are mostly (above 95%) possessed donor specific chromosomes.



X,Y FISH analysis show donor-derived hepatic cells only possess female (X, red) chromosome (magnification 1K)

Interestingly, these cells turned-off hematopoietic genes, whereas express hepatocyte-specific genes. In one such experimental model, cellular changes are found to be associated with distinct epigenetic changes with respect to donor cells, and the expression of various chromatin modifying enzymes.

In another project, we have found that transplantation of primed MSCs can recover more than 80% parkinsonian mice from typical apomorphin-induced contra-lateral rotation and motor neuron function deficit. Incidentally, the donor-derived cells have been identified in the striatum region of the brain. All these changes have been possible due to the elevation of tissue dopamine level in the recipient mice as compared with diseased mice.

C. Ovarian cancer

Studies of mouse ovarian cancer in the past lead us to carefully examining of the ascitic fluid of human ovarian cancer patients. Two major phenotypes of tumor cells have been identified for these samples, one that express only EpCAM (a surface marker of ovarian tumor) and another that express EpCAM with CD45 (putative hemato-

epithelial compartment). Systematic analyses of global gene expression profile reveal that many genes are up-regulated in the hemato-epithelial compartment as compared with EpCAM-expressing cells alone. We have hypothesized that over-expressed genes can cause the gain of functional properties in the hemato-epithelial compartment of human ovarian tumor.

Publications

Original peer-reviewed article

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Reviews

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Patent

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Cell Death Regulation

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Theme of Research

The overall theme of the research program is to elucidate the processes that influence cell death programs under varying physiological conditions in diverse model systems.

Objectives

Regulatory networks driving cell fate decisions are important to investigate in the context of understanding diseases. Broadly, our research programme explores the underlying mechanisms of cell survival and death in diverse intracellular and extracellular conditions.

Studies (Cell death in protozoan parasites) in the current year further confirm the requirement for calmodulin (CaM) for the transport of mTXNPx to the mitochondria through the use of reconstituted import assays. We also demonstrate the prevention of temperature driven mTXNPx aggregation in the presence of CaM. These findings further established the idea that CaM is required for the efficient transport of the protein to the mitochondria. In another project on the role of sterols in *Leishmania donovani*, we show that the major sterol is ergosterol and when anti-leishmanial drug antimony was given, the ergosterol level changed suggesting an effect of the drug on parasite sterols. Sterol biosynthesis inhibition induced parasite death suggesting the importance of the sterol increase in drug induced cell death. In the project of 'Mechanisms underlying cell death in cancer', we studied a complex formed between molecules from apoptosis and autophagy pathways as they present potential targets for chemotherapeutics

design. We demonstrate a new observation that p53 interacts with Beclin-1, an event that plays an important role in the determination of cell fate and the site of interaction was identified as the cytosol. We find that Beclin-1 levels are regulated by p53 through ubiquitination. Possible binding site of p53 to Beclin-1 was identified as 1-150 amino acid of Beclin-1. This region harboring the BH3 domain is essential for binding to other BH3 domain proteins and is therefore important for deciding how the cell will behave during changes in intracellular environments. When p53 and Beclin-1 do not interact there is lesser ubiquitination of Beclin-1. Therefore, we demonstrate a complex interplay between two proteins of the apoptosis and autophagy pathway where apoptosis was preceded by autophagy but autophagy was not required for apoptotic death. Autophagy inhibition pushed the cells towards apoptosis that was measurable in reduced tumor sizes *in vivo* and increased apoptosis of cells *in vitro*.

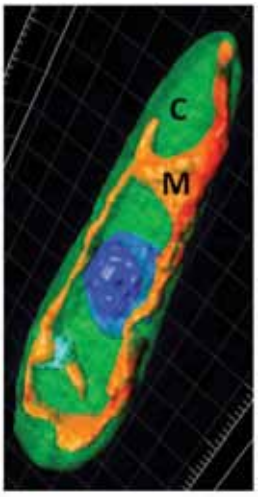

Fig. The single mitochondrion of *Leishmania donovani*

Publications

Original peer-reviewed article

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Review/Proceeding

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Cellular and molecular aspects of reproduction and viral infections

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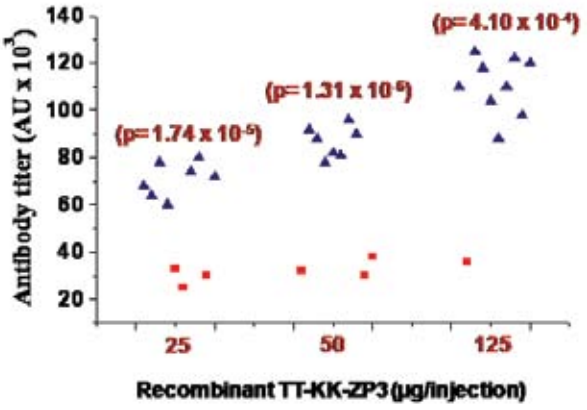
Dvelopment of contraceptive vaccine

Extending the previous year progress on developing the contraceptive vaccine for street dog population management, female mice immunized with varying doses of recombinant TT-KK-ZP3 [T cell epitope of TT (aa residues 830-844) followed by dilysine linker and dog ZP3 (aa residues 23-348)] adsorbed on Alum showed high antibody titers and significant curtailment of fertility ($p < 0.05$). An inverse correlation (r value > -0.8) was observed between antibody titer and number of pups born. Non-pregnant mice showed significantly higher anti-TT-KK-ZP3 antibody titers as compared to pregnant mice. Further, mice immunized with various *E. coli*-expressed recombinant immunogens encompassing sperm specific SP17 and Equatorin proteins revealed that TT-KK-Sp17(C-terminal) [T cell epitope of TT followed by dilysine linker and mouse Sp17 C-terminal (aa residues 76- 126)] led to significant inhibition of fertility.



Scatter plot of serum antibody titer of pregnant *vs* non-pregnant mice immunized with recombinant TT-KK-ZP3 adsorbed on alum: Blue triangles represent serum antibody titer of non-pregnant mice and red squares represent the same for pregnant mice. In all the three groups, non-pregnant mice showed significantly higher antibody titer as compared to pregnant mice.

Molecular mechanisms associated with trophoblast invasion and differentiation

To investigate the relative importance of STAT3 and ERK1/2 activation during LIF-mediated JEG3 cell invasion, LIF treatment led to activation of STAT3 and ERK1/2 signaling pathways. An increase in the expression of invasion-associated molecules like mucin 1 *(MUC1), Fos, Jun* etc was also observed. Abrogation of either STAT3 or ERK1/2 signaling reduced ($p < 0.05$) the LIF mediated invasion of JEG-3 cells and expression of both MUC1 and Fos; suggesting a common denominator in LIF-STAT3/ERK1/2 signaling. To this effect, we observed a decrease in LIF-mediated p-STAT3 ser727 upon blocking either STAT3 or ERK1/2 signaling suggesting that STAT3 ser727 phosphorylation play an important role in JEG-3 cell invasion.

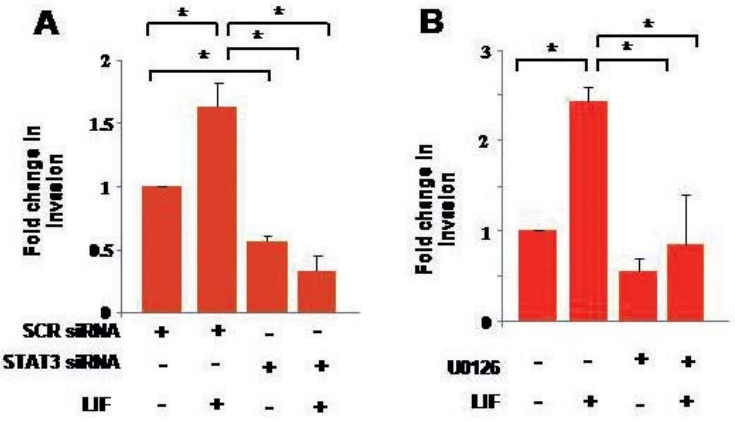
We also investigated the importance of hCG in BeWo cells fusion. The α- or β-hCG knocked-down BeWo cells on treatment with optimized concentration of hCG led to an increase in cell fusion, which however was significantly lower as compared to the control cells. The decrease in BeWo cell fusion was associated with decreased activation of p-PKA and p-CREB in the α- or β-hCG silenced cells.

Evaluation of anti-HIV-1 activity and pre-clinical safety of medicinal plants and semi-synthetic compounds

In our efforts to develop plant-based microbicides for prevention of sexually transmitted HIV-1 infection, the aqueous and 50% ethanolic extracts prepared from *Acocia catechu* as well as n-butanol fraction showed potent anti-HIV-1 activity against both X-4 as well as R-5-tropic viruses. The n-butanol fraction showed good HIV-1 protease inhibitory activity (IC_{50} = 12.9 µg/ml) and also interfered in the Tat-Long Terminal Repeat transactivation mediated HIV-1 transcription. The n-butanol fraction did not lead to any increase in pro-inflammatory cytokines secretion by vaginal keratinocyte cell line (Vk2/E6E7) and was non-deleterious to the intact monolayer formed by Caco-2 and HEC-1A epithelial cells. Using synthetic/natural compounds, potent anti-HIV-1 activity of prostratin, rosmarinic acid and epigallocatechin gallate has also been demonstrated.

Neutralizing monoclonal antibodies (MAbs) against influenza/bird flu virus

Last year, we reported the development and characterization of a humanized antibody with



Significance of STAT3 and ERK1/2 mediated signaling in LIF-mediated invasion of JEG-3 cells: Panels A and B represent the fold changes in invasion after LIF treatment as compared to negative control when STAT3 (siRNA) and ERK1/2 (UO126) mediated signaling pathways were compromised. Data is expressed as mean ± SE from 3 different experiments performed in duplicates.

potent neutralization of 2009 pandemic H1N1 influenza virus. This year, 6 murine MAb's against recombinant HA protein of H5N1 (A/turkey/ Turkey/1/2005) and 10 against recombinant HA protein of H7N9 (A/Anhui/1/2013) virus have been generated. Out of 6 MAbs generated against H5N1, 5 reacted to variable extent with HA protein of different strains of H5N1 and also the whole virus (A/Turkey and A/Vietnam) in ELISA. Interestingly, one of the MAb, MA-12 showed broad reactivity against H1N1, H2N2, H3N2 and H7N9 in addition to H5N1. The initial results using pseudovirus showed that MA-5 neutralized H5N1 A/Turkey virus. Out of 10 MAbs against H7N9, 8 were specific to H7N9 and 2 MAbs addition reacted with H1N1, H2N2 and H3N2.

Publications

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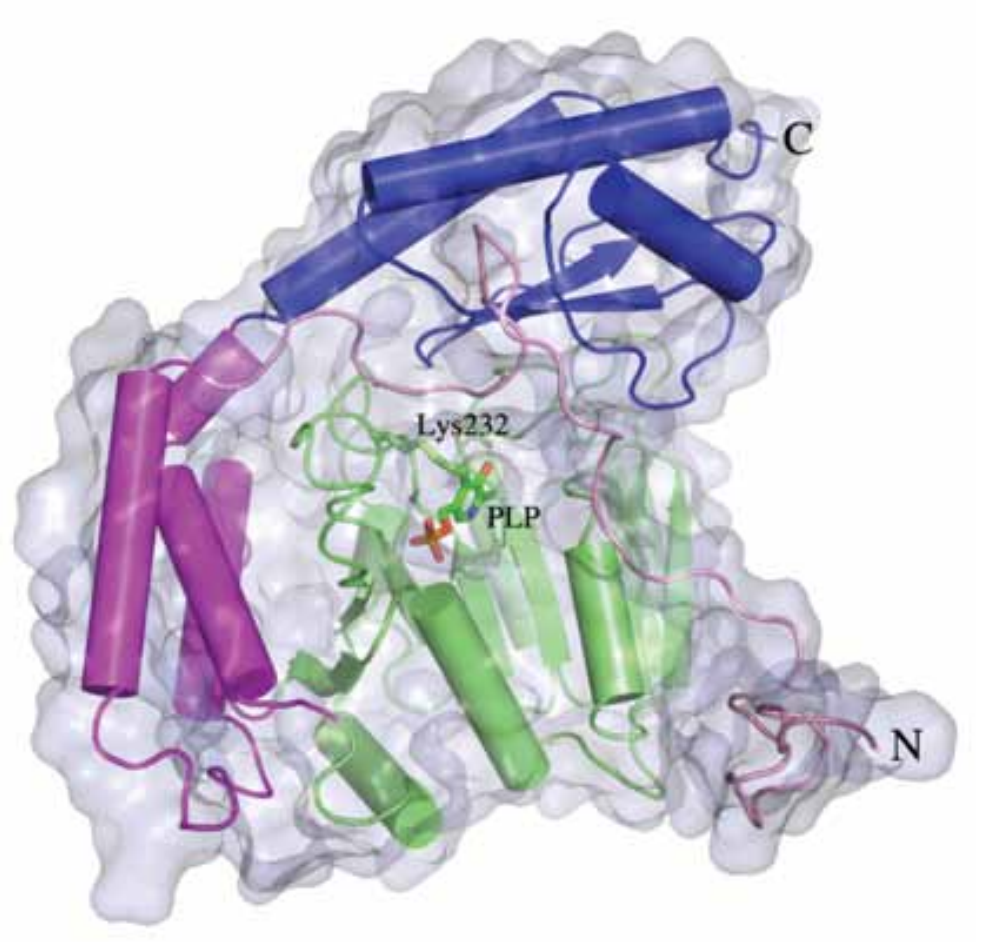
#In press last year, since published.



Monomeric 3D structure of HisC of *Mycobacterium tuberculosis*

MOLECULAR DESIGN

Monica Sundd

Structural studies on proteins, dynamics and ligand interactions using NMR

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The primary objective of our study is to characterize proteins using NMR and other biophysical techniques to understand their function. We are presently pursuing research in two major areas: A) Understanding the structure and function of the type II fatty acid biosynthesis pathway of *Leishmania* major and B) Structural characterization of the proteins involved in fatty acid metabolism. In the first project, we have completed the structure solution of *Leishmania major* holo-acyl carrier protein using NMR and also characterized its acyl-ACP intermediates. Our studies show that the holo-ACP of *Leishmania* is a four helix bundle protein, with a hydrophobic cavity that protects the acyl chain. Careful analysis of the NMR data on the acyl-intermediates led us to conclude that *Leishmania* type II pathway synthesizes in major part short acyl chains as the acyl intermediates longer than C_{8-} are extremely unstable. Figure. 1 shows a worm representation of *Leishmania* ACP highlighting the residues that display C_α and C_β chemical shift changes upon acyl chain elongation.

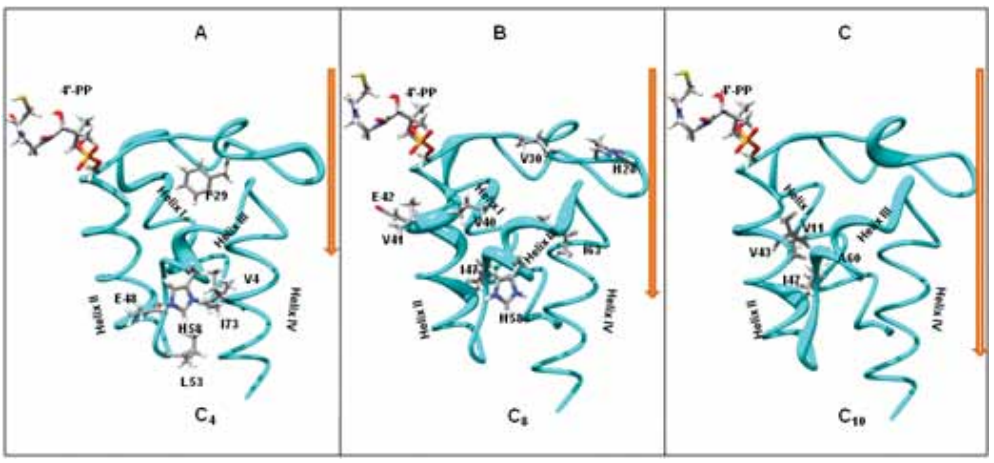

FIGURE 1. A worm representation of the LACP molecule displaying residues that undergo C_α and C_β chemical shift changes upon conversion to A. C_4 -B. C_{8-} and C. C_{10-} LACP. Thickness of the worm at every given point is directly proportional to the C_α chemical shift change. The residues experiencing changes in C_β atoms <2 SD are shown as sticks and labeled.

In order to understand the molecular basis of the unusual specificity of *Leishmania* ACP for its acyl chains, residues that are remarkably different compared to other type II ACPs were identified and mutated to the residues present in *E. coli* ACP. This involved mutations Leu35Ala, Asn36Asp, Phe45Met, His58Asp etc. and the samples were analyzed using NMR. Of these mutations, L35A failed to fold properly, suggesting an important role of Leu 35 in *Leishmania* ACP. Notably, this residue is conserved in all type I ACPs that do not form stable acyl-intermediates in solution. Rest of the mutants displayed a very similar acyl chain specificity equal to the wild type protein. Apart from these residues, we noticed seven additional hydrophobic residues in *Leishmania* ACP compared to other type II ACPs, most of them localized to the hydrophobic core. Combining our NMR data, mass spectrometry data and mutagenesis studies, we posit that the presence of the hydrophobic residues makes the cavity of the ACP rigid, thus preventing it from expansion during acyl chain elongation. As a consequence, *Leishmania* ACP can protect acyl chains only up to C8-. C_8-ACP is a precursor of lipoic acid synthesis, required for the functioning of several enzyme complexes. Based on our results, we speculate that this pathway has been designed by nature to primarily fulfil the lipoic acid requirement in the mitochondria.

In the project involving fatty acid metabolism, we have identified 3 ACBPs (putative acyl CoA binding proteins) that help in the dissemination of the acyl CoAs to their respective site. We have cloned, expressed and purified the three proteins of *L. major* in *E. coli*. A preliminary NMR spectra of the three proteins display good dispersion in an NMR spectrum. The chemical shifts of these proteins have been partly assigned. We also analyzed the binding affinity of these ACBPs towards standard acyl-CoAs like C_4-, C_8-, C_{10}-, C_{12}-, C_{14}- C_{16}-, C_{18}- and C_{20}-CoAs using ITC. The three ACBPs show very different affinities underscoring their importance in *Leishmania*.

Pramod K. Upadhyay

To develop strategies for making sensors and actuators for biological processes

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Differentiation of PBMCs to endothelial-like cells

Peripheral blood mononuclear cells (PBMCs) can be differentiated to endothelial like cell by culturing them in an appropriate angiogenic medium. Different approaches were investigated for differentiating human PBMCs into endothelial cells.

In the first approach, endothelial progenitor cells (EPCs) that are found in very minute amounts (around 0.04%) in the peripheral blood were differentiated into endothelial cells. EPCs were isolated by magnetically labeled antibody against CD133. The $CD133^{+}$ cells were then cultured in a fibronectin coated dish. Characterization of the differentiated cells were done by incorporating DiI labeled acetylated low density lipoprotein (DiI-acLDL). Figure. 1 shows the uptake of DiI-acLDL by differentiated endothelial cells.

In the second approach, the PBMCs were trans-differentiated into endothelial cells by culturing them in EGM-2 medium supplemented with angiogenic cytokines like VEGF, FGF, IGF. The characterization of the cells was done by RTqPCR, western blotting as well as immunocytochemistry. The RTqPCR analysis showed that after 30 days of culture there was a decrease in the

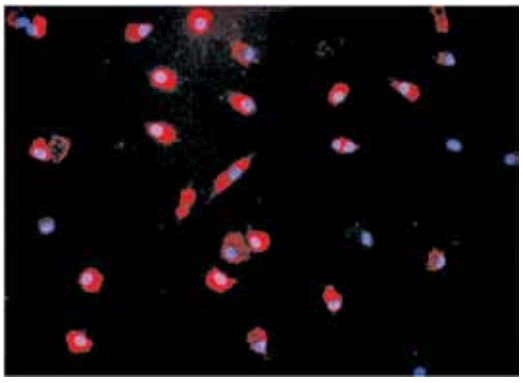

Figure 1 : Uptake of DiI-acLDL by differentiated endothelial cells.

expression of CD14, while there was an increase in the expression of various endothelial markers like CD31, VE-Cadherin, Von Willebrand factor (vWF), VEGFR1 and VEGFR2. The Ki67, a marker for proliferation, showed a 4 fold increase as compared to the Day 1 cells, indicating that the differentiated endothelial cells also show proliferation. The western blot of differentiated endothelial cells also confirmed the expression of vWF and VE cadherin which were absent in day 1 cells. The immunocytochemistry picture of differentiated endothelial cells expressing vWF is shown in Figure. 2.

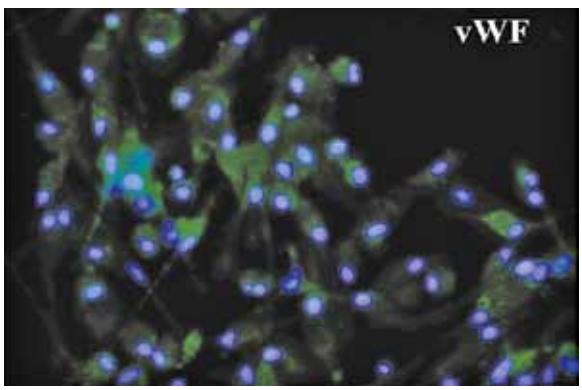

Figure 2 : Immunocytochemistry of differentiated cells for vWF at 20X.

In the third approach, a two steps procedure was followed for the differentiation of monocytes into endothelial cells. In the first step, the monocytes were de-differentiated into stem cell like cells. In the next step, the reprogrammed monocytes were again re-differentiated into endothelial cells by culturing them in medium containing endothelial cell growth supplement (ECGS) for 15 days.

In the RTqPCR analysis, similar to trans-differentiation results were obtained; however in the re-differentiation approach, the Ki67, a marker for proliferation, shows a 10 fold increase as compared to the Day 1 cells, indicating that the re-differentiated endothelial cells are highly proliferative.

Decellularization of a rat blood vessel

Decellularization of the blood vessel removes the cellular component of the graft leaving behind a collagen scaffold. Decellularization was carried out by running 1% triton-x 100 in MQ water through the vessel using a perfusion pump setup. The decellularized rat blood vessel was checked for its potential to elicit an immune response.

Hepatocytes like cells from PBMCs

The reprogramming of PBMCs was further investigated. It was observed that the concentration of serum played a critical role in the degree of 'acquired plasticity' in the Reprogrammed Monocytes (RM). Non activated monocytes were isolated from the peripheral blood of healthy volunteer and cultured with four different types of serums namely, autologous human serum (AHS), human cord serum (HCS), embryonic stem cell grade fetal bovine serum (ESC) and fetal bovine serum (FBS). After six days of culture, the cells were harvested and analysed by flowcytometry for the abundance of CD34 and CD117 (hematopoietic stem cell markers) on the cells having phenotype CD45+CD14+CD16+. The sample size was n= 5. The extent of 'acquired plasticity' in RM, as measured by the percentage abundance of CD34 and CD117, was highest by HCS followed by ESC, AHS and FBS. Similar observations were made in the reprograming of monocytes isolated from HBsAg+ve blood in which n=9.

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Original peer-reviewed articles

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1. Upadhyay P* (2013) Tuberculosis vaccine trials. *The Lancet*. **381**: 2253-2254.
2. Upadhyay P* (2013) New therapeutic approaches for airway hyperimmune response are required. *Indian Pediatr*. **50**:1087.

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Protease-catalyzed splicing of peptide bond

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Our laboratory studies principles and mechanisms associated with enzymatic peptide ligation reactions with a view to facilitate protein semisynthesis and construction of chemically defined bioconjugates that are not easily accessible to routine chemical or genetic approaches of protein engineering. Transpeptidase sortase of gram-positive bacteria has become an excellent synthetic tool in this enterprise. Currently, our group is engaged in studies related to (a) Sortase-mediated protein labeling and conjugation and (b) Structure, dynamics and function of Sortases.

Sortase-mediated protein labeling and conjugation

We had developed a bioorthogonal Sortase-Click reaction suite for defined assembly of multivalent proteins by coupling sortase-mediated ligation and azide-alkyne Huisgen cycloaddtion reactions. We had utilized lysine-based multiple antigenic peptide (MAP) and β-cyclodextrin (β-CD) scaffolds for this purpose. Extending this further, several dendritic scaffolds representing a variety of shapes and pattern were synthesized to display orthogonal groups compatible with labeled proteins. For this, ubiquitin and a immunogenic domain (D3) of RrgB, a major pilin protein of *S. pneumoniae* were expressed with a sortase-recognition sequence. The respective purified proteins were labeled with clickable groups and conjugated to the desired multivalent complementary dendritic scaffolds. Mass spectrometric characterization in each case defined the homogeneity of the respective protein dendrimers. Collectively, these results establish the generality of Sortase-Click reaction as a method of choice for the assembly of well defined multivalent proteins.

