ANNUAL REPORT

2012-2013

NATIONAL INSTITUTE OF PATHOLOGY (ICMR)
PO Box 4909, Safdarjang Hospital Campus, New Delhi 110 029
Email : info@instpath.gov.in • Website : www.instpath.gov.in

annual report

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CONTENTS

NATIONAL INSTITUTE OF PATHOLOGY

Executive Summary

National Institute of Pathology has continued its stride for making significant scientific contributions and achievements during the year 2012-13 also. The scientists at institute worked as a cohesive team for development of new diagnostics, vaccines and therapeutic targets for improvement of health care. The thrust areas of research are tumor biology, infectious diseases including leishmaniasis, tuberculosis, leprosy and chlamydiasis, stem cell biology and environmental toxicology with target of translating research from lab to the bed. Beside human resource development in terms of training PhD and DNB (pathology) students remains the major activity at institute.

In Tumor Biology division, scientists are working mainly on breast, prostate, urinary bladder and brain cancers, lymphomas and leukemias and cancers in northeast region of India focusing mainly on identification of diagnostic, predictive and prognostic biomarkers, new drug targets and gene-environmental links. Study on molecular signature associated with breast carcinogenesis in early breast cancer patients identified differential expression of 578 genes specific and differential methylation of 977 genes in early onset cases. Validation of expression of several differentially expressed genes such as ALDH1B1, ALDH2, CBX2, CCNJ, FOX (3 genes), JMJD2A, MATN, METTL11A, PRMT, SOX genes (3), TMEM41A, WNT5A, WNT3, , HMGN1, JMJD4, MCMs (2), SMARCAs (2 genes) is being done by real time PCR in both early and late onset groups of breast cancer. ALDH-positive breast cancer stem cells (BCSC) sorted from MCF7, SUM159 and HBL100 cell lines showed self-renewal potential by showing mammosphere formation on repeated passages. Treatment of ALDH-positive mammosphere(BCSC) with plant alkaloid elipticine showed significant reduction in the formation of mammospheres while treatment with cytotoxic drug paclitaxel enhanced mammospheres formation. Interestingly treatment of mammospheres(BCSC) with combination of elipticine and paclitaxel also showed significant reduction in mammospheres formation.

In studies on bladder cancer non-invasive urothelial bladder cancer was found associated with MYD88-dependent activation of NFκB pathway and a Th-2 dominant status

while Th-1 polarization was observed in muscle-invasive urinary bladder carcinomas along with upregulation of TLR7/8 and NFκB activation. Immunohistochemistry was performed on Tissue microarray for TBX21, GATA3, PRKCE and SRC. The expression of TBX21 was significantly high in invasive bladder cancers (p value < 0.001) and PRKCE expression was significantly high in non-invasive cancers (p = 0.044).

Study of 33 microsatellite markers from the HLA region by fragment length analysis in Nasopharyngeal carcinoma cases from North east region of India showed significant association (p≤0.05)of two microsatellite markers, HL003 and D6S2704 with the risk. Detection of EBV in tissue/ blood samples by PCR and in situ hybridization showed presence of EBV sequences in more than 90 percent cases of NPC. SNP analysis in esophageal cancer in NE region showed genes in amplified regions (NTRK2, TPO, PLA2G5, PAK1, MAPK10, FGF12 and FGF4) were found to be significantly associated with MAPK signaling pathway. Genes found in deleted regions, several genes were found to be most significantly involved in MAPK signalling pathway (MAPT, CACNA1D, TGFBR2, PPP3CC, CACNB2 and FGF14), cytokine-cytokine receptor interaction (IL22, IL26, CACNA1D, PPP3CC and FLT1) and Wnt signalling pathway (PPP2CB, WNT7A, PPP3CC and NFATC1). The other predominant pathways found were focal adhesion, ECM-receptor interaction, Wnt signalling pathway, ErbB signalling pathway, cytokine-cytokine receptor interaction, Jak-STAT signalling pathway, VEGF signalling pathway and mTOR signalling pathway. FGF12 and PAK1 genes have been selected for further functional genomics studies.

In patients with chronic lymphocytic leukaemia, Reactive Oxygen Species (ROS) modulates regulation of B-cell activation, proliferation and survival of the cells for prolonged period without apoptosis and the cross-talk between B- and T-cells that may lead to clonal proliferation of B-cells and dysfunctional T-cells. Cellular ROS level was analyzed in T- cells, B-cells and NK cells and it was found that these cells have dynamically changing ROS levels. The dynamic behavior of cells due to intracellular redox environment was analyzed and significant changes in cell proliferation and regulation of apoptosis were observed.

In studies on Leishmaniasis first comprehensive insight into the underlying mechanism of miltefosine resistance in L. donovani showed an upregulated DNA synthesis,

transporter activities and downregulated protein metabolic process as determined by BLAST2GO, AmiGO and KEGG pathway analysis in miltefosine resistant parasite compared to wild type parasite. Current results suggest several probable mechanisms by which the parasite sustains miltefosine pressure including (i) increased ABC 1 mediated drug efflux (ii) reduced protein synthesis and degradation (iii) altered energy utilization via increased lipid degradation and (iv) increased antioxidant defence mechanism via elevated trypanothione metabolism and reduced oxidative phosphorylation. Evaluation of paromomycin(PMM) susceptibility in VL and PKDL clinical isolates, including miltefosine pre-treatment isolates and MIL post-treatment isolates including relapses showed all VL/ PKDL field isolates susceptible to PMM suggesting its potential efficacy in VL and PKDL therapy.

Studies on immunological responses to recombinant PSA (Parasite Surface Antigen) revealed that PSA failed to generate significant cellular and humoral responses in healed VL and PKDL. PSA induced poor lymphoproliferation in VL and PKDL and failed to stimulate IFN-γ, TNF-α or IL-10 in any of the study groups. Analysis of Leishmania-specific cell mediated cytotoxicity based on the measure of granzyme B, upon stimulation of PBMCs with TSLA and PSA in vitro revealed significantly high granzyme B level in response to TSLA compared to PSA among the different study, indicating that PSA failed to show any significant cell mediated cytotoxicity even in Leishmania exposed individuals.

Centrin knock-out live attenuated Leishmania parasite in human cells suggested protective immunogenicity. Evaluation of immune responses generated by LdCen1$^{-/-}$ Leishmania parasite e in human PBMCs cells showed predominant Th-1 response with stimulation of IL-2, TNF-α and IFN-γ after infection with wild type as well as with both knock-out parasites in comparison to control uninfected cells. Cytokines that play important role in Th-2 response (IL-4, IL-10) did not show any significant stimulation except IL-6, which showed significant stimulation after infection. Immune response induced by live attenuated parasites LdCen1$^{-/-}$ was similar to that of wild type. Th-1 and Th-17 responses stimulated by LdCen1$^{-/-}$ could provide protective response leading to host resistance to infection. Development of Loop-mediated Isothermal Amplification (LAMP) assay based on kDNA sequence of Leishmania donovani for diagnosis of VL

and PKDL.by naked eye detection. The assay has shown sensitivity of 96.4% in VL, 100% in BMA and 96.48% in PKDL samples. The assay was L. donovani species-specific (responsible for VL in Indian sub-continent) and negative for L. infantum, L. tropica and L. major.

In Chlamydiasis studies have been undertaken to find the prevalence of Chlamydia trachomatis infection in the synovial fluid of patients with reactive arthritis (ReA)/ undifferentiated spondyloarthropathy (uSpA) and to understand the cytokine pattern in the joints of infected patients. 14% ReA/ uSpA patients were found positive for C. trachomatis in the synovial fluid. Serum IFN-gamma and IL-4 concentrations were significantly higher in C. trachomatis-infected ReA/ uSpA patients as compared to uninfected control osteoarthritis patients ($p < 0.05$, for IFN-gamma and $p < 0.02$, for IL-4).