Structure, dynamics and function of Sortases

The elucidation of mechanistic imperatives underlying substrate recognition by housekeeping sortase of *S. aureus* (SrtA) and its linkage to enzyme dynamics is crucial for inhibitor design. Towards this we first examined the chemical and conformational signatures of a 'minimal' sortase substrate. The LPXTG recognition motif located near the C-terminus of bacterial proteins is always followed by a stretch of residues. Therefore it was pertinent to evaluate if an isolated LPXTG pentapeptide would serve as an effective

substrate. The propensity of three LPXTG pentapeptide sequences containing Glu, Ala or Asn respectively at the X position was investigated. Interestingly, the native LPXTG sequences (X= A, E or N) could be converted into a productive substrate only after capping their ends with acetyl and amide groups or by placing an Ala residue (ALPNTGA) at the termini suggesting that charge neutrality of the LPXTG motif was essential for the transpeptidation reaction. Furthermore, this could be interpreted to mean that an amide or peptide linkage (-CONH-) flanking the LPXTG sequence may play a role in recognition of a catalytically competent substrate. The failure of a N-methylated and C-amidated LPNTG peptide to undergo transpeptidation reaction was consistent with this idea.

To further elucidate the chemical features of a minimalist *bona fide* substrate, we reasoned that intrinsic amidase activity of SrtA may facilitate acylation of an amide substrate to form an acyl-enzyme intermediate. Accordingly, a tetrapeptide Ac-LPNT-NH_2 was subjected to transpeptidation reaction. Under standard conditions, SrtA catalyzed the transpeptidation reaction *albeit* at a slower rate but produced higher equilibrium yields relative to the counterpart LPNTG pentapeptide. The reason for the higher conversion is perhaps linked to the reversibility of the transpeptidation reaction because ammonia byproduct will not be a facile substrate for the reverse reaction. SrtA-mediated transpeptidation with another version of the above peptide (Ac-LPNT-NH-CH_3) carrying a methylated amide proceeded with a relatively slower rate but produced similar equilibrium yield as Ac-LPNTG-NH_2. Taken together, our results establish an acetylated and amidated LPNT tetrapeptide as the minimalist recognition motif of SrtA and demonstrate that LPETG peptide seen in the crystal structure of SrtA-substrate complex is not bound to the enzyme in a catalytically competent state.

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Therapeutic Interventions in Chronic Diseases

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Simvastatin induced neurite outgrowth unveils role of cell surface cholesterol and acetyl CoA carboxylase in SH-SY5Y cells

Statins are known to modulate cell surface cholesterol (CSC) and AMP-activated protein kinase (AMPK) in non-neural cells; however no study demonstrates whether CSC and AMPK may regulate simvastatin induced neuritogenesis (SIN). We found that simvastatin (SIM) maintains CSC as shown by Fillipin III staining, Flotillin-2 protein expression / localization and phosphorylation of various receptor tyrosine kinases (RTKs) in the plasma membrane. Modulation of CSC revealed that SIN is critically dependent on this CSC. Simultaneously, phospho array for mitogen activated protein kinases (MAPKs) revealed PI3K / Akt as intracellular pathway which modulates lipid pathway by inhibiting AMPK activation. Though, SIM led to a transient increase in AMPK phosphorylation followed by a sudden decline, the effect was independent of PI3K. Strikingly, AMPK phosphorylation was regulated by protein phosphatase 2A (PP2A) activity which was enhanced upon SIM treatment as evidenced by increase in threonine phosphorylation. Moreover, it was observed that addition of AMP analogue and PP2A inhibitor inhibited SIN. Bio-composition of neurites shows that lipids form a major part of neurites and AMPK is known to regulate lipid metabolism majorly through acetyl CoA carboxylase (ACC). AMPK activity is negative regulator of ACC activity and we found that phosphorylation of ACC started to decrease after 6 hrs which becomes more pronounced at 12 hrs. Addition of ACC inhibitor showed that SIN is dependent on ACC activity. Simultaneously, addition of Fatty acid synthase (FAS) inhibitor confirmed that endogenous lipid pathway is important for SIN. We further investigated SREBP-1 pathway activation which controls ACC and FAS at transcriptional level. However, SIM did not affect SREBP-1 processing and

transcription of its target genes likes ACC1 and FAS. This study highlights a distinct role of CSC and ACC in SIN which might have implication in process of neuronal differentiation induced by other agents. In conclusion, this study unravels a co-ordinated action of CSC and ACC in SIN. We highlight two major events involved during SIN: 1) retention of CSC which acts as stabilizer to orchestrate signaling events necessary to promote neuritogenesis; 2) initiation of fatty acid biosynthesis by ACC activation through PP2A phosphatase dependent de-phosphorylation of AMPK (fig 1).

Methionine down-regulates TLR4/MyD88/ NF-κB signalling in osteoclast precursors to reduce bone loss during osteoporosis

In the present study, methionine, a nutritionally essential amino acid has been employed as a tool to evaluate whether an anti-osteoporotic pharmacological agent can perform similarly at varying lengths of time. Administration of an essential amino acid methionine to a rat osteoporotic system prevented pathophysiological bone resorption. Methionine-inhibited trans-differentiation of blood

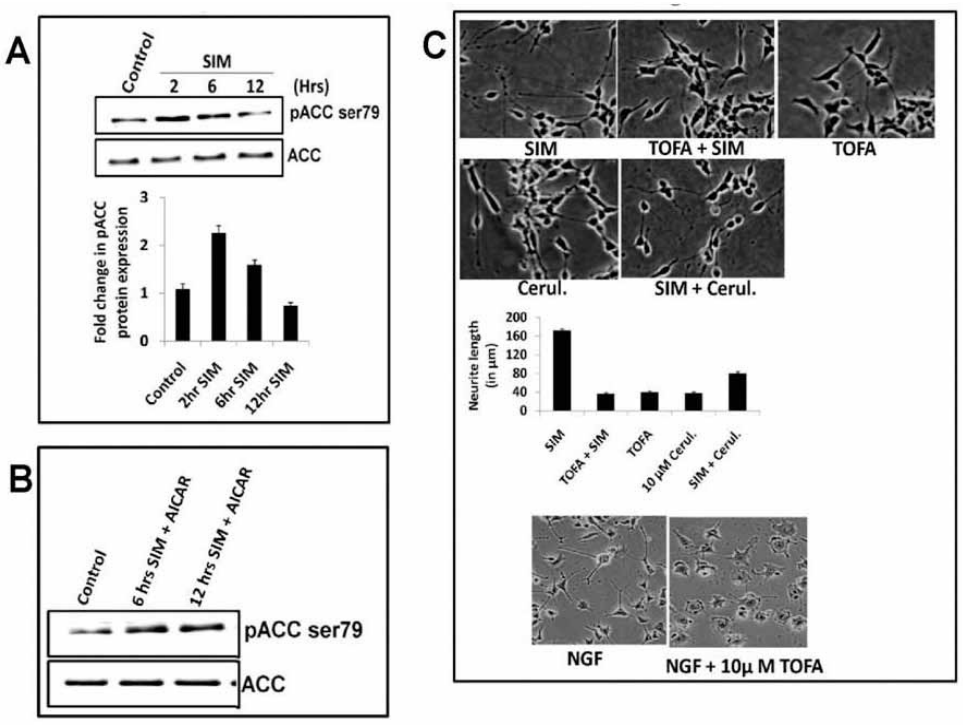

Figure 1: Dephosphorylation of AMPK substrate i.e. acetyl CoA carboxylase (ACC) initiates neuritogenic effect of SIM. A) Immunoblot showing SIM induced change in phosphorylation of ACC over a period of 2 hrs, 6 hrs and 12 hrs. SIM led to a significant ($p < 0.05$*) increase in pACC (Ser-79) levels at 2 hrs which was followed by a gradual decrease. Total ACC levels showed no change during SIM treatment. Error bar graph shows fold change (normalized to control) in pACC (Ser-79) levels at different time points (mean ± SEM; n = 4). **B)** Immunoblot showing modulation of ACC phosphorylation by SIM follows AMPK pathway. As evident, addition of AICAR (an AMPK activator) increased the phosphorylation of ACC either alone or in presence of SIM till 12 hrs. **C)** Morphology of SH-SY5Y cells under light microscope showing significant ($p < 0.001$) inhibition of SIM induced neuritogenesis in presence of acetyl CoA carboxylase (ACC) inhibitor i.e. 10 µM TOFA and fatty acid synthase (FAS) inhibitor i.e. 10 µM Cerulenin (Cerul.). Error bar graph shows the comparative difference in neurite lengths SIM, TOFA, SIM + TOFA, Cerulenin and SIM + Cerulenin treated cells for a period of 12 hrs (mean ± SEM; N = 50 cells per condition from 3 separate cultures). Morphology of PC12 cells under light microscope in presence of NGF or NGF + TOFA for a period of 3 days. Scale Bar = 100 µm.

mononuclear cells to functional osteoclasts and this was due to its ability to down-regulate TLR-4/MyD88/NF-κB cascade in developing osteoclasts, a signaling mechanism that was originally thought to be involved only in pathogen recognition (Fig 2).

A combination therapy of Alendronate (bisphosphonate) + methionine significantly improved bone physiology of osteoporotic rats (even at a significantly lower dose of Alendronate) highlighting methionine as a pharmacological drug for anti-resorptive therapy. Intriguingly, we

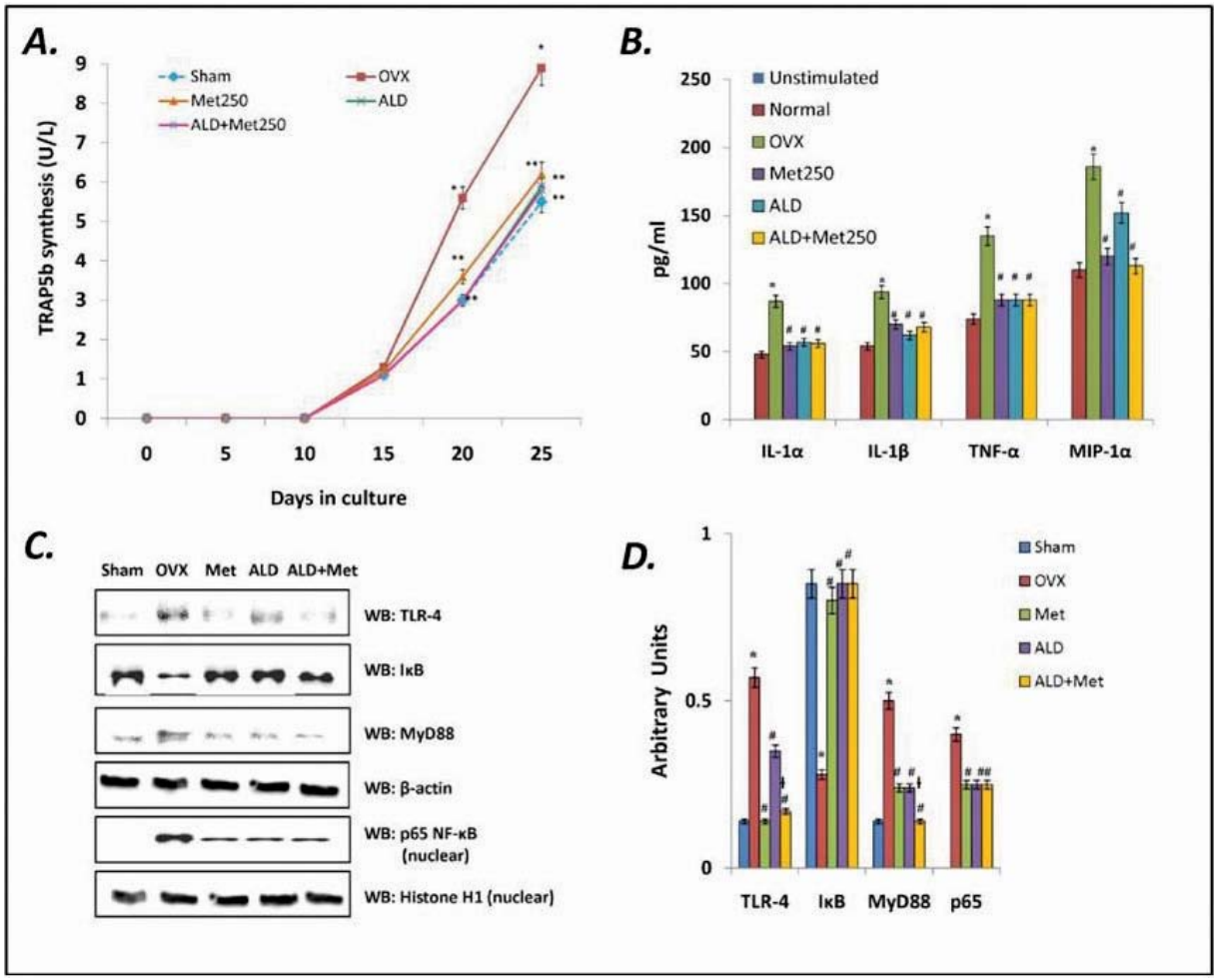

FIGURE 2: *Effect of methionine administration on osteoclastogenesis in vivo.* Methionine at a dose of 250 mg/kg body weight was administered in drinking water for 10 weeks. Alendronate (200 µg/kg body weight) was administered orally to ovariectomized rats at an interval of 5 days for one month. For combination therapy, rats were administered alendronate (100 µg/kg body weight) and methionine (250 mg/kg body weight). Both the treatments were performed 6 hours apart. Blood mononuclear cells isolated from these rats were cultured in the presence of M-CSF (25 µg/L, days 1-11) and RANKL (20 µg/L, days 6-20) *ex vivo*. **A**. Representative histogram showing changes in TRAP5b secretion in response to methionine and alendronate administration. Values are mean ± SEM; n=4. **P*<0.05 compared to normal rat. ***P*<0.05 compared to ovariectomized rat. **B**. Changes in pro-inflammatory cytokine production in blood derived osteoclast cultures *ex vivo*. Pro-inflammatory cytokines were assayed by multiplex kits from Millipore on Bioplex 200™ (Bio-Rad, Hercules, CA, USA). Values are mean ± SEM; n=4. *P<0.05 compared to normal rat. #*P*<0.05 compared to ovariectomized rat. **C**. & **D**. Changes in the expression status of TLR-4, MyD88 and NF-κB in developing osteoclasts following ovariectomy and methionine or alendronate treatment by Western blot. **P*<0.05 compared to basal. Values are mean ± SEM; n=4. #*P*<0.05 compared to ovariectomized rat. ǂ P<0.05 compared to alendronate administered rat.

discovered that after a deflection point (higher doses and prolonged therapy), methionine turned from a pharmacological agent to a hyperhomocysteinemia inducing substance producing many pathophysiological changes similar to that of cancellous osteopenia, the beginning stage of osteoporosis.

Publications

Original peer-reviewed articles

#1. Pathak, CM, Singh RR, Yadav S, Kapoor N, Raina V, Gupta S*, Surolia A* (2014) Evaluation of Benzothiophene carboxamides as analgesics and anti-inflammatory agents. *IUBMB Life* doi: 10:1002/iub.1252

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#4. Vijayan V, Khandelwal M, Manglani K, Ranjan Singh R, Gupta S*, Surolia A* (2013) Homocysteine alters osteoprotegerin/RANKL System in the osteoblast to promote bone loss: Pivotal role of redox regulator forkhead O1. *Free Radic Biol Med*. **61**:72-84.

*Corresponding authors

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Patents

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2. Surolia A, Gautam RK, Singh M, Chattopadhyay T (2013). Composition useful for treatment of diabetes and disorder. *(Patent No. EP2133091; Granted on April 19, 2013).*

3. Surolia A, Gautam RK, Diwedi VK, Gupta S (2013). Synthetic peptides and random copolymers for the treatment of autoimmune disorders. *(Application No. US 14/115,598; Filed on November 04, 2013)*.

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Molecular mechanism of enzymatic reactions and enzyme-ligand interactions

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Stability of wthGBP1 and its mutants in the presence of the substrate analogue and biochemical assays

To investigate whether the stability of the protein upon binding with the substrate is related to the product formation, kinetic assays were carried out. In the crystal structure of hGBP1, Ser157 (globular domain) interacts with and Glu313 (helical domain) through H-bonding and have been mutated to Ala. The catalytic efficiency for GDP formation in Glu313Ala decreased by ~ 2 fold compared to wild type, but for GMP it increased by ~ 1.5 fold. To understand this difference, we performed heat-induced unfolding studies with or without GppNHp. Like wild type the binding of the analogue increases the stability of the mutant proteins. The relative difference in the ΔG_D between the free and bound proteins for Ser157Ala is lower than wild type (1.7 kcal/mol vs 0.9 kcal/mol for wt vs Ser15Ala respectively) but for Glu313Ala it is higher than wild type (2.4 kcal versus 1.7 kcal/mol for Glu313Ala versus wt respectively). This suggests that difference in ΔG_D between the free and bound proteins plays an important role in the product formation; larger difference (higher stability) favors GMP formation, but smaller (lower stability) favors dissociation of GDP-bound enzyme dimer resulting in more GDP.

Mutational studies on the sequence motif of *H. pylori* arginase

H. pylori arginase has a distinct sequence ^{153}ESEEKAWQKLCSL^{165}, which is absent in the analogous enzymes. To examine its role, each residue was individually mutated to alanine and kinetic assays were performed. Among mutants, Glu155Ala, Trp159Ala and Cys163Ala showed remarkable results. Glu155Ala and Trp159Ala resulted in complete loss of the catalytic activity. Cys163Ala showed a decrease in the catalytic efficiency by about 16 fold compared to the wild type with Co^{2+}. However, with Mn^{2+} Cys163Ala failed to show detectable activity. The catalytic efficiency for Glu156Ala decreased by ~ 5 fold

than the wild type, suggesting that Glu156 is also important for the function of the protein. Cys163Ala did not show appreciable cooperativity (n ~1.2) and detectable activity with Co^{2+} and Mn^{2+} respectively, implying that Cys163 may play a role in oligomerization of the protein. Unlike wild type, Cys163Ala showed mainly as a monomer with dimer being a smaller amount, indicating that Cys163 plays a critical role in dimerization. Thus, these results demonstrate that the motif is extremely critical for the function of the protein.

Simulations studies and location of the sequence motif

To get a structural insight into how the motif is important for the function, we performed MD simulations to refine the model structure of *H. pylori* arginase. A snapshot of the simulated structure at 445.7 ns shows that the sequence consists of largely a loop with a small helix in Lys161-Ser164. Trp159 moved closer to the metal binding site and is buried in the protein. The positioning of the motif near the active-site is due to the hydrogen bonding interaction of Trp159 with Asp126 (distance ~ 2.65 Å). This interaction was formed at around 120 ns and remained intact during rest of the simulations, implying that it may have an impact on the structure and function of the protein. To verify this, Asp126 was mutated to Ala and kinetic assay was done. Interestingly, this mutant resulted in complete loss of the catalytic activity. Trp159Ala failed to show detectable activity. The data further suggest that the interaction of Trp159 with Asp126 appears to be critical in positioning the motif near the active site for the function of the protein.

Deletion studies on the sequence motif and evaluation of the metal binding upon mutations

To examine the role of the whole sequence motif, a truncated *H. pylori* arginase was constructed, where the residues 153-165 have been deleted. The deletion protein failed to display activity, further implying that the motif is critical for the function of the protein. Arginases are known to have an intact bimetallic center at the active-site. Like wild type, all mutants except Trp159Ala exhibit similar metal binding (~ 2 metals) in the presence of either Co^{2+} or Mn^{2+}. Trp159Ala exhibits one metal, suggesting that Trp159 is crucial for retaining a metal ion. To verify this, Trp159 was mutated to Phe. Like Trp159Ala, Trp159Phe also showed no detectable activity and contains one metal ion, further verifying that Trp159 is critical for retaining a metal ion and thus in the activity. Interestingly, Asp126Ala also showed one metal ion, indicating that Asp126 is crucial for retaining a metal ion. These studies demonstrate that both Trp159 and Asp126 are close to the metal binding/active-site and critical for retaining the bimetallic center, thus for the function of the protein.

Janendra K. Batra

Ribonucleases and heat shock proteins: Involvement in host defense

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The work is focused on two major themes

1. Investigation of the role of human ribonucleases, particularly eosinophil ribonucleases, eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) in host defense. Also, human ribonucleases, and natural protein toxins targeting RNA or ribosomes are being analyzed for structure-function relationships to understand their molecular mechanism of action, and to explore them to design knowledge-based recombinant toxins.

2. Investigation of crucial housekeeping proteins of *M. tuberculosis* for their role in survival and virulence of the pathogen. We are studying the functioning of caseinolytic protease (Clp) machinery, and RNase P mediated tRNA maturation in *M. tuberculosis*. Clp proteases regulate the expression of virulence genes, and also help bacterial pathogens in countering stress in the host. RNase P is a key housekeeping enzyme involved in tRNA maturation, and is structurally completely different in bacteria and human. Both, Clp proteins and RNase P could be promising drug targets in *M. tuberculosis*.

Anti-HIV activity of ribosome inactivating protein, saporin and ribotoxin, restrictocin

Saporin showed a dose dependent inhibition of HIV propagation in two model cell lines. Further, using three mutants of saporin, namely Y16A, W208A and Y72A it was found that for the anti-HIV activity of saporin its N-glycosidase and DNA fragmentation activities are important.

Restrictocin also showed a dose dependent anti-HIV effect in both the cell lines. Further, using three mutants of restrictocin, namely Y47A, H49A and H136A we demonstrate that the specific RNase activity of restrictocin is involved in its anti-HIV activity. Human pancreatic ribonuclease and RNase A which are more potent RNases did not manifest any anti-HIV activity.

Investigation of the mechanism of apoptosis induction by saporin

Earlier, we have shown that saporin induces apoptosis in mammalian cells through the intrinsic pathway. We have further delineated the mechanism of apoptosis induction by saporin and our study shows that saporin treatment induces a loss of mitochondrial membrane potential accompanied by reactive oxygen species generation in Hela cells. SAP/JNK, ERK and p38 were phosphorylated in Hela cells treated with saporin. The levels of p21WAF/CIP1, a cell cycle protein involved in cell growth inhibition and ER stress regulating protein, Bip decreased with time in saporin treated Hela cells.

Stress regulation and persistence mechanisms in Mycobacteria

In *M. tuberculosis* genome, the upstream region of groES and hrcA genes contains the probable CIRCE DNA binding site for transcriptional repressor HrcA. Using recombinant mycobacterial HrcA protein we have shown that HrcA requires other factors from the mycobacterial cell extracts to be able to bind to CIRCE DNA. Further studies to identify the interacting partner(s) of HrcA, which may be required for its DNA binding and in turn the transcriptional repression activity are underway.

The DNA binding mechanism of the other transcriptional repressor in *M. tuberculosis*, HspR were analysed using a DNA fragment from the HAIR motif, upstream of dnaK gene. Our study shows that the specific binding to HAIR element and temperature sensing activity of HspR are rendered by other molecule(s) that remain to be identified.

Structure-function analysis of ribonuclease P of *M. tuberculosis*

On the basis of multiple sequence alignment, Phe23, Val27, Ala70, Arg72, Ala77, Arg93 and Asp124 were identified to be unique in the protein component *M. tuberculosis* RNase P. Eight variants of the *M. tuberculosis* RNase P protein component were generated that contained the unique residues substituted to those conserved in *E. coli* and *B. subtilis*. On the basis of the enzymatic activities of holoenzymes reconstituted with the variants, they could be grouped in three categories. One group that includes F23A, A70K and R72A variants showed significantly reduced activity; the second group that includes A77F and D124S had partial activity; and the third group comprising of V27F, R72L and R93A mutants showed non-specific activity on pre-$tRNA_{Ala}$. These observations suggest that the selected residues play different roles in different protein components with respect to their interaction with the respective RNA component.

Publications

Original peer-reviewed article

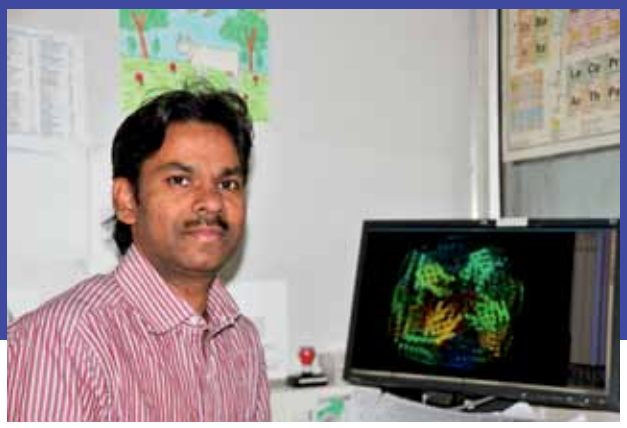
#1. Shah U, Haquea MA, Zaidia S, Hassan MI, Islam A, Batra JK, Singh TP, Ahmad F* (2014) Effect of sequential deletion of extra N-terminal residues on the structure and stability of yeast iso-1-cytochrome-c. *J. Biomol. Struct. Dyn.* doi:10.1080/0739/102.2013.84826.