In tuberculosis Resistin, a proinflammatory cytokine secreted by immune cells and having anti apoptotic potential, has been identified as a TB end point determinant based on ELISA on serum of TB patients and their controls. This protein is NFkB independent, mediated via TLR2 receptors and involved in a cross talk between inflammation and ER stress influencing pathogenesis/disease progression through immune cells. Few novel unique sequences had also been identified bioinformatically from M.Tb and are shown to be highly specific which can further be used for diagnostic purpose. Comparison of functional diversity of PE/PPE protein family of H37Rv (Virulent strain) with H37Ra (avirulent strain) of M. tb using in-Silico tools showed loss or gain of protein binding sites in disordered regions attributing their role in pathogenesis Study on Leprosy reversal type 1 reaction showed increased expression of chemokine receptor CXCR3 mRNA in BT type 1 reaction as well as BB type 1 reaction. CXCL10/IP-10 mRNA shows statistically significant increase in BB type 1 reaction. The CXCR3 /CXCL10 axis may prove to be useful laboratory aids in the diagnosis of type 1 reaction.

The study on a novel arithmetic approach for fool-proof production of growth arrest in 3T3 cells suitable for human epidermal culture to test the hypothesis that feeder effectiveness depends on calculated exposure availability of MMC has almost been completed. The growth stimulatory influence of feeders produced by minimal, medial & maximal inhibitory concentrations and the respective dose per cell combinations of

MMC were verified by growth patterns of epidermal keratinocytes at various passages and at varying feeder-keratinocyte ratios with γ-irradiated feeders as controls. It was demonstrated that feeders inhibited by the medial MMC concentrations produced significant growth stimulation than the minimal and highly toxic concentrations whose influence was comparable to γ-irradiation. BrdU incorporation studies and colony forming efficiencies revealed similar influence. The optimized technique was employed to produce well characterized prototype of cultured epidermis. The present approach identified a cost-effective and optimized growth of epidermal keratinocytes.

The Study on epithelial-mesenchymal interactions using human epidermal keratinocyte stem cells and innovatively growth-arrested fibroblast feeders in 3-D collagen model involved designing of experiments using in vitro 3-D skin model to assess qualitative and quantitative differences in the morphogenesis of epidermis. The minimal, medial and maximal concentrations of MMC treatments resulted in significantly varied feeder cell extinctions in collagen synonymous to 2-D experiments. The basic technique of constructing whole skin model consisting of 3T3 fibroblasts in Type-I collagen gel as Dermal Equivalent and epithelialization by human epidermal keratinocytes followed by complete stratification of the epidermis at air-liquid interface were accomplished. This model will be useful in future studies on the role of differentially attenuated feeders towards healing of experimentally produced wounds in such 3-D models.

The study on pesticide exposure on tea garden workers in north-eastern states of India using placenta and blood of women revealed that pesticides can reach placenta and accumulate there and some of them such as endosulfan β, metribuzine, imidacloprid, Acephate, etc. have potential to cross the placental barrier and enter the foetal bloodstream. They can cause alterations in the development as well as functions of placenta resulting in adverse effects during pregnancy. Ultrastructural study of placenta has revealed that the villi in placenta of tea garden workers exposed to pesticides were comparatively shorter and thinner and less vascularised as compared to non exposed group. Fibrinoid, a homogenous extra-cellular material similar to fibrin was frequently observed in villous stroma. It is plausible that deleterious effect of pesticides on placental barrier of tea garden workers could result of impairment of placental barrier, restrict nutrient supply from mother to foetus and thus could be the cause the Low birth Weight (LBW).

Under translational research activities species specific mono-clonal antibody to C.trachomatis has been produced for diagnosis of C trachomatis infection. Sample kit is under preparation. A DOT –BLOT assay for diagnosis of sequalae to Chlamydia trachomatis infection has also been developed using cHSP60. LAMP assay is being developed as a rapid and effective tool for detection of L. donovani in clinical samples. Validation in clinical samples in endemic area is undergoing. Work on development of Live attenuated Leishmania Vaccines is undergoing in collaboration with US-FDA.

Overall 2012-13 year had been productive through significant contributions in areas of basic and clinical research. I take this opportunity to convey my thanks to Dr. V. M. Katoch, Director General, ICMR and Secretary, Department of Health Research for his support for infrastructure development and encouragement for scientific and academic programs. I acknowledge my sincere thanks to my scientific, technical and administrative staff for their contribution towards enhancement of infrastructure and scientific activities.

Dr. Sunita Saxena
Director

LIST OF ONGOING PROJECTS

TUMOR BIOLOGY

BREAST CANCER

1. STUDY ON GENE EXPRESSION AND HYPERMETHYLATION PROFILES IN EARLY ONSET BREAST CANCER
2. STUDY ON MICRO RNA SIGNATURES ASSOCIATED WITH BREAST CANCER STEM LIKE CELLS (CSCS) AND THEIR ROLE IN DRUG RESPONSE

CANCERS IN NORTH-EAST INDIA

3. IMMUNOGENETIC PROFILE OF NASOPHARYNGEAL CANCER IN A HIGH-PREVALENCE REGION OF NORTHEAST INDIA
4. GENOME-WIDE ANALYSIS OF GENETIC ALTERATIONS IN PATIENTS WITH ESOPHAGEAL CANCER FROM NORTHEAST INDIA USING SINGLE NUCLEOTIDE POLYMORPHISM ARRAYS
5. EPIGENETIC STUDIES IN ESOPHAGEAL CANCER IN HIGH RISK REGION OF NORTHEAST INDIA

HEMATOPOIETIC-LYMPHOID MALIGNANCIES

6. DYNAMIC REGULATION OF LYMPHOCYTE SIGNALING OF ACUTE LEUKEMIA

URINARY BLADDER CANCER

7. CHARACTERISATION OF HOST IMMUNE FACTORS ASSOCIATED WITH PROGRESSION OF SUPERFICIAL TCC OF BLADDER BY MICROARRAY ANALYSIS

PATHOLOGY OF INFECTIOUS DISEASES

LEISHMANIASIS

8. DRUG RESISTANCE IN VISCERAL LEISHMANIASIS
9. STUDIES ON IMMUNOLOGICAL RESPONSES TO RECOMBINANT PSA (PARASITE SURFACE ANTIGEN) - A POTENTIAL VACCINE CANDIDATE
10. DEVELOPMENT OF LIVE ATTENUATED VACCINE CANDIDATES FOR LEISHMANIASIS
11. UBIQUITIN RELATED MODIFIER 1: A POST TRANSLATIONAL MODIFICATION MACHINERY *IN LEISHMANIA DONOVANI*
12. DEVELOPMENT OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY FOR DIAGNOSIS OF *LEISHMANIA* INFECTION

CHLAMYDIASIS

13. IMMUNOPATHOGENESIS OF REACTIVE ARTHRITIS INDUCED BY CHLAMYDIA TRACHOMATIS

TUBERCULOSIS

14. UNDERSTANDING THE UNDERLYING MECHANISM OF MACROPHAGE IMMUNE MODULATION AND IDENTIFICATION OF MARKERS FOR TB TREATMENT END POINT DETERMINATION
15. UNDERSTANDING THE STRUCTURAL FLEXIBILITY AND FUNCTIONAL DIVERSITY OF PE/PPE PROTEIN FAMILY OF *MYCOBACTERIUM TUBERCULOSIS;* IDENTIFICATION AND ROLE OF UNSTRUCTURED/DISORDERED REGIONS IN THIS FAMILY USING *IN-SILICO* TOOLS

LEPROSY

16. CLINICOPATHOLOGICAL DETERMINANTS IN LEPROSY TYPE 1 REACTIONS

ADULT STEM CELL BIOLOGY

17. ***A NOVEL ARITHMETIC APPROACH FOR FOOL-PROOF PRODUCTION OF GROWTH ARREST IN 3T3 CELLS SUITABLE FOR HUMAN EPIDERMAL CULTURE***

18. **STUDIES ON EPITHELIAL-MESENCHYMAL INTERACTIONS USING HUMAN EPIDERMAL KERATINOCYTE STEM CELLS AND INNOVATIVELY GROWTH-ARRESTED FIBROBLAST FEEDERS IN 3-D COLLAGEN MODEL.**

ENVIRONMENTAL BIOLOGY

19. **HUMAN ENVIRONMENTAL BIOMONITORING OF POLYNUCLEAR AROMATIC HYDROCARBONS (PAHS) IN URBAN MEGALOPOLIS OF NCR DELHI AND INVESTIGATE THE ASSOCIATION BETWEEN PAH EXPOSURE AND INTRAUTERINE GROWTH RESTRICTION**

RESEARCH ACTIVITIES

TUMOR BIOLOGY

TUMOR BIOLOGY

1. Study on gene expression and hypermethylation profiles in early onset breast cancer

Scientific staff : Dr. Sunita Saxena, Dr. S. A. Raju Bagadi, Dr. Sujala Kapur, Ms. Shreshtha Malvia

In collaboration with : Dr. Chintamani, Dr. R. S. Mohil, Department of Surgery, Safdarjang Hospital, New Delhi
Dr. A. Bhatnagar, Department of Cancer Surgery, Safdarjang Hospital, New Delhi, India.

Aims, Objectives & Background:

In India breast cancer occurs in young women in more than fifty percent cases. Breast cancer diagnosed at young age shows clinically different characterstics compared to breast cancers diagnosed at older ages. Younger patients more frequently exhibit aggressive features such as large tumor size, high histologic grade, positive lymph nodes, absence of steroid receptors and high S-phase fraction, and young age itself has been shown to be an independent predictor of adverse prognosis. The present study is aimed to study differential gene expression and promoter methylation changes to elucidate genetic and epigenetic factors associated with early onset breast cancer in Indian women.

Work done during the year:

In order to identify unique gene expression profiles associated with breast cancer that occurs at early age (<40 years), we studied gene expression and methylation profile of 20 early (<40 years) and 20 late onset breast tumors (>50 yrs). 1400 genes were found differentially expressed in early onset cancers compared to 2600 genes found differentially expressed in late onset breast cancers showing deregulation of several pathways such as cell adhesion, epigenetic regulation, Wnt signaling pathways, cytokine receptor interaction, toll like receptor growth signaling pathway, cell cycle etc. Methylation analysis showed

672 cpg sites differentially methylated in early onset(443 hyper and 229 hypo) and 689 in late onset (625 hyper and 65 hypo) breast cancers. Among them, hypermethylation of 246 genes was unique to early onset tumors while 347genes were hypermethylated in only late onset cancer and 121 found methylated in both early and late onset cancers (Fig 1). Merging of methylation data was done with gene expression to understand how many genes are transcriptionally deregulated by methylation. Among 443 CpG sites hypermethylated in early onset tumors, 55 genes were found downregulated, 24 upregulated and 364 genes were found unchanged.

Validation of ALDH1B1, ALDH2, CBX2, CCNJ, FOX (3 genes), JMJD2A, MATN, METTL11A, PRMT, SOX genes (3), TMEM41A, WNT5A, WNT3, HMGN1, JMJD4, MCMs (2) genes differentially expressed in micro-array data was done by real time PCR in 20 breast tumors and compared with their normal controls.

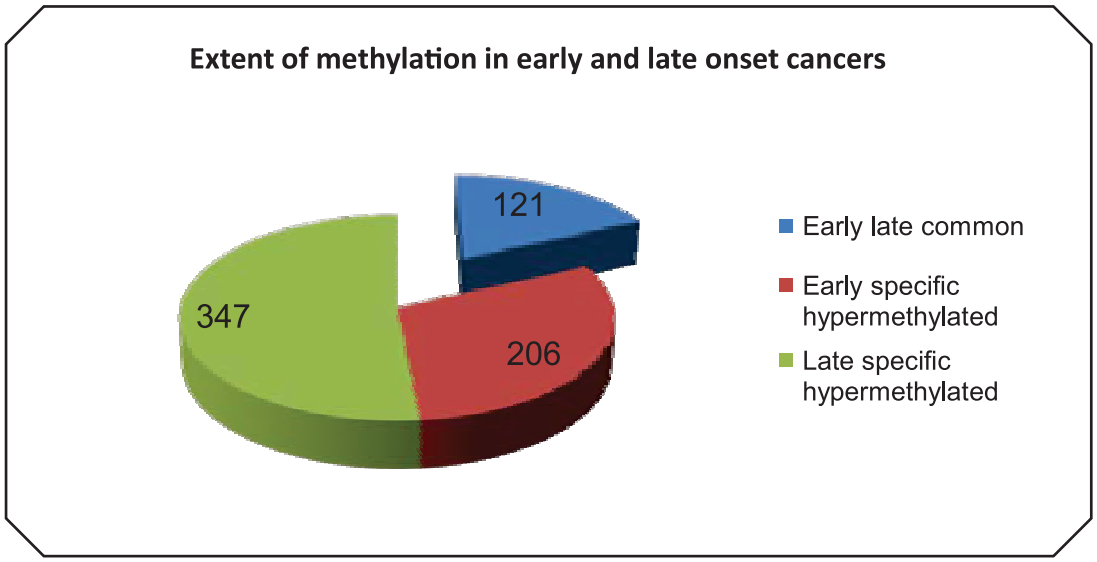
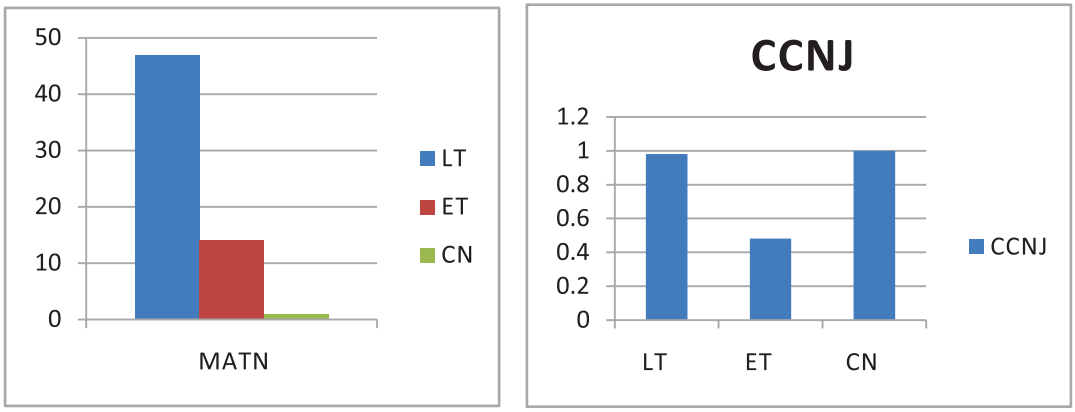


Fig. 1: Showing number of genes differentially hypermethylated in early and late onset breast cancers.