Review/Proceeding

1. Chopra A, Batra JK* (2014) Antimicrobial activity of human eosinophil granule proteins. *Meth. Mol. Biol.* doi:10.1007/978-1-4939-1016-8-23.

*Corresponding authors

#In press last year, since published.



Bichitra K Biswal

Understanding structures and functions of proteins from *M. tuberculosis*

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We have been pursuing two major projects on proteins from *Mycobacterium tuberculosis*. In the first project, we aim at understanding the molecular mechanisms underlying the actions of the enzymes of Histidine biosynthesis pathway. The second project deals with the structural and biochemical characterization of membrane associated proteases (MAPs).

The histidine biosynthesis pathway, leading to the enzymatic synthesis of the histidine from 5-phosphoribosyl-1-pyrophosphate in 10 enzymatic reactions catalysed by 10 enzymes, is conserved among bacteria, lower eukaryotes and plants, but is absent higher eukaryotes including mammals. We have been studying these enzymes, structurally and biochemically, to delineate the mechanisms underlying their action and to design inhibitors for them through a structure based approach. Previously we have determined 3D structures of HisB (imidazole glycerol phosphate dehydratase) and HisC2, an aminotransferase (AT) and have characterized these enzymes biochemically. It was also shown that 3-amino-triazole inhibitor inhibits HisB competitively. In the last one year, we have determined structures of apo and ligand-bound forms of another enzyme, a PLP-dependent histidinol phosphate aminotransferase (HspAT) HisC involved in the catalysis of seventh step, which involves the conversion of imidazole acetol phosphate to L-histidinol phosphate. Each monomer of HisC consists of three domains, palm, thumb and fingers (Fig. 1). The biological functional unit of HisC is a symmetric dimer (Fig. 2). The PLP-binding domain contains a seven-stranded β-sheet sandwiched between four α-helices. Superimposition of ligand-bound and free forms suggests that the N-terminal arm consisting about 40 amino acids undergoes a large conformational change upon ligand binding. Briefly this arm moves closer to the ligand binding region and a few residues including Tyr25 of the loop make hydrogen and van der Waals interactions with PLP and the substrate. The active site is situated in the dimeric interface and residues that line the active site pocket include Tyr25A, Asn37A, Thr38A, Asn39A, Gly101A, Ser102A, Asn103A, Tyr127A, Met129A, His130A, Ala172A, Asn176A, Asp201A, Ala203A, Tyr204A, Thr229A, Ser231A, Lys232A, Arg240A, Leu241A, Gly242A, Arg337A, Arg346A, Tyr67B, Pro260B, and Tyr261B. The active-site cavity possesses a surface area of about 1950 $Å^2$

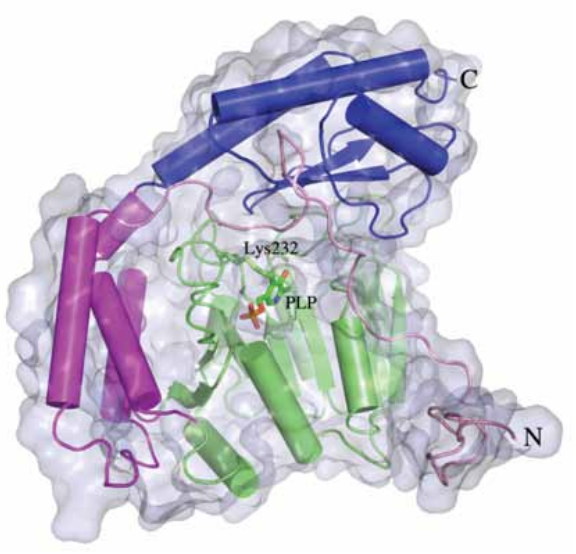

Fig. 1. The monomeric 3D structure of HisC shown in cartoon (with cylindrical helices) and surface representations. The N-terminal arm, palm, thumb and fingers domains are colored in pink, lime, magenta and blue respectively.

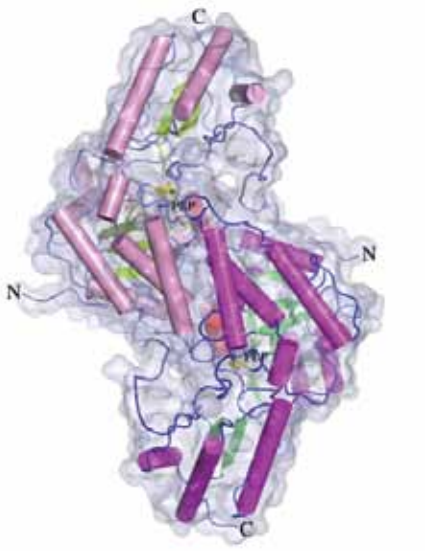

Fig. 2. The dimeric structure of HisC in cartoon and surface representations.

and a volume of about 5500 Å^3. Extensive kinetic studies with various amino acids and Hsp as the substrates show that HisC is an HspAT which also shows AT activity towards aromatic amino acids. Further structural, kinetic and inhibition studies on these enzymes are in progress. We have crystallized a 30-residue truncated version of a membrane proteins (Rv2224), have collected X-ray data and its structure is being determined.

Publication

Original peer-reviewed article

1. Ahangar MS, Vyas R, Nasir N, Biswal BK* (2013) Structures of native, substrate-bound and inhibited forms of *Mycobacterium tuberculosis* imidazoleglycerol-phosphate dehydratase. *Acta Cryst. Section D* **69**: 2461-2467.

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Debasisa Mohanty

Molecular modelling of proteins and protein-ligand complexes using knowledge-based approaches and all atom simulations

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The main theme of the research project is to develop novel computational approaches for prediction of the structures of peptides/proteins and specificities of protein-ligand complexes. Theses prediction approaches for structure and substrate specificity are being used to assign functions to proteins in the various genomes for identifying novel biosynthetic pathways and interaction networks involving protein-protein and miRNA-mRNA.

A. Retro-biosynthetic enumeration of enzymatic reactions leading to a given secondary metabolite

The retro-biosynthetic or reverse approach involves enumeration of various chemical transformations which would generate a given chemical moiety and identifying the enzymes that can catalyze the given biochemical transformation. The reactions associated with assembly line mechanism of biosynthesis of polyketides were stored as generic reaction rules explaining the reaction mechanism. The reaction rules are constructed based on the sub-structural changes occurring in the reaction. In addition to the assembly line reactions, rules were also stored for post PKS reactions like O-, N- and C-glycosyltransfer, O- and N- methyltransfer, N- N dimethylation, transamidation, cyclic carbamoylation, carbamolation, acylation and epoxidation. Corresponding to each generic reaction functional group information of product are stored in a separate database as SMARTS. Functional group here refers to the substructure where the change occurs. Obgrep tool of Open Babel is first used to locate a functional groups present in the given chemical compound. Then using Reactor module of ChemAxon corresponding generic reaction is used to transform given chemical moiety into its precursor molecule. This process is continued till no other functional

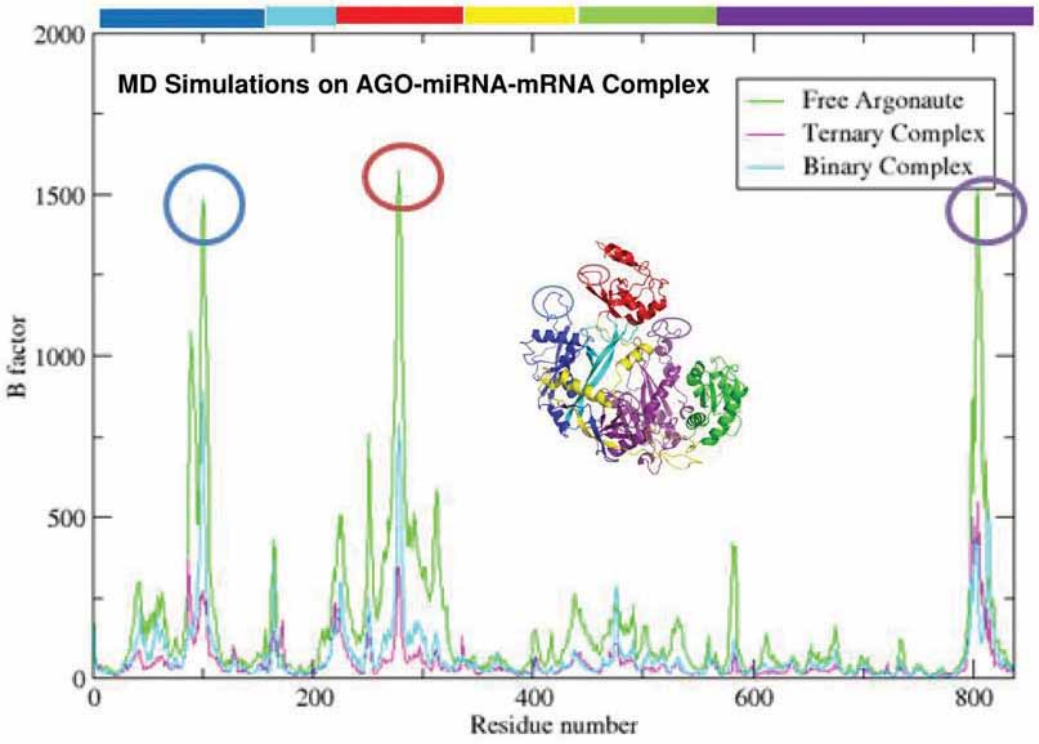
group is detected in the compound. The next step would be to link the biosynthetic reactions to their respective genes using our earlier developed databases of PKSs and NRPSs.

B. Structure based analysis of disease associated nsSNPs on kinases

We have investigated plausible structural rationale for disease associated nsSNPs which are present on catalytic domains of protein kinases in an enriched manner. The novel aspect of our analysis is investigation of the role of nsSNPs in regulation of conformational transitions in kinase catalytic domains. We have explored their role in inducing conformational transitions from inactive to active state by analyzing trajectories obtained from MD simulations on CDK2. Comparative analysis of MD trajectories with respect to the network of contacts involving these residues indicates that there are certain crucial contacts which are differentially maintained in active and inactive forms. Mutations in these conserved residues might destabilize the kinase catalytic core and shift the conformational equilibrium towards catalytically inactive state thus providing structural rationale for some of these disease associated mutations. In fact, we have proposed structural roles for various disease associated mutations based on the MD studies and classified them based on their roles in maintaining structural integrity, catalytic function, interaction with other downstream macromolecules and regulation of catalysis.

C. Structure and dynamics of microRNA-protein complexes

The binding of the miRNA to the target mRNA takes place in the RNA induced silencing complex (RISC) which consists of a ternary complex involving Argonaute (AGO) protein, miRNA and mRNA. Explicit solvent molecular dynamics simulations have been carried out on AGO-miRNA binary complex (PDB ID: 4F3T), free AGO and AGO-miRNA-mRNA ternary complex. Multiple 500 ns simulations revealed readjustments in the miRNA- mRNA interactions in the ternary complex involving bulging out of one nucleotide (U5) at the miRNA side and formation of a non watson-crick G:A base-pairing. Interestingly, in simulations on the miRNA-mRNA duplex in isolation i.e. in absence of the AGO protein, no such readjustments involving non-canonical interactions were observed. Therefore, our simulations highlight the role of AGO in determining specificity of miRNA target recognition involving non-canonical interactions.



Publications

Original peer-reviewed articles

1. Tiwari G, Mohanty D* (2013) An In Silico analysis of the binding modes and binding affinities of small molecule modulators of PDZ-Peptide interactions. *PLoS ONE* **8**:e71340.
2. Damle NP, Mohanty D* (2014) Deciphering kinase-substrate relationships by analysis of domain specific phosphorylation network. *Bioinformatics* doi:10.1093/bioinformatics/btu112.
3. Tiwari G, Mohanty D* (2014) Structure Based Multi-Scale Approach for Identification of Interaction Partners of PDZ Domains. *J. Chem. Inf. Model.* doi:10.1021/ci400627y.

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Dinakar M. Salunke

Structure, interaction and design studies involving regulatory peptides and proteins

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The structural aspects of molecular recognition and its applications in analyzing the mechanisms associated with specific regulatory events and in rational molecular design.

Objectives

1. Understanding the protein architecture and the structural biology of various regulatory events.
2. Analysis of the structural principles of immune recognition and molecular mimicry.
3. Rational molecular design studies based on the above.

Although immune system is shown to be highly specific, degenerate specificity in immune recognition is often been observed. We have been working words understanding degenerate specificity of antibodies using a peptide (DVFYPYPYASGS) and a sugar (methyl α D mannopyranoside) as model antigens. Both, carbohydrate antigen and the corresponding mimicking peptide, were shown to be equivalent in polyclonal responses as well as in the monoclonal antibodies that were generated, in which an anti-sugar affinity matured antibody (2D10) was able to recognize both sugar and the peptide with equivalent affinities. Thermodynamic analysis of antigen-antibody (2D10) interaction had suggested that the flexibility in the antigen combining site may possibly help in the manifestation of molecular mimicry. However, when the structures of 2D10 in apo and the antigen-bound forms (with the sugar as well as the peptide) were determined, it was evident that no conformational flexibility in CDRs of 2D10 existed, instead it was evident that the plasticity in the interaction had helped in the manifestation of molecular mimicry. One interesting aspect of the study as pointed out by authors was that even if the potential for flexibility existed, it was not been utilized while recognizing both ligands.

Therefore, in order to address this conundrum, we began looking for other sugars and peptides which can bind to the 2D10 antibody with comparable affinities. Crystallographic analyses of 2D10 bound to five different sugars (methyl α D glucopyranoside, α D lactose, α1-3-Mannobiose,

α1-6-Mannobiose, α1-3, α1-6-Mannotriose) carried out at 2.5 Å, 2.5A, 2.1Å, 2.75 A and 1.7 Å, respectively have given interesting insights underlying the basics of specificity in molecular recognition. Comparison of the structures has shown that the antigen combining site for sugars is constituted of CDR H3, L1 and L3 only. All the five sugars have an overlapping primary binding site (equivalent to the methyl α D mannopyranoside interacting region). This primary sugar binding site have been shown to accommodate same/ similar as well as dissimilar sugars by utilizing plasticity in the interacting residues available in the antigen combining site. The second sugars of the similar disaccharides (α1-3-Mannobiose, α1-6-Mannobiose) have been adjusted in the same direction but with utilizing different sets of interacting residues of the antibody paratope. However, the second sugar of dissimilar disaccharide (lactose in comparison to α1-3-Mannobiose, α1-6-Mannobiose) exploits different paratope space altogether. The trisaccharide (α1-3, α1-6-Mannotriose) was accommodated in the same site by differential positioning of the second and third sugar rings in the antibody paratope (in comparison to all disaccharides) as well as by utilizing conformational flexibility in the paratope region (mainly in CDR L1). This study had demonstrated that an affinity matured antibody can utilize atleast three different strategies in order to accommodate structurally similar/dissimilar sugars.

The structural proteomics of allergy seed proteome of eggplant (*Solenum melongena*), was explored. A 45 kDa protein, SM80.1 showing weak homology with other 7S vicilins for which preliminary crystallographic studies were presented earlier, was further refined building almost entire model of the protein. The model was refined at 1.5 Å to an Rfree of 0.22 and Rwork of 0.21. The overall crystal structure of SM80.1 indicates that it is a homotrimer consisting of 393 residues in each monomer of which only residues 274-293 are structurally disordered. A monomer subunit is composed of two similar domains further subdivided into a core and a loop sub domain. Each domain consists of 2 elements, a compact eight-stranded beta barrel having the “swiss roll” topology and an extended flexible fragment containing several short alpha helices. This is the first native structure in this family of proteins with only one disordered region. Each domain of SM80.1 forms a central cavity to facilitate ligand binding. It was found that N terminal β-barrel domain has a pyroglutamate molecule whereas an acetate molecule was present in the C-terminal domain. Superimposition of two domains shows that both the ligands exist exactly at the same position. These ligands may have a probable role in structural integrity and formation of swiss-roll topology. This protein might be acting as a depository of pyroglutamate and acetate, which are required in different metabolic pathways or could be a source of carbon and nitrogen in the germination process. Along with above Mg is also present in the structure. Surface electrostatic potential map of the protein showed an uneven distribution of charge on the protein surface. Although there was a little difference, the basic structure was close to those of 7S Adzuki bean, canavalin, AraH1 and phaseolin. All these structures have very low sequence identity, suggesting that multiple and varied sequences can yield similar three-dimensional structure and explaining evolutionary divergence.

Another protein, SM80.2, was also purified from the defatted seed powder by 80% ammonium sulphate fractionation. The purified protein corresponds to a molecular weight of 11.7 kDa as analyzed by mass determination using mass spectrometry. N-terminal sequencing was also done for the purified protein which identified 20

residues of the polypeptide. The purified protein shows homology with other known 2S albumin family proteins. The protein was crystallized to obtain hexagonal shaped crystals which belonged to space group P6, with unit-cell parameters a = b = 87.48, c = 49.67 Å. As no homologous crystal structures of closely related proteins were available, *ab initio* phasing was pursued utilizing eight inherited sulphur atoms (cysteine). Experimental anomalous intensities were used by the *PHENIX* package to obtain initial phase information. Data were collected on single crystal, first to 1.87 Å, which were used for high resolution structure refinement, and then to 2.5 Å, at synchrotron source (BM14, ESRF, Grenoble) which were used for SAD phasing. Program SOLVE successfully identified in total seventeen sulphur sites from the Bijvoet pair differences. A preliminary model was built automatically by *Autosol*. A total of 150 resides were built and 8 chains were placed with R_{work} and R_{free} values of 38.32 and 41.81 respectively.

Publications

Original peer-reviewed articles

1. Tapryal S, Gaur V, Kaur KJ, Salunke DM* (2013) Structural evaluation of a mimicry recognizing paratope: plasticity in antigen–antibody interactions manifests in molecular mimicry. *J Immunol* **191**: 456-463.
2. Sharma R, Lomash S, Salunke DM* (2013) Putative bioactive motif of tritrpticin revealed by an antibody with biological receptor like properties. *PloS One* **8**: e75582.
3. Gill J, Jayswal P, Salunke DM* (2014) Antigen exposure leads to rigidification of germline antibody combining site. *J Bioinfor Comput Biol* doi:10.1142/S0219720014500061.

Review/Proceeding

1. Salunke DM*, Gill J, Dwevedi A (2013) Comparative structural proteomics of allergenic proteins from plant pollen. *J Ind Inst Sci* **94**: 119-126.

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Srinivasa-Gopalan Sampathkumar

Chemical Glycobiology: Glycoform modulation, carbohydrate-based drug design, and Glycomics

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Our laboratory of chemical glycobiology strives to develop carbohydrate-based small molecules as probes, tools, and inhibitors to shed light on the importance of glycosylation in biological processes. We design and synthesize novel molecules that intercept glycan biosynthesis and investigate effects of these molecules *in* mammalian cells and mice.

Our main objective is to harness the power of carbohydrate-based synthetic small molecules to unravel the importance of glycosylation in biological systems, as follows: (I) Development of non-natural analogues for interception and metabolic glycan engineering (MGE); (II) Development of novel carbohydrate-neuroactive (CH-NA) hybrids to achieve MGE of the central nervous system (CNS) across the blood-brain barrier (BBB) for investigations on glycosylation in CNS development and disorders; (III) Development of glycopeptidomimetics (GPM) based small molecules as inhibitors of matrix metalloproteinases (MMP) and anti-metastatic agents; and (IV) Development of inhibitors of human ganglioside neuraminidase, NEU3, and its role in immunological processes.

I. MTOG inhibition:

Our results showed that peracetyl *N*-thioglycolyl-D-galactosamine (Ac_5GalNTGc, **1**) inhibited elaboration of *O*-glycans in a thiol-dependent manner. Upon treatment with **1** (100 μ M, 48 h), but not the controls, binding of *Maackia amurensis* lectin (MAL-II) (NeuAcα(2→3)Gal-) and peanut agglutinin (PNA) (Gal β (1→3)GalNAc-α-S/T) were abrogated in Jurkat cells. MALDI-TOF/TOF mass spectrometry and Glycoworkbench analysis of permethylated O-glycans from Jurkat cells provided evidence for the presence of substantial levels of T- (Galβ (1→3) GalNAcα-S/T), sialyl-T, di-sialyl-T antigen structures. Mass spectrometry revealed reduction in the levels of T- and sialyl-T and absence of disialyl-T structures upon treatment with **1**. Structure activity studies revealed that both sulfhydryl and galactosamine moieties are critical for inhibition. Effect of 1 on MTOG was found to be dependent on the nature of CD antigen. CD43 glycosylation was differentially affected

by **1** in different cell types due to variations in glycosylation machinery.

II. **CH-NA hybrids:** Mice were treated with CH-NA hybrids and non-hybrids (controls) and tissues were analyzed by far-western blotting after Cu-AAC biotinylation. With Ac_4ManNAz, expression of NeuAz was found in heart but not in brain, except when administered intracranially. Strikingly, intravenous administration of CH-NA hybrids resulted in NeuAz expression in both heart and brain thus providing a 'proof-of-concept' of our piggybacking strategy.

III. **MMP inhibitors:** Towards development of MMP inhibitors, we have designed and synthesized a panel of monosaccharide derivatives carrying ZBG. Starting from multiple monosaccharides, several derivatives carrying a hydroxamic acid were synthesized. Selectively protected monosaccharide donor derivatives for glycosidation with Ser/Thr peptides have been synthesized.

IV. **Human sialidases:** Towards studying sialoglycoconjugates modulation, human cells (SH-SY5Y (neuroblastoma) and CaCo-2 (colon adenocarcinoma)) were screened for expression of NEU1–4 by RT-PCR. Cos-1 (African green monkey kidney) cells stably expressing NEU3-FLAG (kind gift from Dr. Takeo Miyagi, Japan), a membrane-bound sialidase known to hydrolyze ganglioside GD3 to GM3, have been established.

Publication

Original peer-reviewed article

1. Agarwal K, Kaul R, Garg M, Shajahan A, Jha SK, Sampathkumar SG* (2013) Inhibition of mucin-type O-glycosylation through metabolic processing and incorporation of N-thioglycolyl-D-galactosamine peracetate (Ac_5GalNTGc). *J. Am. Chem. Soc.* **135**: 14189-14197.

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Kanwaljeet Kaur

Role of carbohydrates in modulating the structure and function of glycopeptides

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The project is aimed for understanding the role of carbohydrate domains in modulating the structure and function of glycopeptides by involving different model systems such as antimicrobial and thrombin-inhibitory glycopeptides.

Objectives

1. Synthesis and structural characterization of glycosylated amino acids,
2. Structure-function analysis of the synthetic glycoconjugates.

Antimicrobial peptides

Earlier, we have reported the effect of different sugars and their linkages on the activity of two Proline rich antibacterial glycopeptides, formaecin and drosocin. In glycoproteins, usually Thr and Ser amino acids are *O*-glycosylated on their side chain hydroxyl groups. It has been reported that the conformational impact of α-GalNAc attached to Thr residue differs significantly from that attached to Ser residue. To obtain insights into the effect of glycosylated amino acid variation on the structural and functional properties of drosocin, an analogue was synthesized by substituting its glycosylated-Thr at 11th position with glycosylated-Ser and named as S^{11}-α-D-GalNAc-Drosocin. For synthesizing this glycosylated peptide, first critical building block, N^{α}-Fmoc-Ser (Ac$_3$-α-D-GalNAc)-OH was synthesized and then used for peptide synthesis. To compare the antibacterial activity of S^{11}-α-D-GalNAc-Drosocin with its non-glycosylated variant, the S^{11}-Drosocin was also synthesized.

In order to analyze the antibacterial effect of Thr substitution with Ser in Drosocin analogues, MIC of each peptide was determined against Gram negative bacterial strains. Native mono-glycosylated Drosocin (T^{11}-α-D-GalNAc-Drosocin) which contained glycosylation at Thr, displayed higher level of antimicrobial activity compared to other peptide analogues. Substituting Thr with Ser in non-glycosylated Drosocin did not affect the MIC significantly, indicating that Ser or Thr alone did not determine differential antimicrobial behaviour of Drosocin peptides. The comparison of antibacterial activity of S^{11}-α-D-GalNAc-Drosocin and M-Drosocin

(T^{11}-α-D-GalNAc-Drosocin) demonstrated that the MIC for S^{11}-α-D-GalNAc-Drosocin was around two fold to twenty fold higher than that of T^{11}-α-D-GalNAc-Drosocin against different Gram negative strains. With just a difference of γ-methyl group, S^{11}-α-D-GalNAc-Drosocin could not display lethal action comparable to that of T^{11}-α-D-GalNAc-Drosocin.

Interestingly, S^{11}-α-D-GalNAc-Drosocin showed around three times higher MIC than its non-glycosylated counterpart, S^{11}-Drosocin, against different Gram negative bacterial strains. This result was intriguing, suggesting that the addition of *N*-acetyl galactosamine was not the only reason for higher antimicrobial activity of T^{11}-α-D-GalNAc-Drosocin. These observations further confirmed that there was a difference between the conformational impact of glycosylated-Thr and glycosylated-Ser on the peptide backbone.

To analyze the structural effects of substitution of glycosylated-Thr, the comparative conformational properties of Drosocin and its analogues were analyzed by CD spectroscopy in PB (10mM, pH7.4), 90% TFE, and 10mM SDS. It was observed that the conformational studies using CD could not resolve the local conformational changes occurring in the peptide backbone due to modifications in Drosocin.

Thrombin-inhibitory glycopeptides

With an aim to study the impact of glycosylation on the inhibitory potential of active site directed, short peptide inhibitors of thrombin, two non-glycosylated tetra peptides were synthesized. The results indicated that D-ChaPβhomoRG was more efficient than fPβhomoRG in inhibiting thrombin's activity.

The inhibitory potential of Hirudin P6 C-terminal 23 residue peptide and its cognate glycosylated analogue (α-GalNAc HP6) was estimated by fibrinogen competitive assays. The results indicated the higher inhibitory activity of glycosylated HP6 (α-GalNAc HP6) than its non-glycosylated form and suggesting the effect of sugar in modifying the functional activity of thrombin-inhibitory peptide.

Publications

Original peer-reviewed articles

1. Lele DS, Talat S, Kaur KJ* (2013) The presence of arginine in the Pro-Arg-Pro motif augments the lethality of proline rich antimicrobial peptides of insect source. *Int J Pept Res Ther* **19**: 323-330.
2. Tapryal S, Gaur V, Kaur KJ, Salunke DM* (2013) Structural evaluation of a mimicry-recognizing paratope: plasticity in antigen-antibody interactions manifests in molecular mimicry. *J Immunol* **191**:456-463.

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Biophysical and biochemical characterization of *Leishmania* phosphoglycerate kinase: an enzyme in the glycolytic pathway of parasitic protozoa

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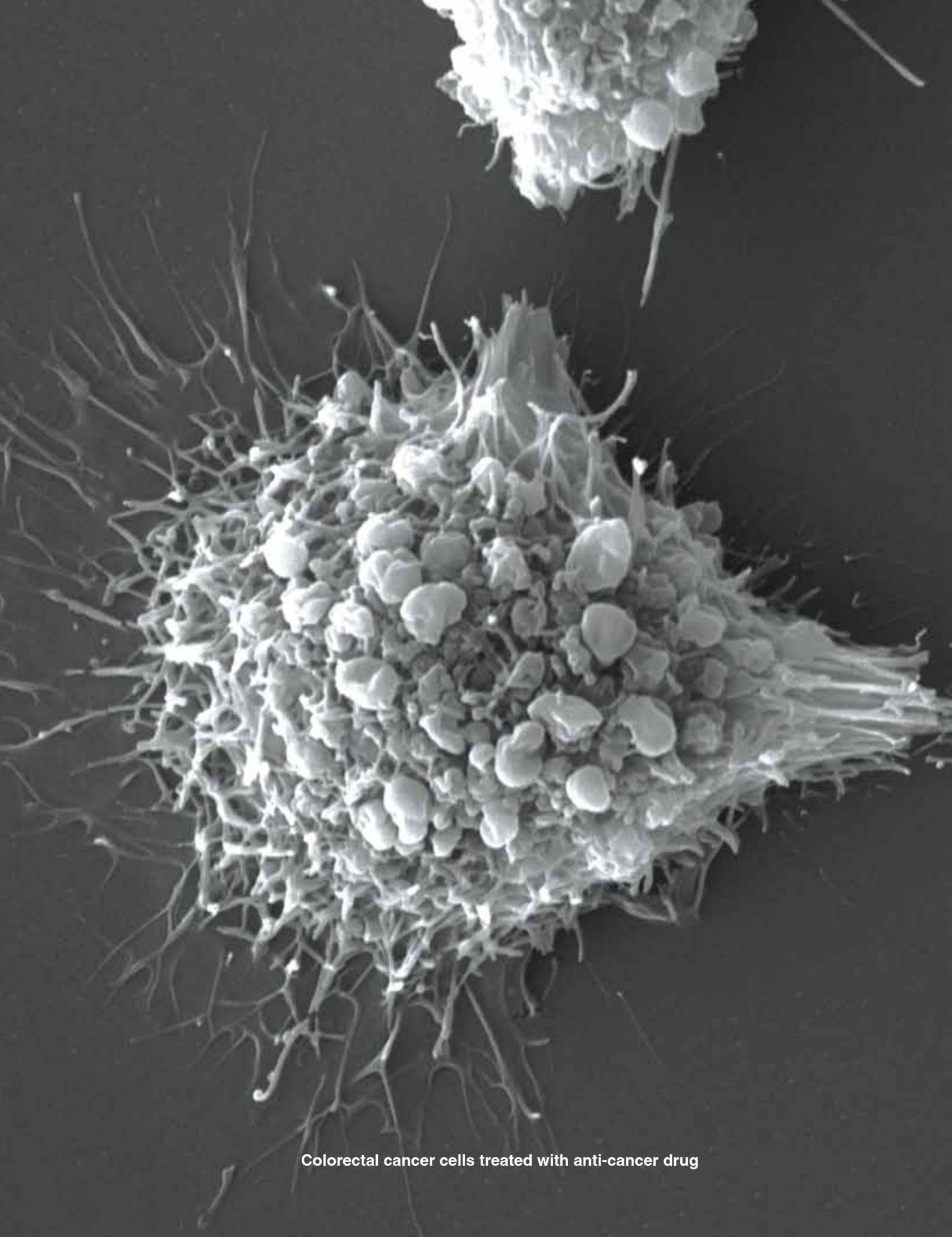
It is known that *Leishmania* sp. unlike mammalian counterparts uses multiple isoforms for many enzymes of the energy pathway, one of which is phosphoglycerate kinse or PGK. *Leishmania* PGK isoforms has some distinct structural features, as PGKB and PGKC differ in a handful of internal residues and in the presence of a long extension at the C-terminus of PGKC. Drug development efforts can be targeted, either at the glycosome itself or at the enzymes present within them for which, targeting unique structural features is critical.

We are interested to use nucleur magnetic resonance spectroscopy to study the enzymology as well as structure of PGK isoforms. We also want to map the metabolic profile of *Leishmania spp* cultures and correlate this to the enzymological studies with purified proteins.

1. Expression, purification and determination of specific activities of PGKB-Lmex and PGKC-Lmex and steady state kinetics study.
2. Comparison between PGKB-Lmex and PGKC-Lmex of, pH optimum of activity and enzyme inhibition by salt and suramin.
3. ^{31}P NMR studies using substrate / enzyme (PGKB-Lmex or PGKC-Lmex) mixtures, with, either no metal, $MgCl_2$, $CaCl_2$, $MnCl_2$ or $CoCl_2$ to determine the change in the dissociation constant of substrate with metal ions. Comparison with data from similar experiments in literature with yeast PGK using Mg-ADP and Mg-ATP.
4. Peptide based studies of glycosomal membrane association of PGKC-Lmex. The peptides used in these studies will be evaluated as useful models to understand the structural basis of the biochemical differences between PGKC-Lmex and PGKB-Lmex.
5. Conformational studies by NMR using site non-radioactive isotope labelling,
6. Using promastigote and amasitoge cultures of *Leishmania spp* for metabolome mapping. The concentration of specific metabolites in the cell at a particular time can be monitored at the micromillimolar level by ^{31}P, ^{1}H and ^{13}C NMR spectroscopy. The metabolites that can be detected are alanine, lactate, acetate, pyruvate, succinate, glycerol, urea, CO_2, oxalate, valine, glutamine and arginine.

In lieu of the structure of the peptide as determined by NMR in solution [S. Kaushik, B. Krishnarjuna, S. Raghothama, S. Aggarwal, V. Raghunathan, A. Ganjiwale. Theoretical and *in vitro* studies of a C-terminal peptide from PGKC of *Leishmania mexicana mexicana*. 185 (2012) 27-35], we have launched into looking at the structure of the entire C-terminal domain extension of PGKC-Lmex by cloning in *E. coli*. The 62 residue domain of the C-terminus of PGKC has been cloned in *E. coli* BL21 (RP) strain. The protein expression is good and western blotting shows the presence of a strong clean band after purification by metal affinity chromatography.

We have already initiated structural as well as biochemical studies using peptides from the 62r domain of C-terminus of PGKC-Lmex.



Colorectal cancer cells treated with anti-cancer drug

GENE REGULATION

Sher Ali

Molecular analysis of the human and animal genome(s)

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Fate of the human Y chromosome linked genes and loci in prostate cancer cell lines DU145 and LNCaP

Prostate cancer is a known cause of mortality in men worldwide although the risk factor varies among different ethnic groups. The prevalence of this cancer is highest in the Western and lowest in the Asian countries. Despite its high prevalence, very little is known about the molecular mechanism of its tumorigenesis. Loss of the Y chromosome is a common abnormality observed in the human prostate cancer. This can cause loss of putative tumour suppressor genes leading eventually to cancer. This hypothesis is supported by the fact that a normal human Y chromosome transferred to a Y chromosome lacking PC cell line PC3, suppressed its tumorigenic property.

We analyzed several Y linked genes and DYZ1 region in the two cell lines namely DU145 (originated from the metastatic site brain of a 69 years old Caucasian male) and LNCaP (originated from the metastatic site left supraclavicular lymph node of a 50 years old Caucasian male). DU145 is hypotriploid having both 61 and 62 chromosomes and carries a single Y per cell. The Y chromosome of DU145 carries a translocated part of chromosome 20. LNCaP is hypotetraploid having 84 chromosomes and carries 2 Y chromosomes per cell. The human Y chromosome harbours 20% of the DYZ1 satellite sequence. DYZ1 was identified as 3.4 Kb band generated upon *HaeIII* digestion of the human male genomic DNA. A normal human Y chromosome contains approximately 4000–4300 copies of the DYZ1 arrays. Since DYZ1 copies do not participate in recombination, it was deduced to have no functional or evolutionary advantage. However, this is now reported to play a crucial role in chromatin folding and maintenance of the structural integrity of the Y chromosome.

With this background, we screened 51 standard sequence tagged sites (STSs) corresponding to a male-specific region of the Y chromosome (MSY), sequenced the coding region of the SRY gene and assessed the status of the

DYZ1 arrays in the human prostate cancer cell lines DU145 and LNCaP. The MSY was found to be intact and coding region of *SRY* showed no sequence variation in both the cell lines. However, DYZ1 arrays showed sequence and copy number variations. DU145 and LNCaP cells were found to carry 742 and 1945 copies of the DYZ1, respectively (Figure 1). The DYZ1 copies are much below the average of that reported in normal human males. Similarly, the number of "TTCCA" repeat and its derivatives within the DYZ1 arrays showed variation compared to those of the normal males. Despite these changes, Y chromosome survived in about 58% of DU145 cells (Figure 2). Most likely, such DU145 cells have managed to retain the critical number of the DYZ1 copies with near normal sequences needed for the sustenance of the Y chromosome. Clearly, the DYZ1 is maximally affected in both the cell lines. We presume that besides susceptibility to PC, males from different ethnic and geographical regions may show sequence and copy number variations in the DYZ1 arrays. However, this warrants a detailed analysis of sufficient number of PC males before a conclusion can be drawn. Based on this study, we construe that copy number status of the DYZ1 may be exploited as a supplementary prognostic tool to monitor the occurrence of prostate cancer using biopsied samples.

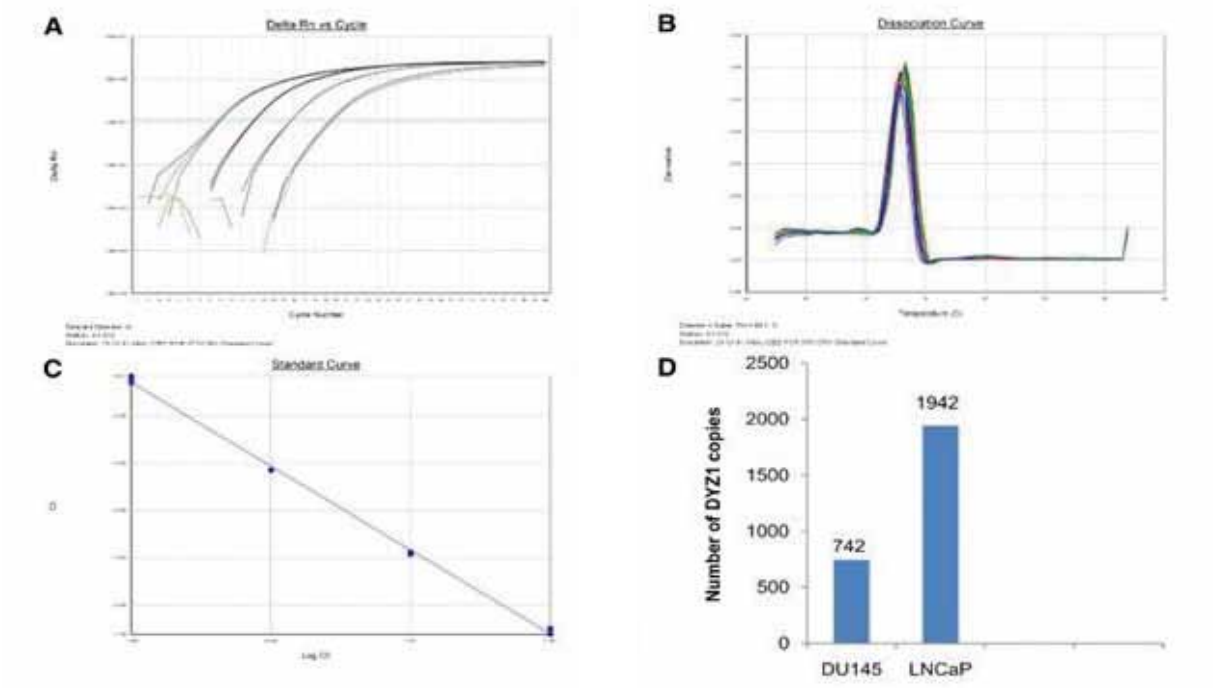

Figure 1 : Copy number estimation of DYZ1 in DU145 and LNCap. (A) represents the amplification plot. (B) the corresponding dissociation curve (C), the standard plot and (D) shows the number of DYZ1 copies in DU145 and LNCaP.

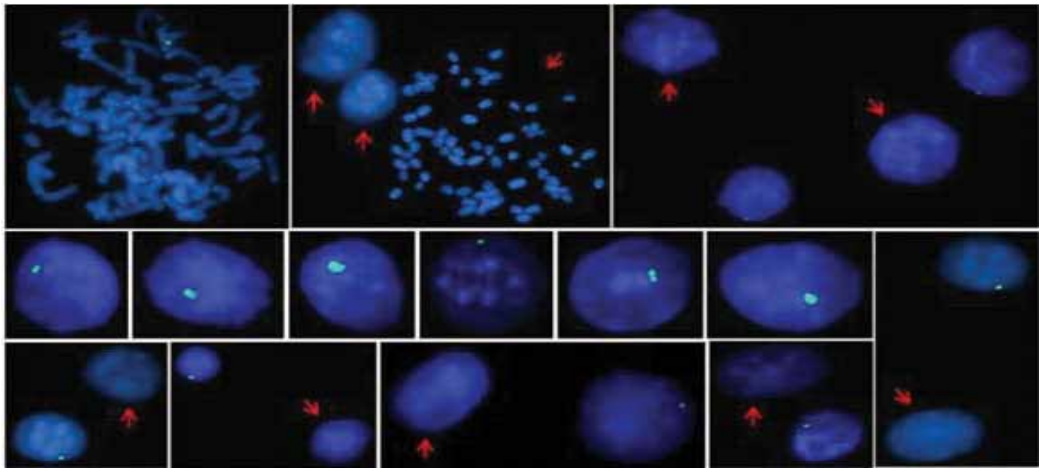

Figure 2 : Localization of DYZ1 in DU145 cells by FISH. DAPI (4', 6-diamidino-2-phenylindole) stained metaphases and interphase nuclei are shown having green signal of DYZ1. Nuclei and metaphases lacking DYZ1 are indicated by red arrow. Note the variation in the DYZ1 signal intensities across nuclei reflecting copy number variation.

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Original peer-reviewed articles

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Deciphering the role of cell signalling in *M. tuberculosis* biology and in the function and dynamics of nucleoporins

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Protein Kinase B (PknB) of *Mycobacterium tuberculosis* is essential for growth of the pathogen in vitro as well as for survival within the host.

Analysis of the *M. tuberculosis* genome sequence suggested the presence of 11 putative eukaryotic-like STPKs and 3 protein phosphatases. The *M. tuberculosis* STPKs affect key mycobacterial processes. Protein kinases A and B, encoded by *pknA* and *pknB* respectively, are part of the same operon carrying cistrons coding for protein phosphatase *pstP*. Though a number of substrates of PknB have been identified, structure-function relationships of the various domains of the protein have not been investigated *in vivo*. The *M. tuberculosis* protein kinase B (PknB) comprises an intracellular kinase domain, connected through a transmembrane domain to an extracellular region that contains four PASTA domains. Our study describes the comprehensive analysis of different domains of PknB in the context of viability in avirulent and virulent mycobacteria. We find stringent regulation of PknB expression necessary for cell survival, with depletion or overexpression of PknB leading to cell death. While PknB-mediated kinase activity is essential for cell survival, active kinase lacking the transmembrane or extracellular domain fails to complement conditional mutants not expressing PknB. By creating chimeric kinases, we find that the intracellular kinase domain has unique functions in the virulent strain, which cannot be substituted by other kinases. Interestingly, we find that although the presence of the carboxy-terminal PASTA domain is dispensable in the avirulent *M. smegmatis*, all four PASTA domains are essential in *M. tuberculosis*. The differential behavior of PknB vis-à-vis the number of essential PASTA domains, and the specificity of kinase domain functions, suggests that PknB-mediated growth and signaling events differ in virulent compared to avirulent mycobacteria. Mouse

infection studies were performed to determine the role of PknB in mediating pathogen survival in the host demonstrate that PknB is not only critical for growth of the pathogen *in vitro*, but is also essential for the survival of the pathogen in the host.

Phosphorylation of nucleoporin Tpr governs its differential localization and is required for its mitotic function.

Nucleoporins are vital components of the nuclear envelope that mediate several critical cellular processes such as transport of macromolecules, progression of cell cycle, gene expression, and chromatin organization. A major constituent of the nuclear basket region of the NPC, nucleoporin Tpr plays a multi-dimensional role in the cell. Studies have implicated a role for Tpr in regulating important processes including chromosome segregation, chromatin organization and unspliced RNA export. Tpr associates with Mad1, Mad2 and the members of the dynein complex during mitosis, and these interactions have been found to be crucial for mediating the proper segregation of chromosomes during the anaphase. We have previously established that Tpr is phosphorylated in both, MAP kinase dependent and independent manner, and found that Tpr acts as both a substrate and as a scaffold for MAP kinase ERK2. Now, we report the identification of S2059 and S2094 as the major novel ERK-independent phosphorylation sites and T1677, S2020, S2023 and S2034 as the minor ERK independent phosphorylation sites found in the Tpr protein *in vivo*. Our results suggest that protein kinase A phosphorylates the S2094 residue, and the site is hyperphosphorylated during mitosis. Further, we find that Tpr phosphorylated at the S2059 residue distinctly localizes with chromatin during the telophase. Abrogation of S2059 phosphorylation abolishes its interaction with Mad1, thus compromising nuclear distribution of Mad2, and results in cell cycle defects. The identification of novel phosphorylation sites on Tpr and the observations presented in this study open fresh avenues for the better understanding of Tpr functions.

Publication

Original peer-reviewed article

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Elucidating the molecular mechanisms of aging and innate immunity using *Caenorhabditis elegans*

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We are interested in understanding the molecular basis of aging using *Caenorhabditis elegans*, in combination with molecular genetics and genomics. We are trying to decipher the complex interplay of transcription factors (TF) downstream of the Insulin-IGF-1 signalling (IIS) pathway in regulating gene expression required for longevity, metabolism, stress- and pathogen resistance. Since Dietary Restriction (DR) is the only intervention that increases life span and delays age-onset diseases, we are trying to decode the molecular events that follow initiation of DR. We are also using chemical genetics to identify and study mechanisms of novel longevity extending compounds.

A. Deciphering coordinate regulation of genes downstream of IIS pathway

We generated a genome-wide recruitment map of FOXO/DAF-16 under low IIS condition. FOXO/DAF-16 binds to 5098 coding and 216 non-coding genes. About 35% of the peaks mapped within 0.5 kb of the transcription start sites, leading to maximum activation of these target genes. We found an overlap of ~35% with existing microarray experiments revealing that microarray data has captured events not directly related to DAF-16 recruitment. About 32% of the DAF-16 peaks have consensus DAF-16 binding sites while 42% possess the related GATA-like site. Additionally, predictive algorithms detected other novel TF-binding sites in the DAF-16-binding peaks, indicating combinatorial control of target genes.

B. Involvement of miRNA-Transcription factor networks in dietary restriction

To study the miRNAome during DR, we performed Next Generation sequencing of DR worms revealing 81 upregulated miRNAs, with no significantly downregulated ones. We also identified the FOXA transcription factor PHA-4

as directly responsible for upregulating these miRNAs during DR. This was interesting as DR requires FOXA/PHA-4 to extend life span. We also performed transcriptome analysis of the DR worms and found that most of the upregulated genes are PHA-4 direct targets. Remarkably, these upregulated genes are also post-transcriptionally targeted by the miRNA transcribed by PHA-4, forming a large feed-forward loop (FFL)-containing network. We propose that these PHA-4-driven FFLs instil robustness in the system, leading to increased longevity during DR.

C. Involvement of novel kinases in dietary restriction

We have identified a novel kinase (IDR-1) that initiates a process similar to DR when knocked down, leading to increased life span. We performed a microarray analysis and found that genes involved in fatty acid oxidation and xenobiotic detoxification are upregulated. We showed that FOXA/PHA-4 regulates these upregulated genes. We also showed that the low levels of ROS produced during DR is due to the increased fatty acid oxidation that shifts the balance of NADH/FADH2 such that more mitochondrial complex II is used. Consequently, *idr-1* RNAi failed to increase life span in a complex II-defective *mev-1* mutant, but could do so in complex I-defective *gas-1* mutant. Additionally, in a beta oxidation-defective mutant, DR did not decrease ROS production. We conclude that during DR, metabolic reprogramming is the reason for low ROS generation and increased longevity. Further, we initiated characterization of an *idr-1*homolog, called *idr-2*. Most of the phenotypes of *idr-1* and *idr-2* knockdown are similar. Interestingly, *idr-2* mutant increases life span in a food-type-dependent manner, the mechanism of which we are currently investigating.

D. Drugs that can extend *C. elegans* life span

We have recently identified a FDA-approved drug that increases life span by preventing glycation. We showed that JNK signalling is involved, as the drug failed to increase life span in mutants of *jnk-1* or its upstream kinase *jkk-1*. Additionally, a drug analog that has anti-glycating activity but not another variant that lacks it can increase life span, connecting the anti-glycating activity to longevity extension. Using LC-MS^{E}, we found that proteins important in metabolism and maintaining musculature were protected from glycation by the drugs. AGE modifications on these important proteins may disrupt normal cellular functions leading to aging, a process that the drug reverses.

Publications

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Molecular biology of infectious diseases

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(A) Development of recombinant vaccine against *Clostridium perfringens*

Clostridium perfringens have been implicated in several diseases of humans and domestic animals and have a significant economic impact on the agricultural industry worldwide. Beta toxin produced by *C. perfringens* type B and C is known to be the primary pathogenic factor of necrotic enteritis and is considered to be a promising chemotherapeutic target.

For the development of vaccine against beta toxin, the protein is needed to be produced in large amounts. Earlier attempts to express the beta toxin in *E. coli* resulted in very poor expression and extremely low yields. Therefore, in order to optimize expression in *E. coli*, mature beta toxin gene was synthesized with codon optimization for *E. coli* expression. SDS-PAGE analysis of induced cultures revealed high level expression of the recombinant protein. The authenticity of the expressed product was established by immunoblot analysis. The recombinant beta toxin was present predominantly as inclusion bodies. Beta toxin was purified from inclusion body fraction and was refolded by pulse refolding method. The biological activity of the refolded protein was evaluated in HL 60 cells *in vitro* and *in vivo* using BALB/c mice.

The anti-sera obtained from BALB/c mice immunized with recombinant beta toxin were able to detect the recombinant beta toxin in immunoblot analysis. The end-point titer was determined to be 100000 by ELISA. Splenocyte proliferation assay using splenocytes isolated from the immunized

mice showed significantly higher proliferation upon stimulation with the recombinant beta toxin.