2a 2b

Fig. 2: Shows differential expression of MATN and CCNJ in breast cancers.
2a. shows MATN over expression in late onset tumors, 2b.shows down regulation of CCNJ in Early onset tumors. (LT-late tumor, ET-early tumors, CN-Control normal tissue)

2. Study on micro RNA signatures associated with breast Cancer Stem like Cells (CSCs) and their role in drug response

Scientific staff : Dr. Sunita Saxena, Dr. S. A. Raju Bagadi, Mrs. Shanti latha Pandrangi, Mr. S. M. K. Muneswarao

Duration : 2013-15

Aims, Objectives & Background:

Tumor recurrence and treatment failure are well known in cancer therapy and recently linked with Cancer Stem Cells (CSCs). Breast CSCs can be sorted out based on the presence of Aldehyde Dehydrogenase (ALDH) enzyme or by using CD44 and CD24 markers to select cancer stem cells ($CD44^+$/ $CD24^-$) and can be enriched and propagated in suspension cultures as mammospheres. The mammosphere system offers an *in vitro* model to study the effect of anti-cancer drugs on breast cancer stem cells. The present study is aimed to identify effect of various drugs on cancer stem cells and bulk cells and characterize microRNAs and genes that are differentially expressed in breast CSCs.

Work done during the year:

Human breast cancer cell lines, MCF7, SUM159 and HBL100 were grown as adherent cultures. The cells were then stained with aldefluor and sorted out using FACS ARIA II. The sorted ALDH+ cells were then cultured in non-adherent, non-serum conditions to form primary mammospheres (termed M1 mammospheres), which were dissociated both enzymatically and mechanically to obtain single cells which were then subjected to serial passaging every 10^{th} day leading to the generation of M2, M3, M4 mammospheres and so on to test the functional definition of stem cells to self renewal. Also, the dissociated cells from these mammospheres were again analyzed for the presence of $ALDH^{+/bright}$ cells and were sorted using FACS at every passage.

The study showed that primary mammospheres contain a distinct population that displays an $ALDH^{-/low}$ phenotype, but fails to generate mammospheres. Instead, the mammosphere-initiating potential rests within the $ALDH^{+/bright}$ cells, in keeping with the phenotype of breast cancer-initiating cells. With increasing passages, mammospheres showed a dynamic increase both in the number of mammosphere forming units and sphere forming efficiency (Fig. 1) until the second passage followed by a dramatic reduction. Also there is an increase in the number of smaller sized spheres relative to the larger ones over multiple generations of mammospheres.

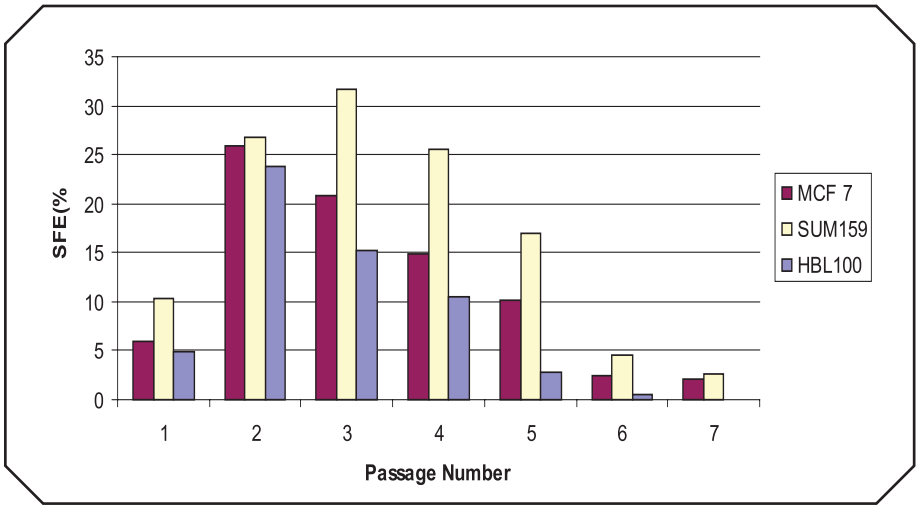


Fig. 1: Sphere Formation Efficiency (SFE) of various breast cancer/ normal cell lines.

Targeting Breast Cancer Stem Cells (BCSCs) offers a promising strategy for breast cancer treatment. We examined the plant alkaloid ellipticine for its efficacy to inhibit the expression of aldehyde dehydrogenase 1 class A1 (ALDH1A1)- positive BCSCs by *in vitro* and *in silico* methods. At 3 mM concentration, ellipticine decreased the expression of ALDH1A1-positive BCSCs by 62 % (p =0.073) in the MCF7 cell line and by 53 % (p =0.024) in the SUM159 cell line compared to vehicle-treated cultures. Ellipticine significantly reduced the formation of mammospheres, whereas paclitaxel enhanced mammosphere formation in both the treated cell lines. Interestingly, when treated with a combination of ellipticine and paclitaxel, the percentage of ALDH1A1- positive BCSCs dropped by several fold *in vitro*. A homology model of *Homo sapiens* ALDH1A1 was built using the crystal structure of NAD-bound sheep liver class I aldehyde dehydrogenase [PDB ID: 1BXS] as a template. Molecular simulation and docking studies revealed that the amino acids Asn-117 and Asn-121, Glu-249, Cys-302, and Gln-350, present in the active site of human ALDH1A1, played a vital role in interacting with the drug. The present study suggests that ellipticine reduces the proliferation and self-renewal ability of ALDH1A1-positive BCSCs and can be used in combination with a cytotoxic drug like paclitaxel for potential targeting of BCSCs.

Currently, we are in the process of standardization of isolation and propagation of cancer stem cells, by using CD44 and CD24 markers to select CSCs ($CD44^+/CD24^-$) by fluorescent activated cell sorting. Two breast cancer cell lines MCF7 and MDA-MB-231have been used; CD44+/CD24- cells cells have been separated from these cell lines and grown them on non-adherent conditions (Fig. 2). MDA-MB-231 has formed large colonies on non adherent conditions, whereas MCF7 has formed few small colonies (Fig. 3).

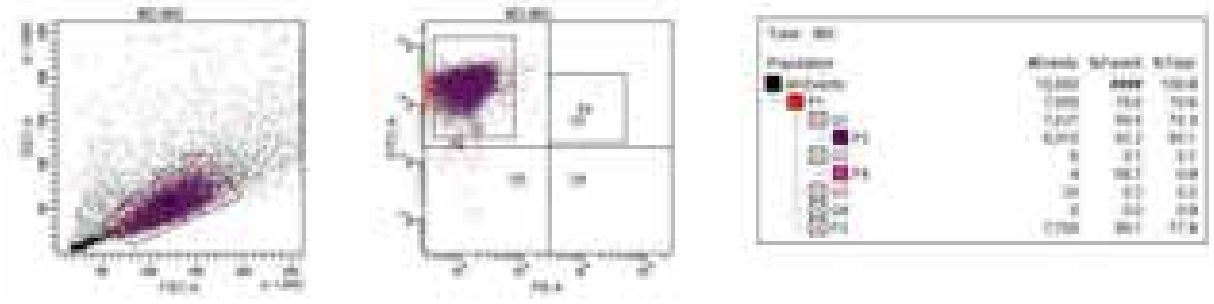
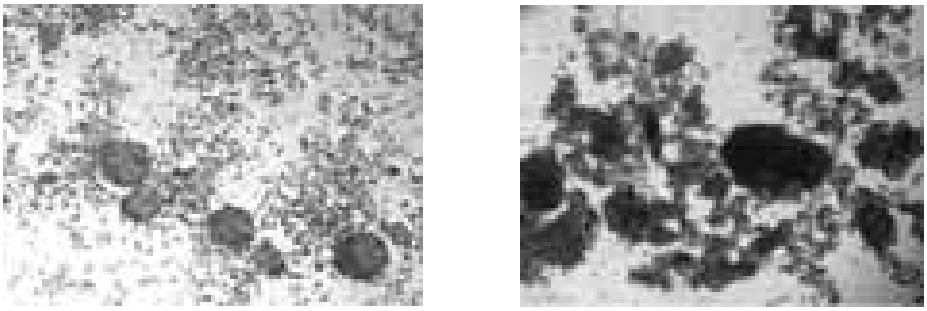


Fig. 2: Showing presence of 78% CD44+/CD24- (cancer stem) cells in MDA-MB-231



a b

Fig. 3: Showing formation of mammospheres on non-adherent surface when 2000 cells were seeded a. MCF7 formed small and few number of colonies b. MDA-MB-231 cells formed large and several colonies on non-adherent plates.

3. Immunogenetic profile of nasopharyngeal cancer in a high-prevalence region of northeast India

Scientific Staff : Dr. Sujala Kapur, Dr. Sunita Saxena, Dr. L.C.Singh, Dr. Saurabh Verma, Dr. A.K. Mishra, Ms Meena Lakhanpal

In collaboration with : Dr. A.C. Kataki, B. Barooah Cancer Institute, Guwahati, Assam
Dr. Y. Mohan Singh, RIMS, Imphal, Manipur
Dr. Tashnin Rehman, B. Barroah Cancer Institute, Guwahati, Assam
Dr. Madhumangal Singh, RIMS, Imphal, Manipur
Dr. Jagannath D. Sharma, Deptt. of Pathology, BBCI, Guwahati, Assam

Duration : 2010-13

Aims, Objectives & Background:

Nasopharyngeal Carcinoma (NPC) is a rare tumor in most parts of the world, but occurs at relatively high rates in some geographic regions and among certain ethnic groups, with the highest incidence worldwide being reported from southeast Asia and southern China. High incidence of NPC in NorthEast (NE) region of India has been reported where it is the eighth most common cancer. The etiological factors of NPC include a complex interaction of genetic, viral, environmental and dietary factors. Antigenic presentation of EBV-derived

peptides is suspected to be involved in the pathogenesis of EBV-associated diseases. In addition, polymorphisms in the HLA region, particularly in the class I region, are also known to be associated with the occurrence of the disease. The aim of current study is to analyze if Epstein Barr viral sequences in the tumor tissue alongwith host imunogenetic factors can explain the high prevalence of nasopharyngeal carcinoma in different ethnic groups of northeastern states.

The project had been undertaken with the following aims :

1. To study the allelic variation in HLA class I and class II loci in patients with nasopharyngeal carcinoma as compared to the control population from northeast India and analyze if there is a correlation of specific HLA alleles to disease susceptibility in this region.
2. To study if there is any variation at the genetic level in HLA class III region that contains genes encoding the pro-inflammatory cytokines (TNFα and TNFβ) and heat shock proteins HSP, gp 70.
3. To analyze if the presence of Epstein Barr viral sequences in the tumor tissue can explain the high prevalence of nasopharyngeal carcinoma in this region.

Work done during the year:

During the year, 120 blood samples from newly diagnosed cases of NPC were collected. Of these, 56 were from Guwahati and 64 from Imphal. 35 tissue samples from these 91 cases were also collected. Genomic DNA was extracted from blood samples from patients and contros (Table 1).

Table 1: Blood and tissue samples received from the two centers of northeast India

Centres	Blood Samples received		Tissue samples received	
	Cases	Controls	Cases	Controls
RIMS, Imphal	64	45	07	1
BBCI, Guwahati	56	55	28	-
Total	**120**	**100**	35	1

We have done genotyping of the HLA region at 6p21.3 using 33 polymorphic microsatellite markers. These markers have previously been identified by use of sequence data from the US National Center for Biotechnology Information map. 33 markers were selected from the HLA region *(Diepstra A, Lancet 2005)*, and were standardized for PCR conditions by using *6-Carboxyfluorescein* (6-FAM) labeled forward primer and unlabelled reverse primer at different gradient temperatures. After PCR amplification, amplicons were subjected to Fragment Length analysis in a DNA sequencer using a mastermix containing GeneScan -500 LIZ Size Standard and Hi-Di. The capillary electrophoresis was performed using the *Applied Biosystems ABI PRISM 3130xl Genetic Analyzer with Data Collection Software Version 2.0*. Analysis of collected data was done using *Gene Mapper software* (Fig. 1).The frequencies of the different alleles and genotypes were compared between patients and controls by use of chi-square tests. When there was a significant difference, specific allele or genotype causing this difference was assessed. Odds ratios and their 95% CI were calculated by logistic regression. Statistical significance was set at $p < 0.05$. The probability of an association was corrected with the Bonferroni Inequality method, i.e., by multiplying the 'p' values obtained by the number of alleles compared (Pc).

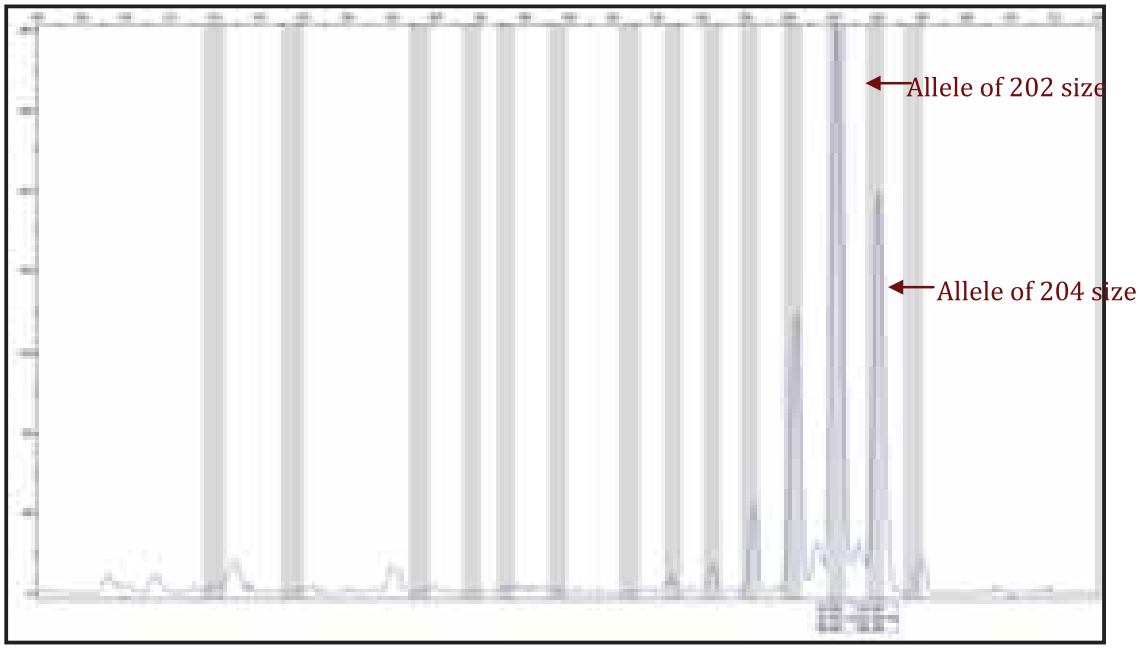


Fig.1: Genotyping for D6S2673 analyzed by Gene Mapper software; This sample shows heterozygous alleles of 202 and 204 sizes.

Analysis of preliminary results was done using Gene Mapper software. It showed many allelic variants for the all 31 micosatellite markers. All the markers showed many allelic variants. Five markers showed 5 different allelic variants, 3 markers with 6 allelic variants, 4 with 7 allelic variants, 3 markers each with 8, 9 and 10 allelic variants, 1 marker each with 11, 12, 13 allelic variants, 3 for 14 allelic variants, 5 for 15 variants and 1 for 17 allelic variants. The most polymorphic marker was D6S2701 with 17 allelic variants. The level of polymorphism was measured considering the number of alleles and genotypes and by heterozygosity. Further statistical analysis is being done to confirm susceptibility loci for NPC in NE India.