In vitro antibody neutralization assays indicated that serum obtained from beta toxin immunized mice significantly neutralized the toxin *in vitro* using HL-60 cells. The toxin-antisera formulation reduced the HL60 cell death compared to serum from the PBS immunized mice. Complete protection was observed against minimal lethal dose of beta toxin in animal group immunized with heat inactivated recombinant beta toxin.

(B) Studies on the functional characterization of PE_PGRS and PE_PPE proteins of *Mycobacterium tuberculosis* H37Rv

Mtb employs numerous intelligent strategies to persist inside host and evade the host defence mechanisms. One such strategy involves the modulation of cytokine profile of macrophages to deviate anti-microbial T-cell responses. The pro-inflammatory cytokines, IL-12, TNF-α and IL-6, play an important role in protective immunity to tuberculosis infection by regulating T-cell activation and stimulating macrophage-mediated microbicidal mechanisms. In order to assess the role of PE_PGRS30 in modulation of host immune response, *M. smegmatis* cells expressing PE_PGRS30 were used as this mycobacterial species naturally lacks PE_PGRS family of genes. Infection of PMA-differentiated human THP-1 macrophages with *M. smegmatis* expressing PE_PGRS30 resulted in reduced production of inflammatory cytokines. However, no change was recorded in the survival ability of PE_PGRS30-recombinant *M. smegmatis* in macrophages or in macrophage viability. Down-regulation of pro-inflammatory cytokines by PE_PGRS30 was not mediated through IL-10.

Further, to dissect the PE_PGRS30 protein into distinct functional domains, THP-1 macrophages were infected with *M. smegmatis* expressing deletion mutants of the protein. Profiling of pro-inflammatory cytokines illustrated that, similar to the full length protein, only PE + PGRS mutant caused reduction in the levels of IL-12, TNF-α and IL-6. However, greater reduction in the TNF-α levels by PE+PGRS mutant as compared to complete PE_PGRS30 might be attributed to the anti-inflammatory effect of the C-term domain.

The role of PPE14 in modulating host macrophage effector functions was evaluated. The purified recombinant-PPE14 stimulated the production of pro- inflammatory cytokines particularly TNF α and IL12 by PMA-differentiated THP-1 macrophages and human peripheral blood mononuclear cells. The induction of pro-inflammatory cytokines by PPE14 was found to be TLR2- and MyD88- dependent.

Publications

Original peer-reviewed articles

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Patent

1. Garg LC, Bhatia B (2014). Recombinant subunit vaccine against beta toxin of *Clostridium perfringens*. *(Application No.58/DEL/2014; Indian Patent Filed on January 08, 2014).*

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Epigenetic regulation of the eukaryotic genome: Role of transcriptional insulators in organizing chromatin

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Mechanisms underlying interactions between *cis*-acting regulatory elements in context of chromatin are incompletely understood even though such interactions are crucial for appropriate regulation of nuclear processes like transcription and VDJ recombination. CTCF dependent insulators play an important role in the functional organization of the mammalian genome as they can coordinate intrachromosomal and interchromosomal contacts and thus influence *cis*-DNA interactions. Our efforts are directed towards understanding the influence of CTCF on chromatin domain organization and its relevance for transcription and VDJ recombination.

To explore the mechanisms underlying insulator activity, antigen receptor loci like IgH, TCRa/d, TCRb etc, are particularly interesting. Transcription as well as RAG mediated VDJ recombination are exquisitely regulated during development at these loci and underscore the importance of appropriate enhancer-promoter interactions. Further, recombination requires physical interaction between RSS elements associated with the V, D and J segments. These segments are located at large distances from each other on the chromosome and higher order chromatin reorganization is necessary to bring them together prior to recombination. By exhibiting long range interactions between different types of elements, the antigen receptor loci present a useful framework to explore the role of CTCF in defining independently regulated chromatin domains. Taking advantage of this, we are currently investigating the chromatin structure and organization of the wild type and genetically manipulated TCRb loci to understand various aspects of insulator function as well as VDJ recombination.

Organization of an ectopic CTCF dependent insulator at the TCRb locus was observed to impair enhancer Eb dependent transcription and D-to-J recombination in the genetically manipulated TCR-ins allele. Chromatin structure at the nucleosomal level is the crucial determinant of transcription and recombination. Allele specific ChIP analysis to detect the presence of

H3K4me3 and H3K9Ac demonstrated that the acquisition of activating histone modifications at the PDb1 promoter was almost completely abolished in the mutant TCR-ins allele compared to the wild type TCRb allele. The enrichment of these marks was also enormously reduced in the gene segments Jb1 and Cb1 that are proposed to be independent of Eb-PDb1 interaction. Eb has been proposed to regulate the locus by "looping" as well as by some form of "tracking." Based on the 3C analysis, it was evident that Eb makes a physical contact with PDb1 as well as PDb2 on the wild type TCRb locus. However, presence of a functional CTCF dependent insulator in TCR-ins prevents the formation of Eb-PDb1 loop while leaving the Eb-PDb2 loop unaffected. Together, our analysis of enhancer-promoter-insulator interactions and histone modifications suggests that Eb employs "facilitated tracking" to activate PDb1 and PDb2 and the CTCF dependent H19-ICR insulator has the ability to abrogate several aspects of facilitated tracking.

In addition to blocking the enhancer activity at TCRb locus, the inserted H19-ICR also altered the choice of Vb segments used for VDJ recombination in a CTCF dependent manner suggesting the ability of CTCF to modulate interactions between *cis*-regulatory elements other than promoters and enhancers. We have identified a few regions of the TCRb locus that bind CTCF and have standardized chromosome conformation capture (3C) assay to investigate the higher order chromatin organization defined by CTCF binding. The 3C was carried out in thymocytes derived from Rag1 deficient mice i.e. on TCRb alleles prior to VDJ recombination. ProB cells from Rag1 deficient mice were used as controls. Clearly, the interactions amongst CTCF binding sites were specific to T cell lineage and were significantly lower in ProB cells. Our analysis suggests that CTCF based chromatin loops are relevant for V-to-DJ recombination at TCRb locus and get altered under the influence of ectopic CTCF binding sites.

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Role of cell signaling in eukaryotic development

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We are interested in dissection of signaling and trafficking mechanisms that operate in diverse cell types. Here is a brief description of our recent studies:

I. Dissection of intracellular signaling and trafficking cascades of *Plasmodium falciparum.*

a. cAMP and calcium signaling in the blood stage development of malaria parasite.

Calcium Dependent Protein Kinases (CDPKs) are important calcium effectors that regulate diverse parasitic processes. Some CDPKs are essential for parasite, therefore, are refractory to gene disruption. We used a new strategy to knock down PfCDPK1 in P. falciparum, which involves its fusion to FKBP domain. PfCDPK1 was successfully knocked down and seemed to have a role in erythrocyte invasion. In order to decipher the mechanism via which calcium signaling and PfCDPK1 may regulate invasion, we need to identify substrates of this kinase. A comparative phosphoproteomics is being used to identify PfCDPK1 substrates. We further dissected the mechanism via which cAMP and calcium signaling pathways crosstalk. Parasitic cAMP levels seem to modulate calcium levels in the parasite, thereby may regulate calcium signaling. In this context, the role of CDPKs was investigated and the modulation of cAMP levels were found to alter CDPK activity.

b. Role of phosphoinositides in parasite signaling and trafficking

PfCDPK7 interacts with PI(4,5)P2 via its PH domain. Preliminary studies had suggested that it is important for the development of asexual parasites as the growth rates of PfCDPK7 knockout (PfCDPK7-KO) were significantly attenuated in comparison to the 3D7 parasite line. Strikingly, the transition of a significant number of PfCDPK7-KO rings failed to mature to trophozoites. These aborted parasites exhibited distorted morphology, which explained the slower growth rate PfCDPK7-KO line. Furthermore, comparison of the number of merozoites per segmenter/schizonts revealed that PfCDPK7-KO parasites had significantly fewer nuclear bodies per schizont/segmenter. These observations suggest that PfCDPK7 may be important for the development of rings and may also have a role in schizogony. Various biochemical and cell biological studies suggested the involvement of PfCDPK7 is TVN formation and nutrient uptake. The mechanism via which it regulates this process is being investigated.

Toxoplasma gondii, an apicomplexan parasite, shares several similarities with *Plasmodium* in processes like host cell invasion. *Toxoplasma gondii* ranks among the best experimental models to study obligate intracellular parasitism. The CDPK family, which is absent in the mammalian host, is conserved in apicomplexan parasites. TgCDPK7 is an orthologue of PfCDPK7 in *Toxoplasma gondii*. To study the function of *TgCDPK7*, gene disruption studies were carried out which suggested that it may be essential for parasite growth. As indicated above, conditional or inducible gene knock out (iKO) is possible in *Toxoplasma*. Attempts to generate TgCDPK7-iKO using tetracycline (Tet)-repressor based system were successful.

II. Molecular mechanisms that regulate Cell Cycle Related Neuronal Apoptosis (CRNA)

The cell cycle of terminally differentiated cells like neurons is arrested in response to neurotrophic factor mediated signaling. However, the cell cycle of neurons is reactivated in response to neurotoxic insults, which leads to their apoptosis. Recently, we identified a novel mechanism via which the levels of cyclin D1 are upregulated, which results in Cell Cycle Related Neuronal Apoptosis (CRNA). We are interested in investigating the role of miRNA in CRNA. Our studies suggest that miR34a expression is elevated during neuronal differentiation and may promote it. Cyclin D1 was identified as a target of miR34a in neurons. The levels of miR34a upon treatment with neurotoxic amyloid peptide $A\beta_{42}$, which causes CRNA, were measured. When miR34a was over expressed in neurons, $A\beta_{42}$ induced CRNA was significantly reverted. Interestingly, the levels of cyclin D1, which increase upon $A\beta_{42}$ treatment, reduced significantly upon miR34a overexpression. The decrease in CRNA caused by miR34a was significantly reverted by cyclin D1 over expression. These data suggested that the decrease in miR34a expression contributes to CRNA and it may achieve this by targeting cyclin D1. Further studies to decipher the mechanisms involved in the regulation of miR34a in CRNA are in progress.

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Understanding mechanisms underlying biology of depigmenting disorder Vitiligo

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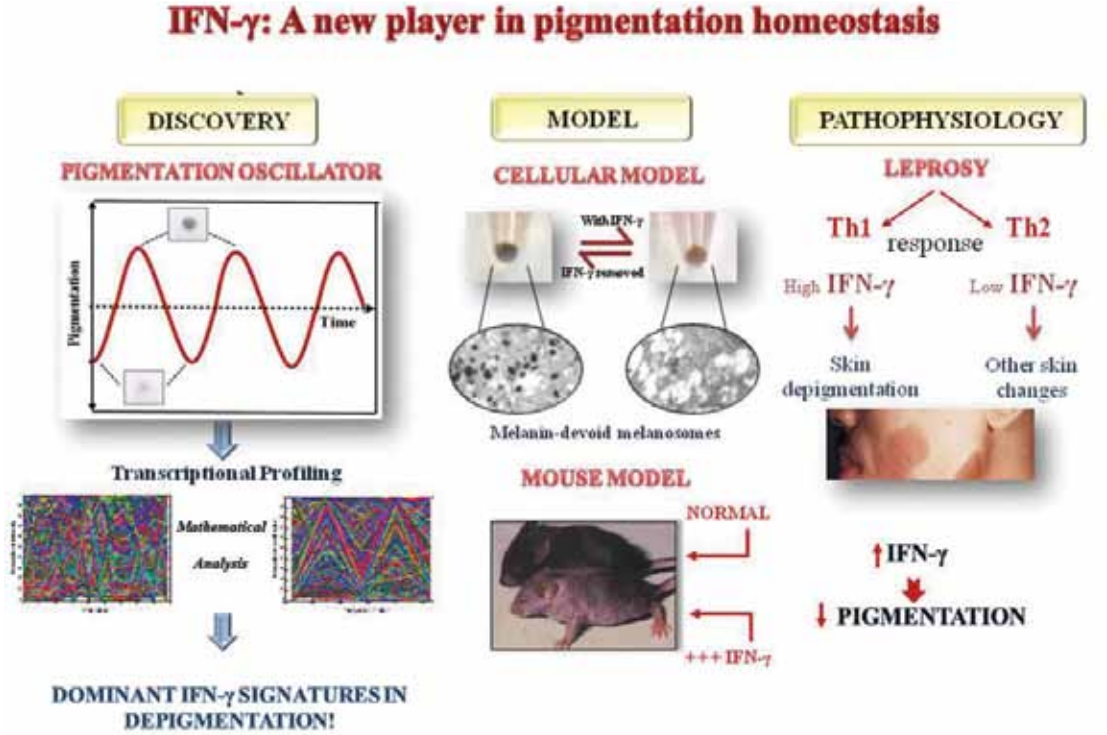
The paradoxical interaction of sunlight with skin forms the fundamental basis of human existence. The apparent resilient impression of human skin camouflages incessant sensitization processes occurring at the interface. Pigmentation is one such adaptive response that protects epidermal cells from persistent solar radiation. Due to strong environmental influence, subtle changes accumulate throughout the life-span of an individual. Ease of identification of altered phenotypes has led to a lot of information regarding genetic aspect of controlling skin color. However, molecular mechanisms regulating dynamicity of this complex trait need to be deciphered.We are interested to elucidate molecular mechanisms underlying this homeostatic process since many of the skin diseases are likely to be an outcome of perturbed microenvironment of skin epidermis. While on one hand there are skin cancers that are common amongst lightly pigmented Caucasian skin, disorders like vitiligo result in white depigmented patches in dark pigmented skin.Since latter is of serious concern for Indian skin, our laboratory is interested to understand dynamic aspects of pigmentation biology to delineate critical nodes that could trigger progressive loss of melanocyte cells from epidermis. Using a systematic approach involving cellular, molecular and pathophysiological studies, we have unravelled a new temporal function of an immunomodulatory factor, IFN-γ, in skin pigmentation homeostasis (Figure 1). Our study establishes direct association between immune system and skin pigmentation, providing new leads to understand pigmentary diseases.

UV-mediated skin tanning is a protective response of epidermal cells involving increased melanin formation that maintains genomic integrity. However, melanin synthesis itself is an energy intensive and pro-oxidant process. Uncontrolled melanogenesis is thus detrimental to the cells. While several pathways of tanning have been studied, recovery mechanisms have not been deciphered. To identify active regulatory networks underlying melanogenesis, we developed a pigmentation oscillator model using B16 melanoma cells that can reversibly undergo continuous cycles of pigmentation-



depigmentation without exogenous addition of any factors. A mandatory requirement to maintain oscillatory function is the presence of negative feedback loop with a time delay. To delineate such negative regulatory mechanisms underlying melanogenesis, we performed periodogram analysis of the oscillatory transcriptome dataset from pigmentation oscillator. Our unbiased mathematical analysis revealed an inverse correlation of IFN-γ signaling with melanogenesis. Exogenous addition of IFN-γ to normal human melanocytes indeed decreased expression of key melanogenic genes with concomitant accumulation of immature melanosomes. We established the canonical JAK-STAT1 pathway, together with downstream transcriptional mediator IRF1, as the central mechanism driving transcriptional suppression of pigmentation genes. This effect is independent of MITF, the central transcription factor governing melanocyte biology. Remarkably, melanocytes regain their normal cellular programming on withdrawal of the cytokine, a scenario that makes this regulatory network tenable to function in physiological context.

Surprisingly, prior to our study, significance of IFN-γ in melanogenesis has been underappreciated. IFN-γ signatures in mouse epidermis become apparent after six days of UV exposure. In such transient temporal responses, biological function can be understood by utilizing chronic models.We thus probed for IFN-γ signatures in epidermis of leprosy patients, an infectious human disease that manifests in form of a spectrum. Our studies show an association of increased IFN-γ signaling and hypopigmentation phenotype in leprosy patients. Interestingly, transgenic mice with chronic expression of IFN-γ in epidermis were earlier reported to show complete penetrance of hypopigmentation phenotype. Our studies with IFN-γ null animals likewise show

increased pigmentation in knockout animals. This difference is substantially enhanced on UVB exposure where knockout animals display a clear delay in regaining basal pigmentation levels, exemplifying the requirement of IFN-γ in maintenance of pigmentation homeostasis after sun exposure.While further studies are required to establish this physiological relevance, our data suggest a novel possible role of IFN-γ in the detanning process.

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Determining the signaling and repair pathways that are altered in human cancer using RecQ helicases as the model system

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Project A: Decipher the role of ubiquitylation of BLM helicase in DNA damage response and repair

In order to ascribe a biological function to the RNF8/RNF168-dependent ubiquitylation of BLM, the sites of ubiquitylation needed to be identified. To narrow down the lysine residues within BLM potentially targeted for ubiquitylation, two independent ubiquitylation site prediction programs (Ubpred and Ubipred) were used, which both highlighted lysines at 105, 225 and 259 (K105, K225 and K259) as being high confidence target resides. To investigate whether these lysine residues were ubiquitylated by RNF8/RNF168, recombinant BLM containing each individual lysine mutated to arginine or all three sites were mutated in combination (3K-R) and purified. Loss of any of the three predicted lysine residues individually resulted in a reduction in the level of *in vitro* BLM poly-ubiquitylation. The RNF8/RNF168-dependent ubiquitylation was completely abrogated in 3K-R BLM mutant indicating that RNF8/RNF168 can target multiple lysine residues within BLM for ubiquitin chain conjugation *in vitro*. More importantly, loss of these three critical N-terminal lysine residues resulted in a significant decrease in the overall level of K63-

linked BLM ubiquitylation after HU-treatment, supporting the notion that they represent the major sites of K63-linked BLM ubiquitylation *in vivo*. In stark contrast to the WT BLM, the single mutants substantially compromised the ability of the exogenous BLM to form HU-induced foci. This defect was exacerbated when all three sites of ubiquitylation on BLM were lost. A similar lack of BLM 3K-R localization was also observed after ionizing radiation. Combined, these data support the concept that RNF8/RNF168-dependent ubiquitylation of BLM promotes its recruitment to sites of DNA damage. Importantly, in keeping with an essential requirement for the RNF8/RNF168-dependent ubiquitylation of BLM after DNA damage for its function, wild type BLM but not the BLM 3K-R mutant was able to suppress HR in BS cells.

Project B: Determine the functions of RECQL4 and p53 in mitochondria

We have recently demonstrated that RECQL4 and p53 are required for optimal *de novo* mtDNA replication. To further characterize the interaction of RECQL4-p53-PolγA with mitochondrial control region *in vivo*, we carried out mitochondrial chromatin immunoprecipitation (mtChIP) assays with a combination of five primer sets spanning the entire control region. The mtChIP results indicate that both RECQL4 and p53 are required for optimal binding of mitochondrial polymerase PolγA to specific regions within the control region and thereby regulate mtDNA replication. To elucidate the role of RECQL4 and p53 in mtDNA replication, we first determined whether RECQL4 and p53 were complexed *in vivo* with the PolγA/B2 holoenzyme. For this purpose, co-immunoprecipitations of endogenous proteins was carried out in two isogenic pairs of cell lines, namely RTS patient fibroblast AG05013 and its isogenic counterpart expressing wild-type RECQL4 namely AcGFP-RECQL4 (1-1208) Clone 1. In addition we also performed co-imunoprecipitations of the endogenous proteins from cell lines HCT116 and HCT116 p53-/-. Immunoprecipitation with anti-RECQL4 antibody revealed the presence of a complex consisting of RECQL4, p53, PolγA and PolγB in AG05013 AcGFP-RECQL4 (1-1208) Clone 1 cells. Such a complex was absent in the co-IPs of AG05013 cells, which do not express RECQL4, indicating the specificity of the reaction. A similar complex was also observed in HCT116 cells when immunoprecipitated with PolγA antibody. The PolγA immunoprecipitate in HCT116 $p53^{-/-}$ cells contained only RECQL4 and PolγB (and not p53), thereby again indicating the specificity of the complex formation. The lack of any specific signal when the immunoprecipitations were carried out with IgG beads further confirmed the presence of the RECQL4-p53-PolγA/B2 complex *in vivo*.

Publications

Original peer-reviewed articles

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Understanding the regulation of DNA replication

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Our laboratory is working towards understanding the mechanisms by which checkpoint proteins stall the cell cycle and DNA replication, preventing genomic instability and cancer. The objective is to identify yet unknown checkpoint pathways that monitor the replication apparatus. Thus, we are attempting to unravel the protective regulatory control of mammalian cells, failure of which is likely to cause genomic instability.

Role of alternate single-stranded DNA-binding proteins in checkpoint signaling

The damaged single stranded-DNA is rapidly coated with the primary single strand binding protein complex, Replication Protein A (RPA) which enables the recruitment of ATR and its interacting partner, ATRIP to the sites of DNA damage. We observed that in the absence of RPA complex, an alternate single-stranded DNA-binding protein complex, hSSB1-INTS3 associates with the single-stranded DNA and elicits ATR–ATRIP mediated checkpoint response (Fig. 1). Furthermore, similar to the canonical checkpoint pathway this alternate pathway requires the sensor, Rad17-9-1-1 complex. Therefore, we identified an alternate pathway for the activation of ATR-ATRIP complex, modifying the fundamental understanding of checkpoint activation where recruitment of RPA is considered obligatory.

Role of non-coding RNAs in regulation of cell-cycle and DNA replication

The mechanism of regulation of cell cycle and DNA replication genes during quiescence is not completely understood and we have analyzed the changes in microRNA and gene expression during serum starvation and predicted the targets of the upregulated microRNAs. We are manipulating the expression of stress-regulated microRNAs and assay the effect on cell cycle and replication factors. We will also address if disruption of the regulated microRNAs alters the cell cycle progression. Therefore, in this part we aim to determine the role of non-coding RNAs in regulating cell-cycle and DNA replication genes during transition between proliferation and quiescence.

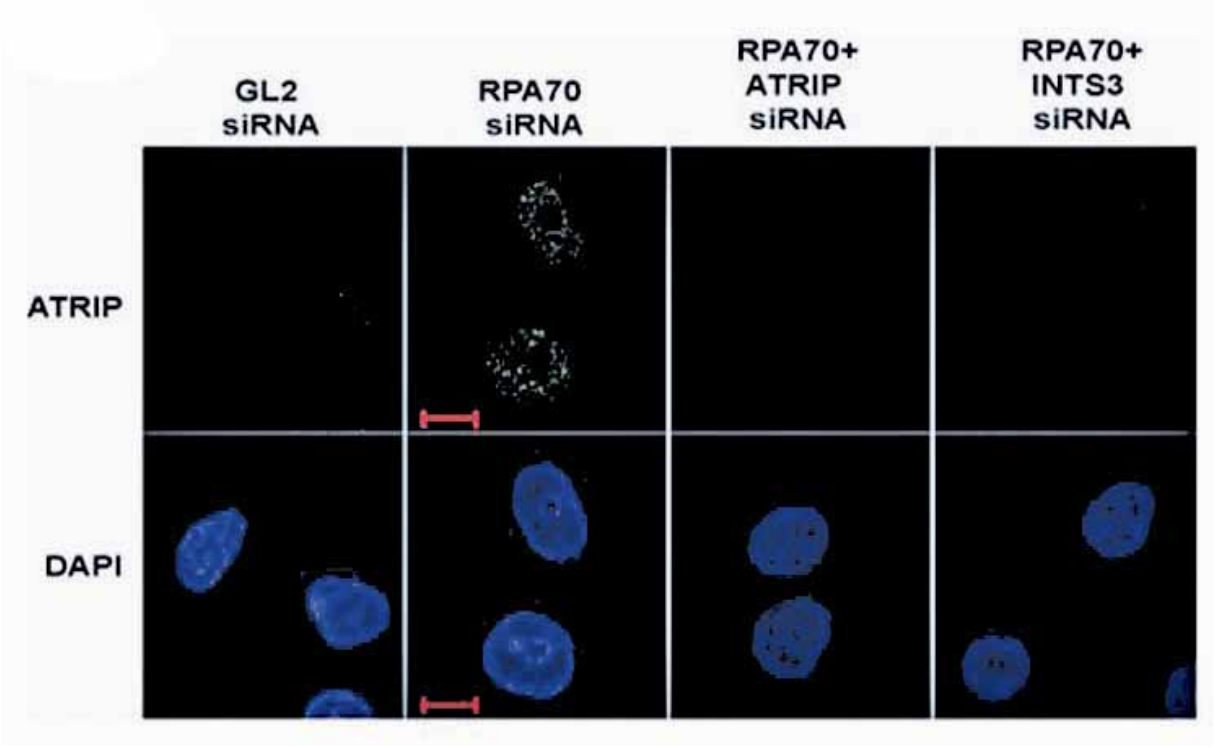

Figure 1. ATRIP foci formation after RPA depletion. HeLa cells were transfected with siRNA duplexes against *GL2, RPA70* alone or in combination with *ATRIP* or *INTS3* and the cells were visualized for ATRIP foci. Scale bar is 10 micron.

Role of replication proteins during mitosis

Recent studies have shown that some replication proteins localize to centrosomes and kinetochores. We systematically depleted replication proteins by RNAi and assayed for mitotic defects: we identified that depletion of a GINS subunit results in multipolar spindle formation and increased centrosome number in mitotic cells (Fig. 2). By utilizing two different antibodies we also established that the GINS subunit localizes to the centrosomes. Controlled depletion of GINS subunit did not trigger a DNA damage checkpoint response indicating that the observed phenotypes are not due to DNA damage induced cell cycle checkpoint. Moreover the aberrant centrosome number was not observed in interphase cells indicating that the GINS subunit localizes to the centrosomes and is required for mitotic progression. We would now like to determine the role of GINS subunit in coordinating the mitotic events.

In summation. we aim to define the role of the alternate SSBs in the activation of ATR-ATRIP after different genomic stresses. We are evaluating the role of non-coding RNAs in regulating cell-cycle and DNA replication genes during proliferation and quiescence. Finally, we are ascertaining the role of replication proteins during mitosis.

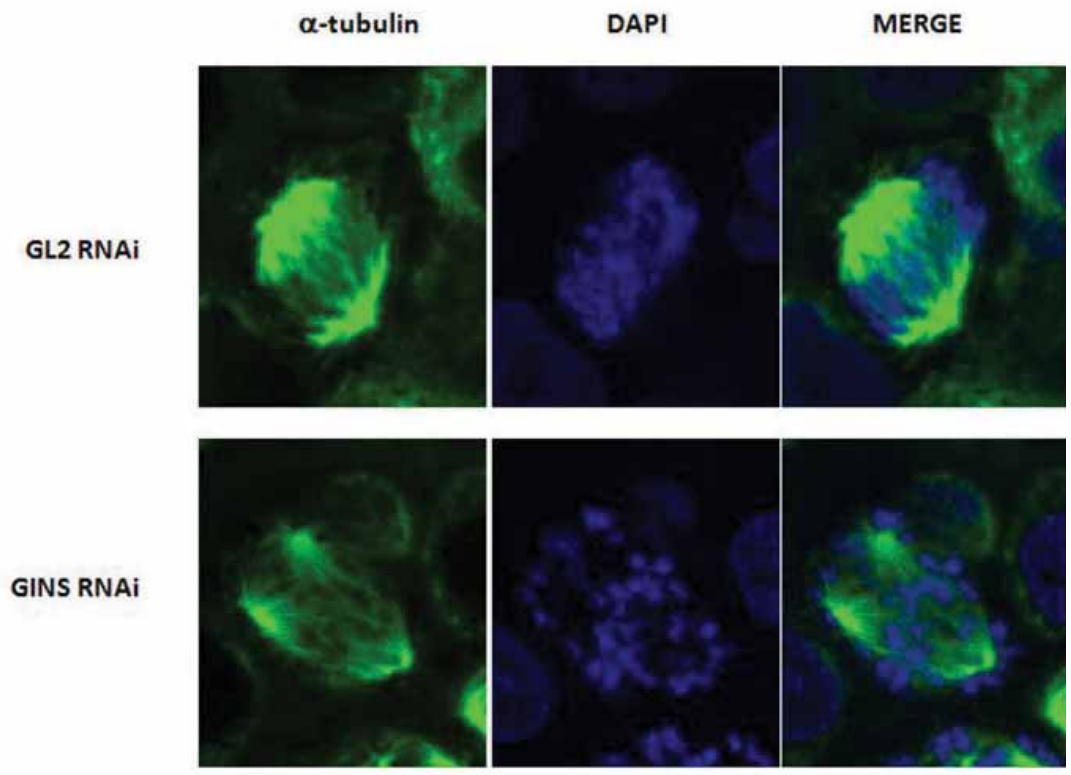

Figure 2. RNAi depletion of GINS subunit induces mitotic aberrations. HeLa cells were transfected with *GL2* or *GINS* subunit siRNA followed by immunofluorescence with mouse anti-alpha-tubulin antibody. Nuclei were stained with DAPI. Note that depletion of GINS subunit results in multipolar spindles.

Sanjeev Das

The role of tumor suppressors in stress response

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In response to various intracellular and extracellular stresses p53 is rapidly stabilized and activated thereby inducing cell cycle arrest, apoptosis or senescence depending upon the extent of cellular damage. Temporal regulation of diverse sets of target genes is thought to be achieved by post-translational modifications and through its interaction with other cellular proteins. To investigate p53 regulation, we performed a proteomics screen to identify p53 interacting proteins. We are now conducting further studies to understand the function of HDAC5 as a p53 interacting protein. HDAC5 belongs to the class IIa HDAC (histone deacetylase) subfamily. By performing coimmunoprecipitations as well as *in vitro* binding assays we found that HDAC5 directly interacts with p53. Since HDAC5 is a member of class II family of deacetylases, we examined the effect of HDAC5 on p53 acetylation upon genotoxic stress. Using immunoblotting as well as mass spectrometry based assays we showed that HDAC5 deacetylates p53 with specificity for lys120 site of p53. Since our results indicate that HDAC5 undergoes nuclear-cytoplasmic shuttling upon genotoxic stress, we further investigated the underlying molecular mechanism. We found that with increased accumulation of ROS at extended periods of genotoxic stress, there is a concomitant activation of CAMKII (Ca^{2+}/calmodulin-dependent protein kinase II), which phosphorylates HDAC5 leading to its nuclear export. We also found that HDAC5 is required for the selective induction of p53 proarrest and antioxidant target genes at early phase of genotoxic stress while at extended periods HDAC5 undergoes nuclear export resulting in downregulation of p53 proarrest and antioxidant target genes and induction of proapoptotic target genes. These findings were further corroborated by analyzing cell cycle profile and ROS levels. To corroborate the role of HDAC5 in genotoxic stress response *in vivo*, we used RNAi to knockdown HDAC5 expression in mice which were then subjected to genotoxic stress. These results demonstrate that HDAC5 plays a key role in modulating p53-mediated genotoxic stress response *in vivo* that augments prosurvival functions of p53 over apoptosis.

p73 is one of the tumor suppressors of the p53 family of nuclear transcription factors. p73 exhibits many p53-like properties: it can bind to p53 DNA target sites, transactivate p53-

responsive genes and induce cell cycle arrest or apoptosis. However the molecular mechanisms underlying p73 regulation remain unanswered. To address the lacunae in the understanding of p73 stability and function, we carried out a proteomics screen to identify p73 interacting proteins under normal and genotoxic stress conditions. We have now identified TRIM28 and MED15 as potential p73 interacting proteins. TRIM28 has been shown to exhibit E3 ubiquitin ligase activity. Our results indicate that coiled coil domain of TRIM28 binds to the N-terminal transactivation domain of p73. We also examined this interaction under genotoxic stress conditions. Our results show that TRIM28-p73 interaction is curtailed with increased duration of genotoxic stress and is completely abolished at late time points. Moreover, upon genotoxic stress p73 gets phosphorylated in c-Abl-dependent manner leading to abrogation of its interaction with TRIM28. MED15 is a component of the activator-recruited cofactor (ARC) complex or the Mediator complex. The Mediator complex is one such multiprotein complex that functions as a bridge between regulatory proteins and Pol II, thereby regulating Pol II-dependent transcription. Since MED15 serves as a key coactivator in various transcriptional complexes, we investigated whether MED15 can serve as a p73 coactivator. We found that the p73 transactivation function upon genotoxic stress was significantly increased in presence of Med15. Further, we determined the effect of MED15 on p73-mediated transactivation of its target genes. We found out that upon genotoxic stress the activation of p73 downstream targets is severely compromised in the absence of MED15. These results establish that MED15 is an indispensable coactivator of p73.

Publications

Original peer-reviewed articles

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2. Sen N, Kumari R, Singh MI, Das S* (2013) HDAC5, a key component in temporal regulation of p53-mediated transactivation in response to genotoxic stress . *Molecular Cell* **52**:406-420.

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#In press last year, since published.

Subeer S. Majumdar

Production of transgenic and other animal models for biomedical research

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Theme of the research is to generate transgenic animals for using them as a system for the study of functional genomics and mammalian development and other animal models for use in Biomedical research.

Objectives

1. To develop new easier techniques for making transgenic animals.
2. To develop transgenic animal models using genes relevant to human health and diseases as well as to use this technology for making large animals expressing therapeutic products in their milk for increasing affordability of such therapeutics.
3. The other objective is to extend collaborative help in providing specific animal models (transgenic or non transgenic) for Biomedical research.

Generation of various transgenic mice for other investigators

All collaborative work for making various transgenic animals for other investigators were undertaken as and when the constructs were given and fore founder animals were provided to Principal Investigator's for generating transgenic lines to address their respective scientific goals. Since a lot of them were just initiated last year, no major confirmatory results are generated yet. Work is being continued.

Attempts to generate transgenic buffalo expressing therapeutic protein in the milk

Isolation of buffalo β-casein promoter region and transcriptional regulatory element of this important udder gland specific milk protein gene of Indian river buffalo by us provided an elegant opportunity for guiding the expression of recombinant therapeutic proteins in milk. We have isolated the β-casein genomic region (BuCSN2) which contains promoter region, exon1, intron1 and exon2 (NCBI Accession No. KF612339) from the genome of Indian river buffalo (*Bubalus bubalis*). We had reported the generation of transgenic mice in which milk glands expressed EGFP when EGFP cloned under this promoter (BuCSN2-EGFP) was used to make the transgene.

There are difficulties and high cost of making transgenic large animals. In present day scenario,

transgenic animals may also be blamed to carry potential risk of germ line transmission of introduced transgene to normal traits if not kept isolated, making them as possible biohazard. Keeping all these in view, it is worth trying to transfect Mammary Luminal Epithelial Cells, the cells which express and secrete milk proteins in the time of lactation, *in-vivo* to have a possible bioreactor by somatic genomic modification. In spite of several attempts using various gene delivery methods people have failed to generate a efficient method for *in-vivo* gene delivery in mammary gland. It was shown before that virosome mediated targeted delivery of transgene in liver cells is possible in vivo. Taking clue from this, we have used reconstituted Sendai Viral Envelope to generate a gene delivery vehicle for easy, efficient and cost effective delivery of transgene in mammary gland *in-vivo*. Sendai virus is a negative stranded RNA virus of the family *Paramyxoviridae*. Sendai viral envelope devoid of its nucleic acid which is known to contain F and HN glycoproteins, was used to entrap gene of Enhanced Green Fluorescent Protein under control of Buffalo Beta-casein Promoter (BuCSN2) which was

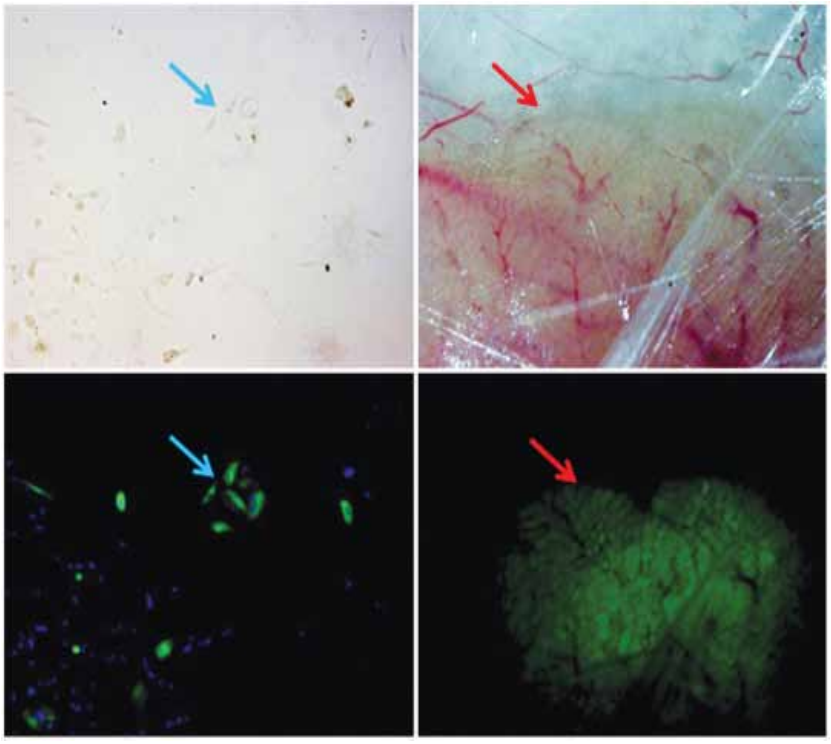

Figure1: BuCSN2-EGFP transfected buffalo mammary epithelial cells expressing EGFP (left panel). Breast gland of virosome entrapped BuCSN2-EGFP treated female mice during lactation (right panel). Note: Upper panels represent light microscopic images and lower panels represent same area under UV light.

isolated and functionally characterized by us, for generating Sendai Virosome (HNF-V) for transfection. DNA entrapped Sendai Virosome was prepared as per standard procedure. In brief, membrane of Sendai Virus is solubilized using detergent followed by removal of viral nucleic acid by centrifugation. Desired transgene constructs are mixed into supernatant which contain only viral lipid bilayer along with HN and F glycoprotein. Removal of applied detergent by SM20 Bio Beads start the reconstruction of the viral membrane making gene entrapped HFNV virosome. Dilutions of HNF-V was done in sterile PBS for mammary gland intra ductal delivery. Perfusion of such virosomes through the teat canal in various female mice at different time of mammary development helped us target mammary luminal epithelial cells preferentially. Lactating breast gland of such HNF-V treated female mice were analyzed for detection of EGFP expression. EGFP expression was discerned in the breast gland of some of the treated females but best results were obtained when HNF-V was delivered in mammary glands during advanced stage of pregnancy (Figure 1, right panel). This work can be extrapolated to buffalo or cow which can potentially serve as a rich source of therapeutic proteins when EGFP is replaced with other gene(s) coding therapeutic Proteins. This may avoid the risk of uncontrolled dissemination of gametes. *In-vitro,* BuCSN2-EGFP transfected buffalo mammary epithelial cells expressed EGFP (Figure 1, left panel).

Generation of transgenic rat through testicular route

Transgenic mice serve as surrogate models of animal and human diseases for evaluating occurrence of the disease and treatment modalities using sufficient number of individuals which may not necessarily be possible in case large/ farmed animals are to be considered or for that matter human beings, who cannot be experimented. Several disorders can be efficiently and meaningfully studied only in rat models which are 10-12 times bigger in size than mice. Mouse cannot be bled frequently for determining humoral changes and it cannot withstand major surgeries under anesthesia unlike, rat. Structure – function relationship of various organs for toxicological evaluation is vastly studied in rats and parameters to evaluate teratogenic effects are better established in rats, mainly due to rat's extensive use in drug testing in the past. It is important to note that developmental process of fetus is fast ,similar to mice, in rats and 10-12 offspring are born within 21 days of mating in rats making them suitable for teratological studies also. Since rat is considered as more closer to human than mice in terms of genetics, physiology and pathology, rat models bear more significance than mice.

Hence, establishing an easy method of rat transgenesis will open a great scope to study several disease conditions more easily addressing issues of animals and man both. The genetic manipulation in rat model remained limited due to lack of tools for the efficient transgenesis in this system mainly because of inefficient embryo manipulation unlike mice. Therefore, there is a persistent need to develop methods to produce transgenic rat which should be user friendly, less time consuming and relatively inexpensive. In recent past, we reported transgenesis in mice by exploiting the ability of undifferentiated spermatogonia to integrate foreign gene. We are in the process of establishing methodologies to generate the transgenic rat by exploiting the spermatogonial germ cell in the testis for electroporation with transgene. As a proof of

principle, we tried to generate α-thalassemia disease model by over expressing the beta globin chain in a physiologically superior transgenic rat system. We found a decrease in hemoglobin concentration, mean cell volume (MCV) and mean cell hemoglobin (MCH) in blood and also detected the HbH inclusion body by Briliant cresyl blue staining of blood of the alpha thalassemic rat model mimicking the silent carrier state of human α-thalassemia. Deformities of RBCs were also noted. Following this new procedure, we wish to interrupt expression of specific genes *in vivo* by shRNA and evaluate physiological effects of such RNA interference by knock-down in transgenic rats.

Generating a better model for post chemotherapeutic germ cell transplantation

A model for post chemotherapeutic infertility alleviation is made routinely by intraperitonial injection of busulfan. Busulfan doses, less than 40 mg/kg, given to adult mice do not result in prolonged depletion of endogenous spermatogenesis in most tubules; on the other hand, higher doses are often known to induce severe hematopoietic suppression requiring bone marrow transplantation or causing death, as shown before. Data on the effects of busulfan on spermatogonia in other species are limited and the doses used were close to lethal. To overcome such disadvantages of this model, we attempted to standardize busulfan injection directly to testis, locally, in order to deplete the germ cells without compromising animal's health or animal's life. F1Bl6SJL mice were used for standardization of busulfan treatment directly in the testis. For this purpose, various doses ranging from 25µg to 100 µg/testis was tested to determine the most effective dose for germ cell depletion of testis. The standardized dose resulted in reduction of the testis size and killing of most of the germ cells of testis within 12-15 days post injection. The animals were observed to be healthy with no signs of health deterioration even after several months. This procedure helped in preparing animal model with less pain and suffering to animal while objective of germ cell depletion was achieved. This also generated such germ cell depleted testis in shorter duration of time (12 days vs 70 days).

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Patent

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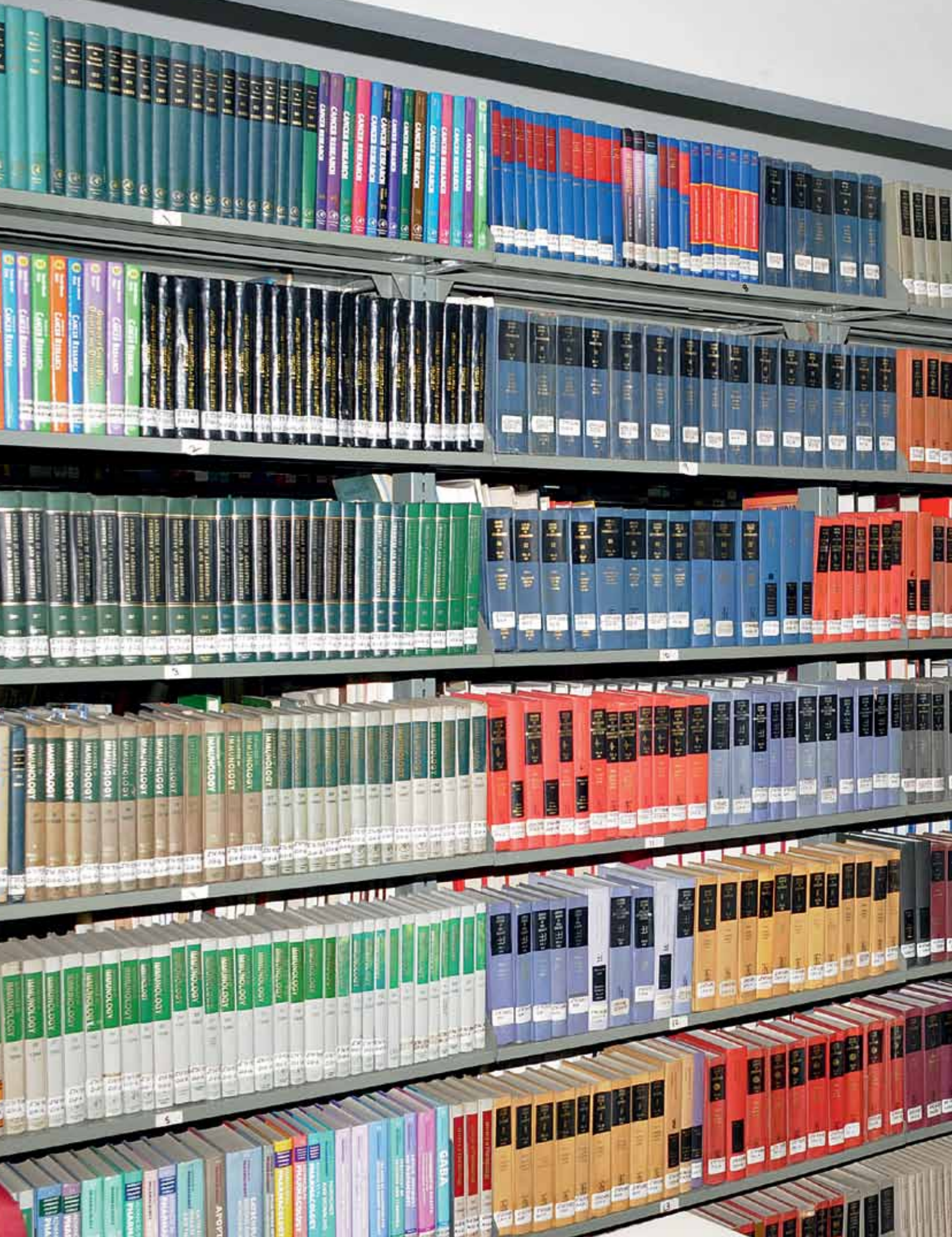
CANCER RESEARCH
IMMUNOLOGY

PUBLICATIONS, PATENTS AND TECHNOLOGY TRANSFER

A. ORIGINAL PEER-REVIEWED ARTICLES

#1. Agarwal S, Saini S, Parashar D, Verma A, Jagadish N, Batra A, Suri S, Bhatnagar A, Gupta A, Ansari AS, Lohiya NK, Suri A* (2013) Expression and humoral response of a-kinase anchor protein 4 in cervical cancer. *Int J Gynecol Can* **23**: 650-658.

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B. REVIEWS/ PROCEEDINGS

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C. PATENTS / TECHNOLOGY TRANSFER

1. Baliger P, Teckman J, Mukhopadhyay A (2013). Bone marrow-derived cells ameliorates the pathological consequences of the liver in case of alpha1-antitrypsin deficiency. *(Application No. US 14/109212; Filed on December 17, 2013).*

2. Garg LC, Bhatia B (2014). Recombinant subunit vaccine against beta toxin of *Clostridium perfringens*. *(Application No.58/ DEL/2014; Indian Patent Filed on January 08, 2014).*

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compositions and method of producing said vaccines. *(Application No. 1210/DEL/2013; Indian Patent Filed on April 25, 2013).*

4. Majumdar SS, Ganguli N, Usmani A (2013). Isolation, Cloning, Sequencing and Functional analysis of ß-casein promoter along with the regions of exon1, intron1 and exon2 using mammary gland derived cell line of Buffalo. (Bubulus bubalis) *(Application No. US 13/898137; Filed on May 20, 2013).*

5. Majumdar SS, Usmani A, Ganguli N (2013). A shortcut procedure of transgene integration by hypotonic shock into male germinal cells for gene expression and transgenesis. *(Application No. US 14/096,634; Filed on December 04, 2013).*

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7. Panda AK, Gopimohan R and Anish CK (2014). Biodegradable polymer scaffold & process for preparation thereof. *(Patent no. AU 2008315318; Granted on February 06, 2014).*

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11. Surolia A, Gupta S, Singh M, Chattopadhyay T (2013). Composition useful for treatment of diabetes & disorder. *(Patent No. US 8,426,362; Granted on April 23, 2013)*.

12. Surolia A, Gautam RK, Singh M, Chattopadhyay T (2013). Composition useful for treatment of diabetes and disorder. *(Patent No. EP2133091; Granted on April 19, 2013).*

13. Surolia A, Gautam RK, Diwedi VK, Gupta S (2013). Synthetic peptides and random copolymers for the treatment of autoimmune disorders. *(Application No. US 14/115,598; Filed on November 04, 2013)*.

14. Suri A (2013). siRNA useful in inhibiting cellular growth/proliferation of cancerous tissues. *(Patent No. 257434; Granted on October 1, 2013).*

TECHNOLOGY TRANSFERRED

The hybrid cell clone secreting monoclonal antibody having specificity for β-subunit of human chorionic gonadotropin (hCG) along with the technical knowhow for its maintenance and production of antibody has been transferred to Dhiti Life Sciences Private Limited, New Delhi. This will facilitate production of indigenous pregnancy detection kit more economically

Gulmohar in full bloom at the NII campus

AWARDS AND DISTINCTIONS

Dr. Chandrima Shaha was awarded Prof. Archana Sharma Memorial Award of the National Academy of Sciences and Chandrakala Hora Memorial Medal of the Indian National Science Academy of the year 2013.

Dr. Subeer S Majumdar was given the Dr. TC Anandkumar Gold medal award by the Indian Society for study of Reproduction and fertility in February 2014. He was elected a Fellow of Indian Academy of Science, India.

Dr. Ayub Qadri has been elected as a Fellow of the Indian National Science Academy from January, 2014.

Dr. Pushkar Sharma received Shanti Swarup Bhatnagar Prize for the year 2013 in Medical Sciences for his contributions towards the understanding of signaling pathways in malaria parasite.