Detection of EBV

By *in situ* hybridization:

Epstein - Barr virus (EBER) PNA probe/fluorescein was obtained from DAKO and used for the detection of EBV infection by *in situ* hybridization. For this, formalin-fixed, paraffin-embedded tissue sections were processed as per manufacturer's instructions. Briefly hybridization was followed by a post-hybridization wash with a stringent wash solution, followed by incubation with alkaline phosphatase-conjugated rabbit F(ab') anti-FITC substrate (enzyme substrate, BCIP/NBT). Thereafter, the specimen was incubated at room temperature followed by a wash.

Finally, the specimen was mounted. Positive staining was recognized under a light microscope as a dark blue/black colour at the site of hybridization (Fig. 3)

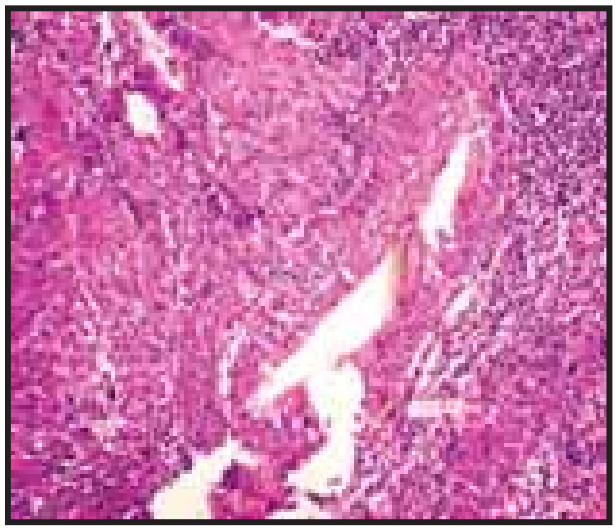


Fig. 3a: Haematoxylin and eosin staining showing tumor cells in nasopharyngeal biopsy.

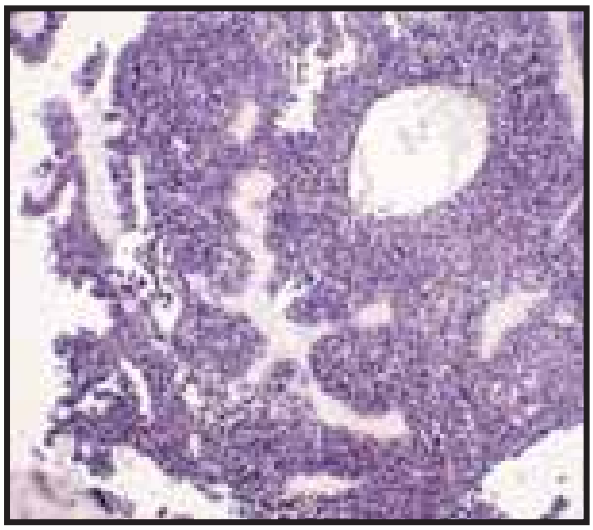


Fig. 3b: *In situ* hybridization showing positive sample with dark blue/black colour at the site of hybridization with EBER probe.

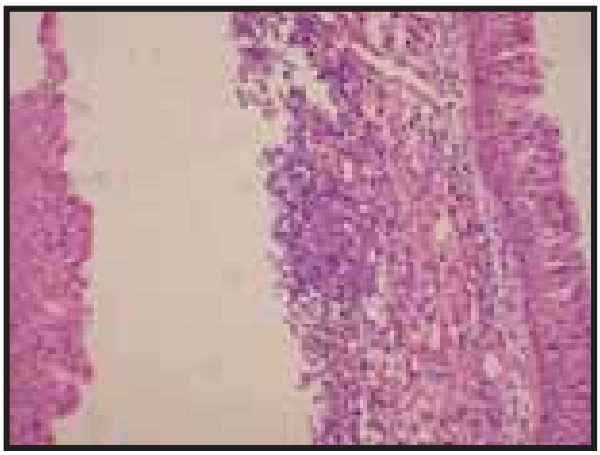


Fig. 3c: Haematoxylin and eosin staining showing normal epithelial cells in nasopharyngeal biopsy.

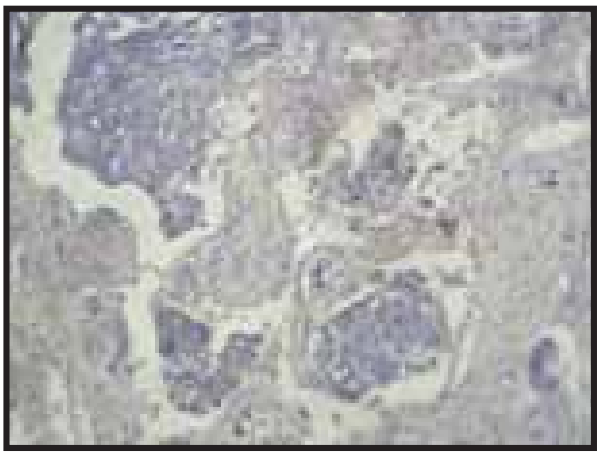


Fig. 3d: *In situ* hybridization showing negative sample with no hybridization for EBER probe

Fig. 3: Images showing standardization of conditions for *in situ* hybridization and H&E staining in paraffin embedded tissue sections

Table 3: Distribution of EBV RNA sequences in NPC and non-NPC patients

S.No.	Disease Status	Total	EBV Positive (N)	EBV Negative (N)	P value
1	NPC Patient	25	23(92%)	2(8%)	=0.0004
2	Non-NPC	14	0(0%)	14(100%)	

NPC Patient : Patient's biopsies found histopahtological-positive for nasopharyngeal cancer. Non NPC : Patients suspected of NPC but after histopathological examination they were found negative for NPC. N = Number of samples

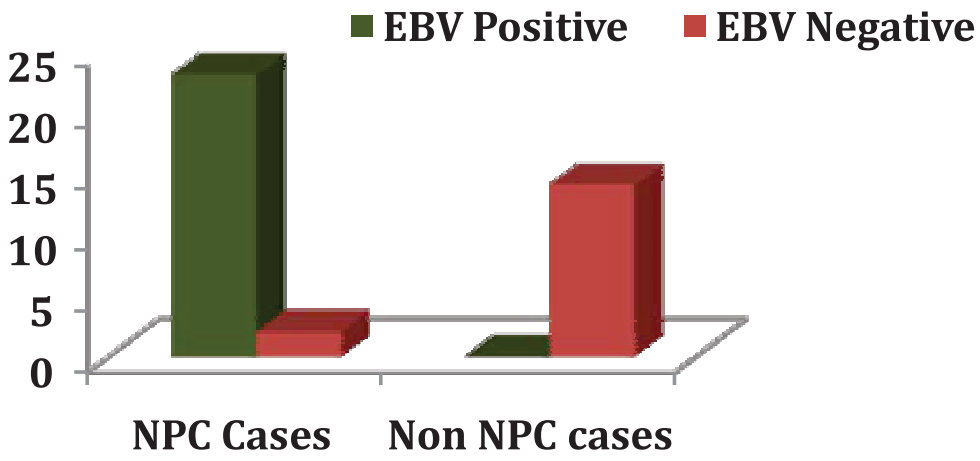


Fig. 4: Bar graph showing frequency of EBV in NPC and non-NPC patients

EBV RNA was detected in 23 (92%) of the 25 biopsies of NPC patients while it was not detected in any control samples (P=0.0004) (Table 3 and Fig. 4). In positive samples, virtually all malignant cells were strongly labeled with probes whereas, EBV was absent in two NPC specimens and in all of the specimens from patients without NPC. This study showed statistically significant differences in the presence of EBV sequences between the NPC patient group and the control group. This confirms the etiological association of EBV with NPC and may help to understand the biological mechanisms of NPC and the correlation of EBV infection with this disease, in high risk, endemic region of NE India.

4. Genome-wide analysis of genetic alterations in patients with esophageal cancer from northeast India using single nucleotide polymorphism arrays

Scientific Staff : Dr. Sunita Saxena, Dr. Sujala Kapur, Mr. Ashish Bhushan

In collaboration with : Dr. Amal Chandra Kataki, Director, BBCI, Guwahati, Dr. Jagannath D. Sharma; Deptt. of Pathology, BBCI, Guwahati, Dr. Avadesh Rai, Research Scientist, BBCI, Guwahati, Dr. B. B. Barthakar; Dept. of Surgical Oncology, BBCI, Guwahati, Dr. Jagdish Mahanta; Director, RMRC, Dibrugarh, Dr. R. K. Phukan; RMRC, Dibrugarh, Assam.

Duration : 2011-14

Aims, Objectives & Background:

Esophageal cancer represents the third most common Gastro-Intestinal (GI) malignancy and ranks among the ten most common cancers worldwide. The incidence of esophageal cancer varies between populations, with a greater than 50-fold difference in high and low risk populations. The highest incidence of this cancer in India has been reported from Assam in the north-east region, where it is the second leading cancer in men and third leading cancer in women. A familial aggregation of esophageal cancer has been reported in Assam region of north-east India alongwith high consumption of tobacco paste and tobacco water, suggesting that environmental carcinogens in addition to geographic and genetic factors play major etiologic roles.

The current study aims to investigate a link between genetic variations including chromosomal changes, LOH and copy number alterations associated with ethnic variation in NE populaton of India showing familial clustering of esophageal cancer associated with a high incidence of tobacco-associated cancers. This would also help in identifying suitable biomarkers for preclinical screening, early diagnosis in familial cases and target for biological intervention. This study may explain our basic research questions, *viz.*: what is the contribution of copy number variation to esophageal cancer in high-incidence

regions and can suitable genetic markers be identified that will help in screening and early detection of cancers in these regions?

Work done during the year:

Endoscopic biopsy specimens from 56 tumor and adjacent normal tissue distant to the tumor have been collected from patients with esophageal cancer during diagnostic endoscopy at Dr. Bhubaneshwar Borooah Cancer Institute (BBCI), Guwahati, Assam.

Tumor and normal tissue samples were preserved in RNA Later s *(Ambion, Austin, USA)* and stored at −70°C until processed. In addition, 5 ml of peripheral blood was collected in EDTA vials from the patients and frozen. Informed consent was obtained from all the patients to use their surgical specimens and clinicopathologic data for research purposes.

In a preliminary study, 15 tumor tissue and blood samples (germ line DNA) from the same patients were processed during this year. Germ-line and tumor tissue DNA was extracted using the Qiagen QIamp DNA Mini kit *(Qiagen, Hilden, Germany)* as per manufacturer's instructions. Each DNA sample was processed followed by *StyI / NspI* Restriction Enzyme Digestion, *StyI / NspI* Ligation, by *StyI / NspI* amplification, fragmentation, labeling, Target hybridization, washing and scanning was assayed according to the standard protocol *(Affymetrix® Genome-Wide Human SNP Array 6.0)*.

43 genes in amplified regions and 50 genes in deleted regions were detected with low and high number of SNPs regions at different arms of chromosomal position. Chromosomal regions with significantly high SNPs were found at the following chromosomal positions: chr1q21.1–q44 (*ENAH*, *KCNH1*, *KCNK2*, *OBSCN*, *PTPRC*, *RGS1*, *S100A3*, and *TNR*), chr5p15.2–p12 (*RNASEN*, *SLC1A3*, and *TRIO*), chr6p25.3–q11.1 (*CD83*, *CDKAL1*, and *TXNDC5*) chr9q13–q34.13 (*GSN*, *NTRK2*, *TLR4*, *TNC*, and *TSC1*), chr11q22.1–q25 (*GRIK4*, *NCAM1*, and *TRIM29*) and chr20p13–p11.21 (*PLCB1* and *PRNP*) in amplified region.

Chromosomal regions with significantly high SNPs in deleted regions were found at the following chromosomal locations: chr3p26.3–p14.3 (*CACNA1D*, *DOCK3*, *FBLN2*, *GRM7*, *WNT7A*, *KCNH8* and *TGFBR2*), chr8p23.2–p21.3 (*ANGPT2*, *DLC1*, *LZTS1*, *MTUS1*, *PPP3CC*, *PSD3* and *MCPH1*), chr10p15.3–p11.21 (*AKR1C3*, *CACNB2* and *PARD3*), chr13 q12.11–q34 (*CDK8*, *CLDN10*, *COL4A1*, *DIAPH3*, *DGKH*, *FGF14*, *FLT1*, *GPC5*, *GPC6*, *HTR2A*, *POLR1D*, *TNFSF11* and *WASF3*) and chr18 q21.2–q23 (*NFATC1* and *DCC*).

Thirteen of 43 genes in amplified regions and 18 of 50 genes in deleted regions were found to be significantly associated with cancer-associated pathways. Of these 13 genes in amplified regions, six genes (*NTRK2*, *TPO*, *PLA2G5*, *PAK1*, *MAPK10*, *FGF12* and *FGF4)* were found to be significantly associated with MAPK signaling pathway. Of 18 genes found in deleted regions, several genes were found to be most significantly involved in MAPK signaling pathway (*MAPT*, *CACNA1D*, *TGFBR2*, *PPP3CC*, *CACNB2* and *FGF14*), Cytokine-cytokine receptor interaction (*IL22*, *IL26*, *CACNA1D*, *PPP3CC* and *FLT1*) and Wnt signaling pathway (*PPP2CB*, *WNT7A*, *PPP3CC* and *NFATC1*). The other predominant pathways found were Focal adhesion, ECM-receptor interaction Wnt signaling pathway, ErbB signaling pathway, Cytokine-cytokine receptor interaction, Jak-STAT signaling pathway, VEGF signaling pathway and mTOR signaling pathway. All these pathways are reported to play a potential role in tumorigenesis.

Biological GO analysis found amplified and deleted regions associated with cell adhesion, cell differentiation and cell signaling play a role in cell apoptosis, cell proliferation, cell migration and cellular morphogenesis, to be significantly associated with esophageal cancer. Six genes (*WNT7A*, *TNC*, *FGF14*, *TNR*, *MAPK10*, and *FGF12*) were recognized as candidate genes which were involved in common function showing high associations with signal transduction (Fig. 1).

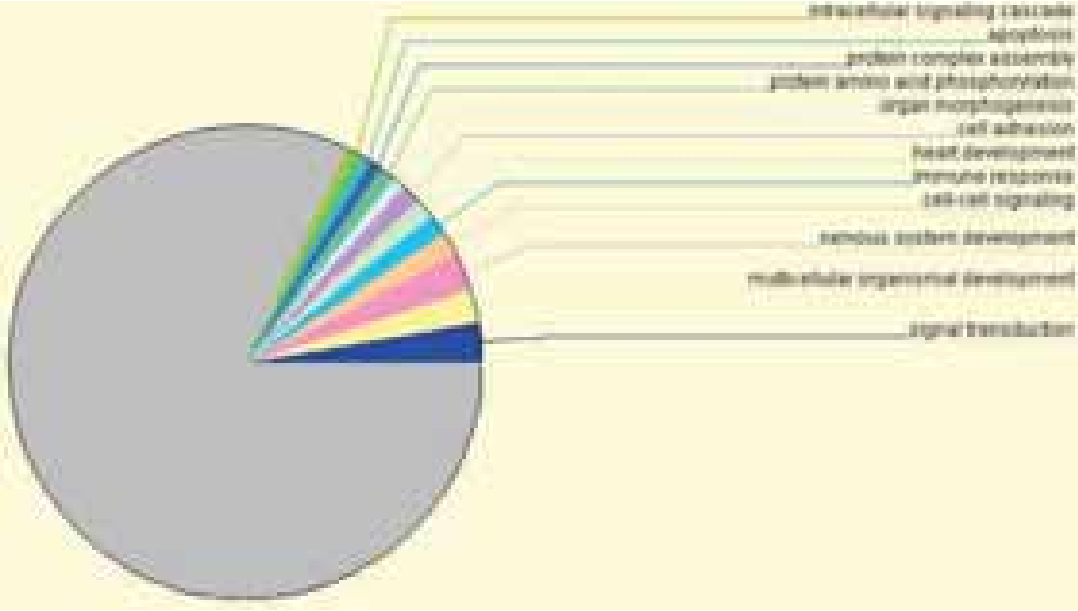


Fig. 1. : Candidate genes involvement in biological function.
Pathways in cancer identified 9 potential candidate genes that were differentially expressed (*COL4A1*, *CTNNA3*, *DCC*, *FGF12*, *FGF14*, *FGF19*, *MAPK10*, *TGFBR2*, *WNT7A*).