Ph.D Degrees Awarded to NII Scholars

Twenty four scholars of the Institute were awarded the degree of Doctor of Philosophy by the Jawaharlal Nehru University on the completion of their work. The details are as follows:

Student's Name	***Topic of Research***	***Guide***
Ms. Ananya Sadhu	Analyzing the role of CIS-Regulatory elements in defining nuclear organization and gene regulation.	Dr. Madhulika Srivastava
Mr. Avinash A. Kumar	Understanding cellular and post-transcriptional regulation in vitiligo.	Dr. Rajni Rani
Mr. Dinesh Giri Goswami	Studies on adjuvant activities of polymeric particulate delivery systems.	Dr. Amulya K. Panda
Ms. Divya Bajaj	Caseinolytic(Clp) protease of *Mycobacterium tuberculosis*: Role in pathogenesis.	Dr. Janendra K. Batra
Ms. Garima Tiwari	Computational analysis of substrate specificity of peptide recognition modules.	Dr. Debasisa Mohanty
Ms. Manpreet Kaur	Study of proteins and their interactions that regulate DNA replication.	Dr. Sandeep Saxena
Mr.Rakshamani Tripathi	Apoptosis and autophagy: fine balance during response to DNA damage.	Dr. Chandrima Shaha
Ms. Shweta Chatrath	Functional characterization of PE_PGRS proteins of *Mycobacterium tuberculosis* H37RV.	Dr. Lalit C. Garg
Ms. Sonali Mitra	Role of Vps like protein in Endocytosis in *Leishmania donovani*.	Dr. Amitabha Mukhopadhyay
Ms.Tora Biswas	Studies on structural aspects of sortase catalysis.	Dr. Rajendra P. Roy
Mr. Amit Kumar	Study on therapeutic potential of Fetal Liver mesenchymal stem cells in Parkinson's mice model.	Dr. Asok Mukhopadhyay
Ms. Anju Kumari	Analysis of repeat tagged mRNA transcripts in patients with different Spermatogenic status.	Dr. Sher Ali

Student's Name	*Topic of Research*	*Guide*
Ms. Kavita Agarwal	Metabolic glycan engineering (MGE) as a tool for probing structure and functions of glycoconjugates.	Dr.S. Gopalan Sampathkumar
Ms. Lele Deepti Shripad	Investigating the role of carbohydrates on structure and function of antimicrobial glycopeptides.	Dr. Kanwaljeet Kaur
Mr.Damle Nikhil Prakash	Development of novel computational methods for analysis of protein phosphorylation networks by combination of structure based approach with evolutionary and context dependent information.	Dr. Debasisa Mohanty
Mr. Nirmalya Sen	Regulation of p53 mediated stress response.	Dr. Sanjeev Das
Mr. Sanket Singh Ponia	Modular organization of host-pathogen interaction in HIV-1 mediated RNAi perturbation.	Dr. Akhil C. Banerjea
Mr. Shembekar Nachiket Satish	Generation and characterization of antibodies against hemagglutinin protein of H1N1 influenza virus.	Dr. Satish Kumar Gupta
Ms. Shikha Saini	Studies of A-kinase anchoring protein 4 expression association with various histotypes of breast carcinoma.	Dr. Anil Suri
Ms. Suchismita Sahoo	Effect of human chorionic gonadotropin on tumorigenesis.	Dr.Rahul Pal
Mr. Bhola Shankar Pradhan	Functional Genomics of some developmentally regulated Sertoli Cell genes putatively relevant to germ cell division or differentiation.	Dr. Subeer S. Majumdar
Mr. Ritesh Kumar Tiwari	Influence of impaired vesicular transport on B cell responses.	Dr. Anna George
Ms. Rupali Gund	Quantitative modulation in T cell responses.	Dr. Satyajit Rath
Ms. Vidya Ranganathan	Study of heterogeneity in the functional commitment of naive $CD4^+$ T cells.	Dr.Vineeta Bal

LECTURES AND SEMINARS

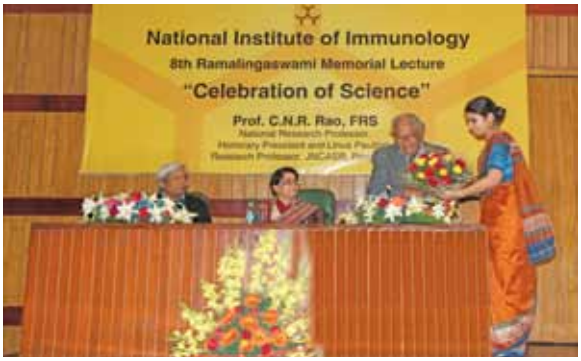
COLLOQUIUM

Prof. Ullas Karanth, Director for Science-Asia, Wildlife Conservation Society, New York delivered a Colloquium lecture on "**Conservation as a scientific enterprise- the tiger as a case study**" on 23rd May, 2013.

RAMALINGASWAMI MEMORIAL LECTURE

Prof. Ramalingaswami, a pioneer and visionary who laid the foundations of Biomedical Research in India, also was one of the founding members of NII. The RAMALINGASWAMI MEMORIAL LECTURE, named after him and awarded each year to a distinguished scientist, was organized on 17th January, 2014.

On this occasion, **Prof. C. N. R. Rao**, FRS., National Research Professor, Linus Pauling Research Professor & Honorary President, JNCASR, Bangalore was invited to deliver the lecture entitled "**Celebration of Science**".



Prof. C. N. R. Rao being felicitated during Ramalingaswami Memorial Lecture.

Prof. C. N. R. Rao interacting with students at NII Campus

FOUNDATION DAY LECTURE

On 6th October, 2013, the 27th Foundation Day of NII was celebrated. **Prof. K. N. Ganesh**, Director, IISER, Pune was invited as the Guest of Honour. He delivered a lecture on "**Peptide**" **Nucleic Acids (PNA) to "Pune" Nucleic Acids "PuNA**".

Prof. K.N. Ganesh at the Foundation Day Lecture

SCIENCE DAY LECTURE

National Science Day was celebrated on 28th February, 2014 at the Institute. **Prof. Deepak Pental,** Professor, University of Delhi, South Campus, New Delhi was invited to deliver a lecture on **"Transgenic Crops: Opportunities and fear"**.

Prof. Deepak Pental delivering the National Science Day Lecture.

PUBLIC LECTURE

Prof. Noyori Ryoji, Noble Laureate in Chemistry (2001), President Riken, Japan delivered a Public Lecture at the Institute on **"Science Shapes our Future"** on 14th September, 2013.

Prof. Noyori Ryoji delivering a Public Lecture at the NII auditorium.

Prof. Noyori Ryoji with Prof. K VijayRaghavan, Secretary, Department of Biotechnology, Govt. of India at the NII auditorium.

SEMINARS

Sl. No.	*Topic*	*Presented by*	*Date*
1.	Counting the dead worldwide	**Dr. Prabhat Jha,** University of Toronto Chair in Disease Control Director, Centre for Global Health Research, St. Michael's Hospital, Toronto	1st April 2013
2.	Molecular basis of hepatitis C virus induced insulin resistance and response to IFN-based therapy	**Dr. Gokul C. Das** Assistant Professor Baylor College of Medicine Houston, Texas, USA	3rd April, 2013
3.	Innate sensing, inflammasomes and NLRs: New insights revealed by imaging and systems approaches	**Dr. Naeha Subramanian** Laboratory of Systems Biology, Lymphocyte Biology Section, NIAID/ NIH, Bethesda, MD, USA	22nd April, 2013
4.	Glutathione and apoptosis-discovering a missing link	**Dr. Anand Bachawat** Dean, IISER, Mohali	29th April, 2013
5.	Applications on SPR	**Dr. Anette Persson,** Manager, Knowledge and Training, GE Healthcare Life Sciences, Sweden	21st May, 2013
6.	Early life determinants of chronic disease	**Prof. Nikhil Tandon,** Department of Endocrinology and Metabolism, AIIMS, New Delhi	12th June 2013
7.	Host defense pathways – TLRs, inflammation and inflammsome	**Dr. Chandrashekhar Pasare,** University of Texas-Southwestern Medical Center, Dallas, USA	3rd July 2013
8.	Membrane-induced folding of alpha-synuclein: A protein chameleon	**Dr. Samrat Mukhopadhyay,** Assistant Professor, Department of Biological Sciences & Department of Chemical Sciences, Indian Institute of Science Education and Research Mohali, Punjab	23rd July, 2013
9.	Antibody drug conjugates for cancer therapy: Past, present and future	**Dr. Jagath Reddy Junutula,** Senior Scientist Genentech Inc. IDNA Way, MS # 231B, South San Francisco, CA, USA	25th July, 2013
10.	Dalia or Dal Makhani-the competitive interplay between host and pathogen in TB infection	**Dr. Kanury Rao,** ICGEB, New Delhi	26th July, 2013
11.	Viewing the immune system through the lens of gene regulatory networks	**Prof. Harinder Singh,** Genentech, USA	7th August, 2013

Sl. No.	*Topic*	*Presented by*	*Date*
12.	Experimentation in Indian Music	**Ms. Shubha Mudgal** and **Dr. Aneesh Pradhan** Mentor, The True School Of Music, Mumbai	8th August 2013
13.	How is the MHC class II peptide repertoire selected by HLA-DM?	**Dr. Dhruv Sethi,** Dana-Farber Cancer Institute, USA	14th August, 2013
14.	ZKSCAN3 is a novel master transcription regulator of autophagy	**Dr. Santosh Chauhan,** Department of Microbiology and Microbial Genetics, University of New Mexico, USA	3rd September, 2013
15.	Human CD8 T cell responses to the yellow fever live virus vaccine	**Dr. Rama S. Akondy,** Emory University, Atlanta, USA	4th September, 2013
16.	Genomic packaging and epigenetic regulation of Hox Clusters	**Dr. Rakesh Mishra,** Center for Cellular and Molecular Biology, Hyderabad	6th September, 2013
17.	Influence of the protein environment on the mechanistic function of Cytochrome P450 (CYP) enzymes	**Dr. Dandamudi Usharani,** Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel	11th September, 2013
18.	Structural basis for conformational coupling across the plasma membrane in activation of EGFR	**Dr. Rahul Das,** HHMI, University of California, Berkeley, USA	18th September, 2013
19.	Master class on bio-entrepreneurship accelerating innovation to market place	**Dr. Krishna M. Ella,** Managing Director & CEO, Bharat Biotech Pvt Ltd. Hyderabad	20th September 2013
20.	Mucin homeostasis in health and respiratory diseases	**Dr. Vivek Malhotra,** CRG, Barcelona, Spain	26th September 2013
21.	Behind the scenes at Cell Press	**Dr.Sri Devi Narasimhan,** Scientific Editor, Cell, Cell Press, USA	21st October 2013,
22.	Interleukin-7 mediated NFAT activation is critical for early thymocyte development	**Dr. Amiya Patra,** University of Wurzburg, Germany	22nd October, 2013
23.	Proofreading during translation of the genetic code	**Dr. Rajan Sankarnarayanan,** CCMB, Hyderabad	6th November 2013
24.	Master coregulator in cancer and beyond	**Dr. Rakesh Kumar,** Dept of Biochemistry and Molecular Medicine, George Washington University, USA	15th November 2013

Sl. No.	*Topic*	*Presented by*	*Date*
25.	The human chorionic gonadotropin: One hormone, multiple pathologies; Studies in transgenic mice	**Dr. Susana Rulli,** Researcher, Instituto de Biologia y Medicina Experimental, Buenos Aires, Argentina	19th November 2013
26	Modulation of chromatin structure by chromatin remodeling complexes-mechanisms, consequences and implications	**Dr. Punit Prasad,** Department of Biosciences and Nutrition, NOVUM Karolinska Institutet, Stockholm, Sweden	25th November 2013
27.	Challenging the dogmas: In the search for a cure for Cancer	**Dr.Shiladitya Sengupta,** Harvard Medical School, Boston, USA	27th November 2013
28	Immunity and Immunopathology to acute viral infections	**Dr. Shalini Sharma,** St. Jude's Children Research Hospital, Memphis, USA	29th November 2013
29.	Living on the edge: scientific careers at the frontiers of research	**Dr. Carmen Gervais,** HFSP, Strasbourg, France	06th December 2013
30.	Architects of the flowering stem and flowers-insights from molecular genetics using model plant species	**Dr. Usha Vijayraghavan,** Indian Institute of Science, Bangalore	9th December 2013
31.	Coordinating genetics and epigenetics to regulate antigen receptor gene rearrangements	**Dr. Rajan Sen,** National Institute on Aging, NIH, USA	10th December 2013
32.	Role of long noncoding RNAs in cell cycle progression and cancer	**Dr. Kannanganattu V. Prasanth,** Associate Professor, Department of Cell and Developmental Biology, University of Illinois at Urbaba-Champaign, Urbana, USA	16th December 2013
33.	Biomimetic microenvironments for stem cell differentiation and tissue engineering	**Dr. Nathaniel S. Hwang,** Seoul National University, Seoul	16th December 2013
34.	Theragnostic significance of Osteopontin and associated genes in Cancer	**Dr. Gopal Kundu,** Scientist F, National Centre for Cell Science, Pune	3rd January, 2014
35.	Regulation of NFkB in inflammation and Cancer	**Prof. Sankar Ghosh,** Dept. of Microbiology and Immunology, Columbia University, NY, USA	16th January, 2014
36.	Inflammation, Insulin resistance and immunity are linked by lipid	**Prof. Samir Bhattacharya,** Emeritus Professor, NASI Senior Scientist, School of Life Science, Visva-Bharati, Santiniketan	5th February,2014

Sl. No.	*Topic*	*Presented by*	*Date*
37.	The translocation mechanism of the conserved viral DNA packaging motor and its regulation	**Dr. Aparna Dixit,** EGE Laboratory, NII, New Delhi	7th February, 2014
38.	cGMP signaling in plasmodium pre-erythrocytic stages	**Dr. Purnima Bhanot,** Associate Professor, Rutgers New Jersey Medical School, New Jersey, USA	18th February, 2014
39.	Role of cohesion in chromosome segregation and its implication in microbial pathogenesis	**Dr. Mohan Chandra Joshi,** Baylor College of Medicine, Houston, USA	21st February, 2014
40.	The molecular basis of regulated exocytosis	**Dr. Shailendra Rathore,** Deptt of Molecular, Cellular and Developmental Biology, University of Colorado, USA	26th February, 2014
41.	Non-coding and coding function of a single mRNA dictates skin homeostasis in wound healing and cancer	**Dr. Gopinath Meenakshisundaram,** IMB, Biomedical Science Institute, Singapore	27th February, 2014
42.	Linking sumoylation to chromatin structure and differentiation of skeletal muscle cells	**Dr. Reshma Taneja,** Associate Professor, Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore	12th March, 2014
43.	Emerging determinant role of airway epithelium in lung diseases	**Dr. U. Mabalirajan,** IGIB, New Delhi	14th March, 2014
44.	Design of artificial proteins to aid recognition of relationship between remote homologues	**Prof. N.Srinivasan,** MBU, Indian Institute of Science, Bangalore	21st March, 2014

CONFERENCE/SYMPOSIUM/ WORKSHOPS ORGANIZED AT NII

Ist BIO-Accelerator program focused on "Accelerating Innovation to Marketplace", Master Class on Bio-entrepreneurship with the theme "Accelerating Innovations to Marketplace" – A small step towards strengthening India's Bio-economy".

A joint initiative of the National Institute of Immunology [NII], Foundation of Innovation and Technology Transfer [FIIT] and BIORx Venture Advisors (BIORx), the program was devised for Ph.D. scholars, Post-doctoral scientists and working executives aspiring to accelerate the path of their discovery to the marketplace. The Master Class was held from 20th to 23rd September 2013 at the National Institute of Immunology.

Indo-German Workshop on "Chemical Biology of Infectious Diseases"

The National Institute of Immunology (NII) and the Max-Planck Institutes (MPI) jointly organized an Indo-German Workshop on Chemical Biology of Infectious Diseases on Jan 20 and 21, 2014 at NII, New Delhi. The goal of this workshop was to discuss respective research expertise of both sides and identify both complementary and synergistic areas in the frontiers of infectious disease research for collaboration between scientists from India and Germany. Scientists from multiple MPI as well as several Indian research institutes actively participated in this workshop. Dr. Chandrima Shaha, Director, NII highlighted the long-term strength of Indian research community in infectious diseases and urged the scientists to fully utilize the opportunity for fruitful collaborations.

Indo-German Workshop on Chemical Biology of Infectious Diseases.

Canada-India: Training the next generation of innovators and researchers.

Shastri Indo-Canadian Institute in collaboration with High Commission of Canada to India, MITACS and National Institute of Immunology organized a special Panel Discussion with His Excellency the Right Honourable David Johnston, Governor General of Canada on "Canada-India: Training the Next Generation of Innovators and Researchers" on February 25, 2014 at National Institute of Immunology. The discussion highlighted issues relating to education cooperation and mobility between Canada and India, and focused on

the important next step of training new researchers and scholars and the creation of new modes of collaboration in an increasingly inter-connected world.

Prof. Shanthi Johnson, President, Shastri Indo-Canadian Institute, while giving Vote of thanks highlighted the importance of the discussions and laid due focus on the 'way forward' in this direction.

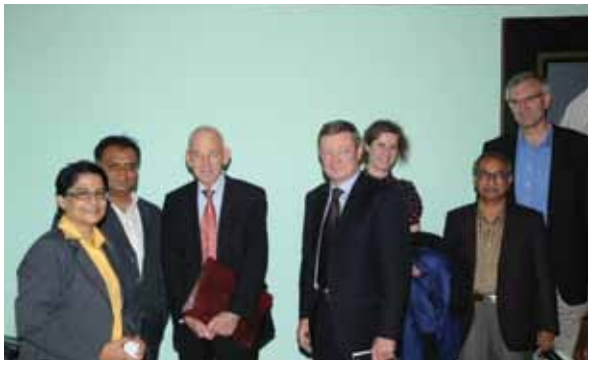
Governor General of Canada His Excellency the Right Honourable David Johnston with Dr. Chandrima Shaha, Director NII and other senior officials from Govt. of India.

Round table discussion with the High Commission of Canada, MITACS, the Shastri Indo-Canadian Institute, NII and DBT.

VISITORS TO THE INSTITUTE

1. French delegation headed by **Dr. Franc Pattus**, Delegue Scientifique, Institut des Sciences Biologique, CNRS visited the Institute on 25th October 2013.

2. Danish delegation headed by **Dr. Mogens Hoerder** visited the Institute on 26th November 2013.



Danish delegation with Dr. Chandrima Shaha.

3. Three members delegation from Vietnam headed by **Ms. Pham Thi Mai Huong**, General Editors of Science and Develop Newspaper (Vietnam's MoST) visited the Institute on 16th December 2013.

4. Delegation from Erasmus University Medical Centre, Netherlands headed by **Prof. Gabriel Paul Krestin**, President European Society of Radiology, Professor and Chairman, Radiology Dept. Erasmus Medical Centre, Rotterdam visited the Institute on 31st January 2014.

5. Governor General of Canada His Excellency the Right Honourable **David Johnston** visited the Institute on 25th February 2014.

Governor General of Canada His Excellency the Right Honourable David Johnston with Dr. Chandrima Shaha, Director, NII.

OTHER NOTABLE ACTIVITIES

ACADEMIC COURSES, TRAINING PROGRAMMES AND INTERACTION WITH OTHER ACADEMIC INSTITUTES

The Institute imparts long term residential training leading to Ph.D. degree of the Jawaharlal Nehru University, New Delhi. Every year 35-40 scholars are admitted to this programme on competitive basis after an examination and interviews amongst a large number of applicants from all over the country.

The Ph.D. programme of the Institute was launched in the academic year 1986-87. Since then, the Institute has admitted a total of 590 students in 28 batches. So far 269 students have been awarded the Ph.D degree including 24 that have obtained the degree in academic year 2013-14. Many others are at various stages of their research work for the degree.

In addition, the Institute accepts students from various Universities/Institutions as 'Summer Research Fellowship' awardees and provides them facilities and guidance. Besides, the Institute also accepts students for the project work during the last semester of their post graduation course.

IMPLEMENTATION OF OFFICIAL LANGUAGE POLICY

The Official Language policy of the Govt. of India is followed in the Institute in letter and spirit:

To promote Hindi as Official Language in official work, Hindi Pakhwara (Hindi Fortnight) was celebrated in the Institute with great zeal from 1st to 14th September 2013. During this period, various Hindi competitions such as Hindi Sulekh (Hindi Writing), Hindi Nibandh (Hindi Essay), Hindi Shrutlek (Hindi dictation), Hindi Vaad-vivad (Hindi Debate), Hindi Samanya Gyaan (General Knowledge Competition) and Hindi Kavita Pathan (Hindi Poetry recitation) were organized in the Institute, wherein a large numbers of faculty members, staff members and students participated. Hindi Diwas (Hindi Day) was celebrated on 13th September, 2013 on the culmination of Hindi Pakhwara.

Hindi Workshop on "How to work in Hindi on Technical and Scientific Subjects" has been conducted for technical and scientific staff to remove their hesitation of carrying out Official work in Hindi on Computers. As NII is a scientific

Dr. D.D. Ojha delivering a Lecture on "World of chemicals in our life".

NII staff participating in "World of chemicals in our life" lecture.

Institution, a lecture on "World of chemicals in our life" in Hindi was organized on 10th January 2014 in the Institute.

CULTURAL ACTIVITIES

During the cultural activities program, in-house competitions like Quiz, Antakshri, Rock show, Treasure hunt, Mixed bag, Rangoli, Marathon, Street play were organised for scholars and

Rangoli competition at NII.

Fancy Dress competition for Staff's Children at Cultural Night.

staff of the Institute whose participation was very enthusiastic. The special feature of the activities included the sports events and fancy dress competitions by the children of staff members.

INDEPENDENCE DAY CELEBRATION

Independence Day was celebrated in the Institute on 15th August 2013. The event was marked by Independence Day Message from the Director, followed by the National Anthem by the students and children of the staff of the Institute.

FAREWELL TO Ph.D. STUDENTS

Farewell function of 2008 batch of Ph.D. Students was held at the Institute.

Farewell function of 2008 batch of Ph.D students which was marked by planting of tree by the student at the Institute.

ANTI-TERRORISM DAY, SADHBHAVNA DIWAS AND COMMUNAL HARMONY WEEK

Anti-Terrorism Day was observed by all employees of the Institute on 21 May 2013 by taking anti-terrorism/violence pledge stating: 'We, the people of India, having abiding faith in our country's tradition of non-violence and tolerance, hereby solemnly affirm to oppose with our strength, all forms of terrorism and violence. We pledge to uphold and promote peace, social harmony and understanding among all fellow human beings and fight the forces of disruption threatening human lives and values'.

With the theme to promote national integration and communal harmony among people of all religions, languages and regions, 'Sadhbhavna Diwas' was observed in the Institute on the birth anniversary of late Shri Rajiv Gandhi on 20 Aug 2013 each staff of the institute took the pledge, 'I take this solemn pledge that I will work for the emotional oneness and harmony of all the people of India regardless of caste, region, religion or language. I further pledge that I shall resolve all differences among us through dialogue and constitutional means without resorting to violence'. To promote the idea further a fortnight from 20 Aug to 3 Sep 2013 was observed as Communal Harmony Week.

REPRESENTATIONS OF SCHEDULED CASTES, SCHEDULED TRIBES AND OTHER BACKWARD CLASSES

The Institute follows reservation orders as per directives of Government of India, while making appointments, to ensure representation of Scheduled Castes, Scheduled Tribes, Other Backward Classes and Physically Handicapped persons as per the prescribed percentage. Existing order in force and as amended from time to time by the Government of India, are compiled with.


NEC
NEC

Small Animal Facility Staff with Dr. P. Nagarajan

Primate Research Centre Staff with Dr. Subeer S. Majumdar

INFRASTRUCTURE

RESEARCH FACILITIES

EQUIPMENT

While most of the routine equipment is available in various laboratories of NII, some high-end instrumentation facilities are shared by various research groups and their collaborators. The equipment in these facilities includes Mass Spectrometers, NMR Spectrometers, Confocal Microscopes, Atomic Force Microscope, Scanning and Transmission Electron Microscopes, High Throughput DNA Sequencer, Flow Cytometers, Dual wavelength X-ray Generator and X-ray device for in-vivo imaging.

BSL-III FACILITY

There are three Biosafety Level III facilities at NII- one each for handling Mycobacterium tuberculosis, Streptococcus pneumonia and HIV.

SMALL ANIMAL FACILITY

The Small Animal Facility of the Institute is committed to ensure the humane care of animals used in approved research and cater defined strains of mice and rats to the scientific community of the institute. At present the facility holds 88 mouse strains, 6 rat strains and 1 stock of rabbit.

The propagation of all defined strains is done in a three- tier system i.e., the Foundation Stock (FS), Pedigreed Expansion Stock (PES) and Production Stock (PS).The selection of sibling pairs at these three levels is done in such a way that all the descendants of each strain can be traced to a common ancestral breeding pair within seven generations of inbreeding. In addition to the above, yet another kind of mice stock namely `Non-Pedigreed Identified Pairs' are also raised. These are brother x sister pairs which are bred without maintaining their pedigree records. The progeny thus produced from such mice is used only for the experimentation. Apart from this, Mutant strains of mice are bred either by 1.Homozygous mutant (-/-) x homozygous mutant (-/-) 2. Heterozygous mutant (-/+) x homozygous mutant (-/-) 3.Heterozygous mutant (-/+) x heterozygous mutant (-/+)

Defined breeding protocols and careful management and husbandry procedures are followed to ensure the purity of each strain of mice. To maximize genetic purity and uniformity of mice, inbred strains are propagated in such a manner that minimizes the genetic drift and the number of generations which separate breeding stock in production colonies from their ancestors in foundation colonies. A random sample of few retired breeders of Foundation, Expansion and Production stock are monitored with the help of genetic and biochemical markers to ensure their genetic purity. The facility also gets support from various principal investigators in the genotyping of transgenic and knockout mice strains to confirm the genetic purity based on presence or absence of the selected gene of interest.

Health monitoring procedures include microbiological examination of feed, water, bedding material, stool samples and animal organs from moribund animals. Health monitoring program

includes screening of Mouse Hepatitis virus, Mouse Parvovirus, Mouse Norovirus Pnemonia virus of Mice, Mycoplasma and Sendai virus using Elisa and PCR. Bacterial pathogens such as *Pseudomonas aeroginosa, Streptobacillus moniliformis, Bordetella, Bronchiseptica, Citrobacter rodentitium, Pasteurella pneumotropica, Staphylococci* and *E.Coli* are screened using culture, biochemical and PCR methods. Fecal samples are randomly selected for the presence of endoparasites by sedimentation method for the presence of syphacia and aspicularis species. Also periodic FACS analyses are also done on immunodefficient mice for their leakiness.

The health quality procedures are implemented to prevent the transmission of infection between cages, these procedures include careful handling of animals, washing using automated cage and bottle washer, use of sterilized corn cob bedding, autoclaved cages, and acidified autoclaved drinking water. The breeding colonies are maintained in IVC systems of international standards. Necessary action based on clinical signs is taken by the veterinarian concerning the necropsy/ autopsy of the infected animals. Preventive and recommended schedule of medication is strictly followed to prevent the infection/s.

PRIMATE RESEARCH CENTRE

The National Institute of Immunology has a Separate facility of Primate Research Centre. Macaques are bred and maintained in the Primate Research Centre for generation of in house animals of known ages for approved basic, pre-clinical and toxicological research using sub-human primates.

Under the breeding program, group mating is done, for the production of healthy animals. This helps in providing animals of known age and parentage. We have large open pens which are used for group mating under semi-natural conditions where food and water is provided ad libitum. Infants are weaned at the age of six months after which they are transferred to open semi-natural housing for over-all growth and better development of bones, muscles and coordination. Monkeys are housed in independent cages at around pubertal age. To prevent cross-cage contamination strict procedures are followed. All cages are washed routinely by scrubbing with soap and are painted once a year. Deworming of the colony is done at least once a year. To check outbreak, the routine TB tests are performed because non-human primates are susceptible to this infection. The chest x-ray of animals, with suspected infection, is performed using x-ray machine in dark room of the Centre. The sick animals are isolated and treated properly after pathological investigations and veterinary consultation according to international norms. To treat minor injuries, gastrointestinal disorders and to revive animals during acute cardio-pulmonary crisis, a stock of medicine is maintained at the Centre.

Protein rich pellets containing appropriate content of fat, carbohydrate and vitamin are provided to monkeys ad libitum. In addition to this, bread, germinated gram, vegetables and/ or fruits are also given daily. For change of taste, occasional feast like bread with sauce or jaggary coated groundnuts are given. Breast feeding mothers and pregnant females are given calcium and vitamin supplements on bread. Care is taken to provide excess feed to such females. Drinking water is provided to the animals by pipelines behind monkey cages, which are connected to flexible protective hose-pipe at the top of each cage. Steel nozzles with Teflon interior are fitted at the tip of these hose pipes for the continuous access to drinking water. To make the staff aware of or to remind preventive measures for health safety, occasional meetings are held with the staff and they are mentored very often. The

attendants are provided with overall, jacket, pajama and foot wear for use during animal handling and cleaning. Use of gloves and mask is mandatory during work. Booster of TT is given once every year. The staff also receives boosters of anti rabies vaccine when required. TB test and chest x-ray of staff and the security personnel are performed occasionally. As a preventive measure, persons having injury are given non-animal work. Every precaution is taken to prevent cross species infection; monkey to human and vice versa. High-grade sanitary norms are followed for cleaning in the monkey rooms and area surrounding the building by using disinfectants and insecticides. To prevent colonization of microbes the sewer channels and tiles of room are routinely cleaned.

Major surgeries are performed in the well-equipped operation theatre whereas minor surgeries involving cuts and wounds are performed in the animal prep room adjacent to it. Technical expertise for surgery, immunization, bleeding, biopsy, electro ejaculation and fertility studies is extended in addition to maintaining and providing primates free of microbial pathogens. Surgical linen is washed using a washing machine. Autoclaving facility for surgical equipments and accessories is provided within the building. A research laboratory is situated in the centre for the research related to primates and the samples obtained from them. This provides basic services to various investigators involving primary processing of biological samples in the Centre. Remote blood sampling and infusion unit is successfully working at the centre. These catheters are used for continuous or pulsatile administration of hormones and drugs using a set of pump and chrontrol (a time setting device) to the ambulatory animals. This is a great asset for physiological mimicry and pharmaco-kinetic studies where experiments can be performed without causing any stress to the animals.

At this center, clearance of the research proposals by CPCSEA after primary clearance from the Institutional Animal Ethics Committee, (comprising of scientists from various fields of expertise and member of CPCSEA) is a necessary requirement for conducting research on primates. The macaques at this Centre served research related to infectious diseases, reproduction, endocrinology, immunology and contraception. The staff makes sure that all the procedures involved in animal handling are pain-free and involve minimum stress to the animal. Where ever unavoidable, proper medication is given to reduce the pain. Experimental animals are provided with special feed, whenever needed. A constant effort is made to keep the animals in a comfortable and stress free environment as per the available guidelines. There are seventeen open enclosures with swings and shelters, some of these are used for rotation of monkeys and some for rehabilitation and or socializing. Attempts are made to keep monkeys in groups in the open enclosures.

Administrative Staff with Senior Manager Mr. N.S. Padamanabhan

SUPPORTING UNITS

Establishment, Personnel and General Administration Services

The Division has been providing effective administrative support in terms of manpower employment, liaison, secretarial assistance and infrastructure to meet its goal of coordination of human and material resources. The activities include service matters, policy implementation, preparation and submission of periodic reports to nodal Ministry, postal-mail, foreign visits of scientists for training, conferences, exchange visits etc. The Institute also conducts periodical trainings for its Administrative & Technical Cadre on subjects of relevant to their roles. The activities are performed through the use of modern gadgets and tools such as fax machines, computers, and e-mail. In addition to the routine jobs, Administration was also been involved in other activities such as maintenance of building, residential complex, swimming pool, guest house, organization of various seminars, workshops, training courses, and lectures.

Financial and Accounting Services

The Division has been responsible for preparation of annual budget, management of funds utilization, receipt and disbursement of all payments, internal auditing, getting accounts audited by statutory and CAG auditors, sending reports to funding agencies `recovery and remittance of TDS from salary and contractors, filling institutional income tax return, obtaining required exemptions of the Income Tax department, maintaining bank accounts, management of trust for CPF, Gratuity Fund, and recovery and remittance of subscriptions of NPS.

Stores and Purchase Department

The Stores and Purchase Department of the Institute is responsible for purchases of chemicals, consumables, research equipment and instruments, glassware and other related items. It acts as a lifeline for research activities. Special emphasis is laid on economical and timely procurement of stores and supplies from local as well as international sources. The important function of purchase is overseen by various purchase committees comprising of three or more scientists, the Finance & Accounts Officer and the Stores & Purchase Officer. The officials of the Stores Department carry out the processing of orders and procurement of materials of different types for the Institute and distribute them to the concerned labs on receipt.

Engineering, Maintenance and Instrumentation Services

The Engineering department of the Institute has been entrusted with all the engineering activities involving maintenance, services and capital works. It has always been the endeavour of the department to provide the best of services with use of the latest/modern technology; as a result, systems are being continuously modernized. Major activities under taken during the reporting year are as follows:

i) Installation of sewage treatment plant. ii) Upgradation of cold rooms. iii) Creation of TEM facility "Electromechanical works". iv) Creation of storage space in existing Incinerator Room v) Setting up of new laboratories & offices. vii) Servicing & repairing of various DG Sets to maintain back up supply. viii) Reactivation of water treatment system. ix) Maintenance & refilling of fire extinguishers. x) Creation of TEM Facility "Civil work". xi) Fabrication of laboratory tables for various labs.

The department is currently working on the following projects:

i) SITC of 1250 KVA, 4 stroke D.G. Set. ii) Construction of boundary wall for 2Acres NII land at Sector- 5, Dwarka. iii) Installation of solar system for sharing regular electrical load. iv) Setting up of new laboratories & offices. v) Development/ landscape of grounds opposite to PRC & near STP Plant. vi) Health check up & repairing work for animal house chiller system. vii) Repairing/ Maintenance/testing of HT/LT Electrical system. viii) Repairing cooling towers of Animal House & D.G. Sets. ix) Installation of rain harvesting system. x) Segregation of power supply system i.e. HT Metering system for residence.

Library and Documentation Services

Library And Documentation Department is a service oriented supportive unit known as Information Management Centre. It provides information support to the scientific staff of the Institute using both archival and contemporary digital resources.

NII Library has computerized all its housekeeping activities and these are being maintained and updated regularly. Web-Online Public Access Catalogue (Web-OPAC) is available for searching database.

The library has a rich collection of books and journals. It has made electronic resources available on the desktops of scientists. The E-journals are available under NII subscriptions and DeLCON consortium project to NII members on intranet/LAN. The library has provided number of value added services to its patron. The library is involved in the process of compilation of Annual Report of the Institute and subscription process of print and online journals, books and publications.

The library takes care of all binding and photocopying work of the Institute. A Hindi Library with a good collection of administrative hindi books and magazines has been set up for popularizing the official language amongst the staff of the Institute. The Department is currently working on an institutional repository as well as comprehensive web pages about the library.

Academic and Training Services

The activities of the Academic & Training Department can be grouped under three major groups. Students Affairs, Outside Training, and In-House training. The unit has been involved in the process of Ph.D. admissions and pre-Ph.D. registration courses as well as arraigning academic and doctoral committee meetings. The unit handles and maintains the records of pre and post doctoral fellowships provided by different funding agencies such as Department of Biotechnology, Council of Medical Research, Department of Science and Technology and Wellcome Trust. The unit has been involved in arranging the participation of scientific, technical and administrative officials of the Institute in training courses, workshops and seminars organized by various organizations from different parts of the country.

Computer Centre

The Computer Centre is responsible for providing all Information Technology related support to NII, which involves managing switches and Wi-Fi controllers in a 600 node LAN, system administration of multiple LINUX based E-mail and Web servers, backup services for mail/web servers, managing UTM devices for network security and integrating internet bandwidth from multiple ISPs. The computer centre staff facilitates day to day trouble shooting, and maintenance and anti-virus support of about 700 PCs and other peripheral devices. In addition, the computer centre also provides specialized services such as management of HPC clusters, managing floating licenses for access to Bioinformatics software over LAN, and IT support for developing in house software for Pay Roll and maintenance of the employee database

Vigilance Cell

The Institute has a Vigilance Cell headed by a Scientist nominated as part-time Chief Vigilance Officer (CVO) by the Chief Vigilance Commissioner (CVC). The CVO and the support staff perform vigilance functions as adjunct duties in addition to their primary responsibilities. The Cell follows various instructions issued by the CVC to ensure effective implementation of the measures outlined so as to strengthen vigilance and anti-corruption efforts. Emphasis has been laid primarily on preventive vigilance as it aids in plugging weak and vulnerable areas. The Institute has been constantly reviewing procedures to identify corruption prone areas, making policies more transparent to avoid ambiguity and streamlining procedures to achieve a corruption free environment. Plans for rotation of staff employed in sensitive areas prone to corruption have been implemented. The property returns of all the staff members for the year ending 2013 have been called for and the same are being scrutinized by the CVO. Sizeable purchases of chemicals, consumables and instruments are handled by various purchase committees of the Institute, thus eliminating the possibility of collusion detrimental to quality and price of purchases. The composition of the purchase committees are reviewed and altered every two years. The Institute has thus far been able to maintain a clean slate in corruption matters. 'Vigilance Awareness Week' was observed in the Institute from 28th October to 2nd November 2013 during which a pledge to fight corruption was taken (on 28th October 2013). The cell has been rendering the periodical reports and returns on vigilance activities to the administrative machinery and the CVC.

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Director, MRC Cancer Cell Unit
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(Special Invitee)
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Mr. Roshan Lal
Mr. Sunder Singh Bisht

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Mr. T. Khaling

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Mr. Babu Lal Meena
Mr. Birender Roy
Mr. Kiran Pal
Mr. Nand Lal Arya
Mr. Pradeep Kumar Tiwari
Mr. Rakesh Kumar
Mr. Raj Kumar Peddipaga
Mr. Rajesh Meena
Mr. Ram Prakash Singh
Mr. Shahnawaz Haider
Mr. Vijendra Kumar

Skilled Work Assistants
Mr. Amar Nath Prasad
Mr. Arun Lal
Mr. Bhan Singh
Mr. Chatter Singh
Mr. Krishan
Mr. Jawahar Singh
Mr. Rakesh Kumar II
Mr. Raj Kumar
Mr. Ram C. Singh Rawat
Mr. Sonu Gupta
Mr. Surinder Singh Rawat
Mr. Vijay Pal

SUPPORTING STAFF

ACADEMIC CELL

Administrative Officer
Mr. Madan Mohan

Section Officer
Mr. Rana Chaudhary

Management Assistant
Ms. Daisy Sapra

Skilled Work Assistant
Mr. Suresh Kumar

COMPUTER & BIOSTATISTICS

Technical Officers II
Mr. M. S. V. V .S. Rao
Ms. Sunita Sachdev

Technical Assistant
Mr. Naveen Chander

ENGINEERING AND MAINTENANCE/ INSTRUMENTATION

Executive Engineers
Mr. Harendra Singh
Mr. Raj Kamal Singh

Assistant Engineer (Civil)
Mr. Mukesh Chander

Technical Officers I
Mr. Amar Nath Sah
Mr. Tarsem Singh
Mr. Yogesh Kumar Tripathi

Junior Engineers
Mr. Asok Kumar Basu
Mr. A .K. Thakur
Mr. Iswari Prasad Sharma
Mr. Jose Kunnapally
Mr. Mahabeer S. Panwar
Mr. Meghraj Kandle
Mr. Netra Pal Singh
Mr. Puran Singh Bangari
Mr. Ranbir Singh
Mr. R. K. Bhardwaj
Mr. R. K. Saini
Mr R. K. Sharma
Mr. Sooraj Prakash
Mr .Vinod K. Panchal

Junior Assistants I
Mr. Darban Singh Rawat
Mr. Mohan S. Negi

Technician I
Mr. Awadhesh Mahto

Technicians II
Mr. Akshya Kumar Behera
Mr. Deen Mohd
Mr Sardar Singh
Mr. Sharwan Kumar

Tradesmen
Mr. Rajiv Kumar

Skilled Work Assistants
Mr. Hukum Singh
Mr. Krishna P. Gaudel
Mr. Surender Kumar Kalra

LIBRARY & DOCUMENTATION SERVICES

Documentation Officer
Ms. Prachi S. Deshpande

Technical Officers II
Ms. Meenakshi
Mr. Ranjiv Mahajan
Ms. Vinod Kumar

Technical Officer I (Documetation)
Mr. Phunglianpau

Technician I
Mr. Satish K. Sharma

Junior Assistant II
Mr. Mohan Lal

Technician II
Mr. Babu Lal

PRIMATE RESEARCH CENTER

Technical Officers II
Mr. Kani Ram

Technical Officers I
Mr. J. P. Bhardwaj
Mr. Rajinder K. Thapa

Technical Assistant
Mr. Rajesh Kumar

Skilled Work Assistants
Mr. Balraj
Mr. Charan Singh
Mr. Ram Kumar
Mr. Shambhu Kumar Bhagat
Mr. Subhash Chand I
Mr. Veer Bhan

SMALL ANIMAL FACILITY

Technical Officer II
Mr. Gopal Krishan

Technical Officer I
Mr. Jarnail Singh

Technical Assistants
Mr. Dinesg C.P.S. Negi
Mr. Mohan K. Mandal
Mr. Sadhu Ram
Mr. Shailendra K. Arindkar
Mr. Surender Singh

Technicians II
Mr. Hira Singh
Mr. Jaglal Thakur
Mr. Mukesh Kumar
Mr. Subhash Chand Dogra
Mr. Yash Pal Singh

Skilled Work Assistants
Mr. Kuldeep Kumar
Mr. Mohar Singh
Mr. Nand Kishore
Mr. Prem Chand
Mr. Ram Bhool
Mr. Ram Dev Yadav
Mr. Ram Surat
Mr. Subhash Chand III

ADMINISTRATIVE STAFF
GENERAL ADMINISTRATION
Senior Manager
Mr. N. S. Padmanabhan

Manager (A & E)
Mr. Girish Bharihoke

Administrative Officers
Ms. Anju Sarkar
Ms. Chandresh Bhagtani
Ms. Lalitha Nair

Assistant Director (Official Language)
Mr. Ranbir Singh

Section Officers
Mr. Dev Dutt
Mr. Rajinder Kumar
Ms. Sanju Bisht
Ms. Sheela Satija

Junior Hindi Translator
Ms. Smita Shukla

Management Assistants
Mr. A.K. Dey
Mr. Dev Datt Sharma
Mr. Jagdish Mogha
Mr. Om Prakash
Mr. Sant Lal

Drivers
Mr. Balam S. Rawat

Mr. Budh Ram
Mr. Madan Lal
Mr. Mahender Singh
Mr. Satyabir Singh
Mr Suti Prakash

Technician II
Mr. Puran Singh

Skilled Work Assistants
Mr. Dinesh Singh
Mr. Nand Lal Malakar

FINANCE AND ACCOUNTS

Finance & Account Officer
Mr. Pradeep Chawla

Section Officer
Mr. Rakesh Satija

Management Assistants
Mr. Suresh Chander Chandel
Mr. Aslam Ali
Mr. Pradip K. Sarkar

Technician II
Mr. Brahm Dev

STORES AND PURCHASE

Store and Purchase Officer
Mr. Padam Singh Rawat

Section Officer
Mr. Mahender Pal Singh

Management Assistants
Mr. Dharambir
Mr. Than Singh

Junior Assistant I
Mr. Alam Singh

Junior Assistant II
Mr. Daya Chand

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Professor M.G.K. Menon unveiling the foundation stone of the first building of NII while Professor S. Vardarajan and Professor G.P. Talwar, Founder Director looks on

NII Collaborations

1	Aligarh	JN Medical College, AMU
2	Allahabad	University of Allahabad
3	Bangalore	IISc, NCBS, St. John's Medical College Hospital, IOB IBAB, Christian Blind Mission
4	Bhubaneswar	ILS, Imgenix India Pvt. Limited, CIFA, RMRC, KIIT
5	Hyderabad	CCMB, Indian Immunologicals Limited, BITS, CDFD
6	Kanpur	IIT
7	Kharagpur	IIT-KGP
8	Kolkata	Phyto Biotech Pvt. Limited, IICB
9	Lucknow	NBRI, SGPGI, IITR
10	Jaipur	CAS - University of Rajasthan, MGMCH
11	Mumbai	NIRRH, Mumbai University
12	Delhi NCR	JNU, RMLH, ICGEB, AIIMS, IIT, DU, IOP, VMMC
		Safdarjung Hospital, VIMHANS Hospital, DIPSAR
		Jamia Hamdard,ILBS, TRF, IGIB, Amity University,
		UCMS/GTB Hospital, ACBR, PBC - THSTI, I Care Hospital, RCB, St. Stephen's Hospital, INMAS
13	Pune	NCCS, Serum Institute, CSIR-NCL, BAIF
14	Sagar	DHG Vishwavidyalaya
15	Shantiniketan	Vishva-Bharati
16	Srinagar	SKUAST-K
17	Varanasi	BHU Medical College
18	Vadodara	MS University of Baroda
19	Chennai	Cancer Institute Adiyar (WIA)

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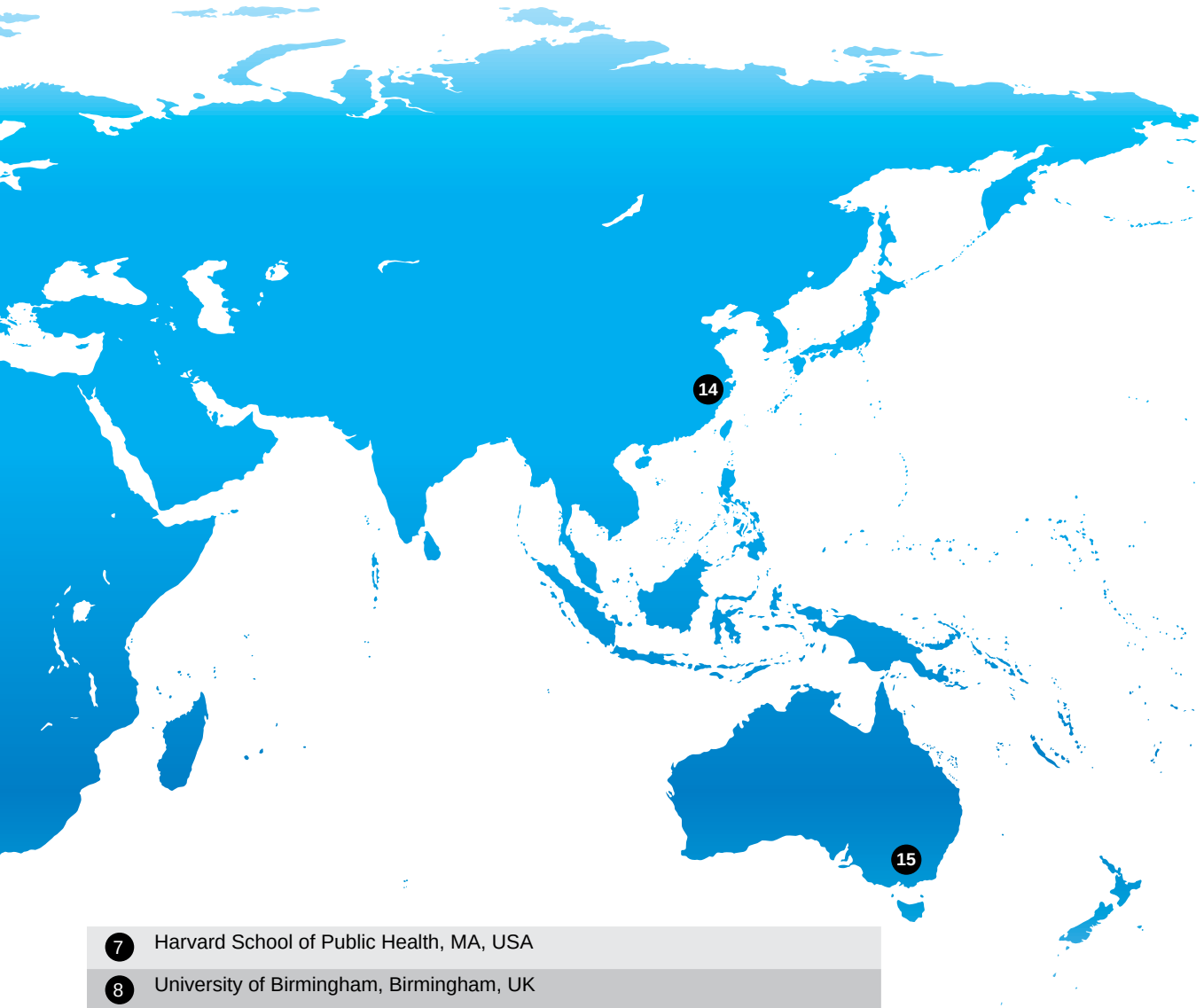
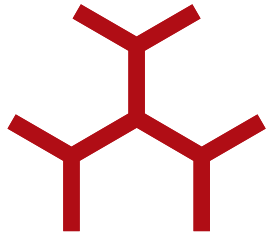
2	University of Arkanasas, Fayetteville, AR, USA
3	St. Louis University, MO, USA
4	Ohio State University, Ohio, USA
5	National Institute of Health, Bethesda, MD, USA
6	University of Delaware, Newark, DE, USA



7	Harvard School of Public Health, MA, USA
8	University of Birmingham, Birmingham, UK
9	Imperial College, London, UK
10	IDIBELL - Bellvitge Biomedical Research Institute, Barcelona, Spain
11	LBCMCP - CNR, Toulouse, France
12	University of Geneva, Switzerland
13	Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
14	Shanghai Institute of Planned Parenthod Research, Shanghai, P. R. China
15	Monash University, Melbourne, Australia



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