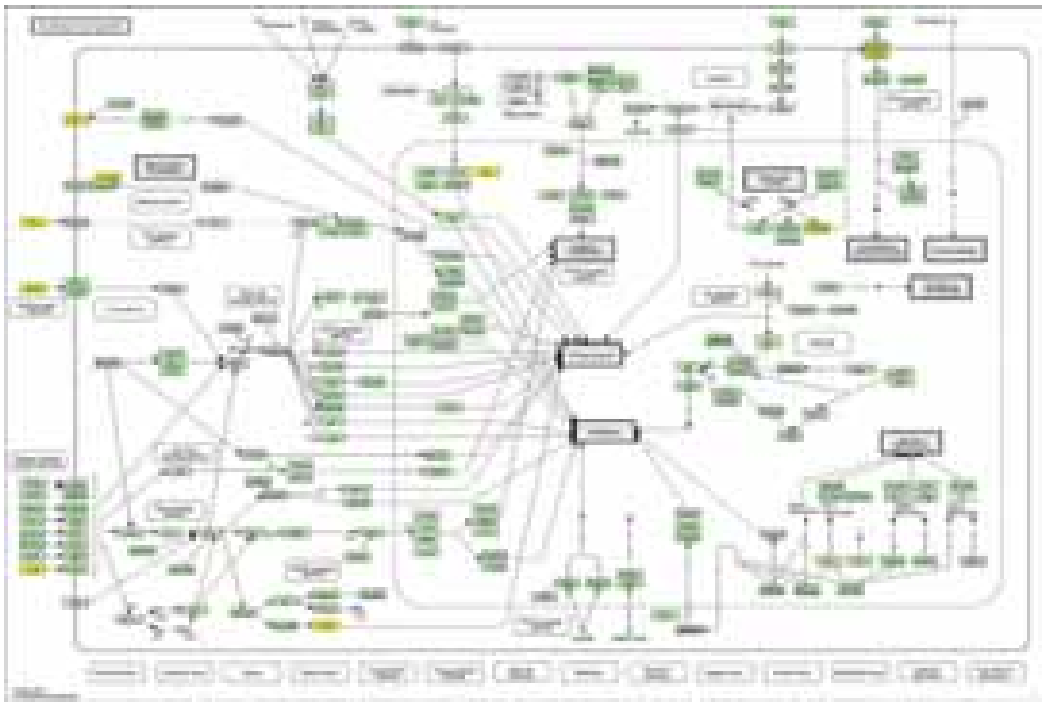


Fig. 2. : Highly significant genes involved in cancer pathway.
Of the 9 potential candidate genes, four genes (*WNT7A*, *FGF14*, MAPK10, and *FGF12*) were involved in common biological function of signal transduction.

Functional Validation of candidate biomarkers by siRNA knockdown of key genes will be done in established esophageal cancer cell lines (KYSE30, KYSE70, KYSE180, KYSE410 and KYSE450). These cell lines are being maintained in a medium containing RPMI 1640 + Ham's F12 (1:1) with 2% Fetal Bovine Serum (FBS) at 37°C with 5% CO2. Doubling time of the cell lines is on an average 30 - 35 hours. At initial stage of functional analysis we have selected one gene from our data, *i.e.*, Fibroblast growth factor 12 *(FGF12)* and protocol for functional genomics is being standardized.

5. Epigenetic studies in esophageal cancer in high risk region of northeast India

Scientific Staff : Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. L.C. Singh, Dr. Virendra Singh

In collaboration with : Dr. Amal Chandra Kataki, Director, BBCI, Guwahati,
Dr. Jagannath D. Sharma; Deptt. of Pathology, BBCI, Guwahati
Dr Avadesh Rai, Research Scientist, BBCI, Guwahati
Dr. B. B. Barthakar; Dept. of Surgical Oncology, BBCI, Guwahati,
Dr. Jagdish Mahanta; Director. RMRC, Dibrugarh,
Dr. R. K. Phukan; RMRC, Dibrugarh, Assam.

Duration : 2011-14

Aims, Objectives & Background:

We have earlier reported several differentially expressed genes in esophaseal cancer in NE region that have also been reported from a high-incidence region of esophageal cancer in China. As DNA methylation, histone modification and miRNA-mediated processes alter the gene expression, the involvement of these processes alongwith our earlier results might be useful to find out epigenetic markers of esophageal cancer risk in northeast Indian population. The present study is aimed to carry out mRNA expression assessment of 84 reported histone modification enzymes and promoter methylation profiling of 94 tumor suppressor genes by quantitative real time PCR arrays in tumor and normal esophageal tissue samples collected from Esophageal Squamous Cell Carcinoma (ESCC) patients.

Work done during the year:

Tissue samples were collected 42 esophageal cancer patients. Genomic DNA and RNA were isolated by using QIAGEN kits following manufacturer's protocols. Quantitative differential mRNA expression profiling of epigenetic chromatin modification enzymes was done by 96-well plates PCR array *(Qiagen)* in 24 tumor and 24 normal tissue samples by pooling 4 matched samples for each experiment. The array profiles the expression of 84 key genes encoding enzymes that modify chromatin accessibility and therefore gene expression. Similarly for tumor suppressor gene promoter methylation analysis, a real time PCR array *(Qiagen)* was used that profiles the methylation status of promoter of 94 tumor suppressor genes. Methylation profiling was done in 6 paired tumor and normal tissue samples collected from age and sex-matched ESCC patients. Real time PCR array data analysis and visualization were done by using online available tools provided by *Qiagen*. In brief, fold change in mRNA expression of chromatin modification enzymes and percentage of hypermethylation of tumor suppressor genes for each gene was calculated by $\Delta\Delta C_T$ data analysis method.

The results indicate statistically significant higher expression of enzymes regulating histone methylation (DOT1L; DOT1-like, histone H3 methyltransferase and PRMT1; Protein arginine methyltransferase 1) and histone acetylation (KAT7; Lysine acetyltransferase 7, KAT8; Lysine acetyltransferase 8, KAT2A; Lysine acetyltransferase 2A and KAT6A; Lysine acetyltransferase 6A) in tumor tissue compared to normal tissue of ESCC patients (Fig. 1).

Tumor suppressor genes OPCML (Opioid binding protein/cell adhesion molecule-like), NEUROG1 (Neurogenin 1), TERT (Telomerase reverse transcriptase) and WT1 (Wilms tumor 1) were found hypermethylated and CDH1 (Cadherin 1, type 1, E-cadherin (epithelial), SCGB3A1 (Secretoglobin, family 3A, member 1), THBS1 (Thrombospondin 1) and VEGFA (Vascular endothelial growth factor A) were found hypomethyalted in tumor tissue compared to normal tissue of ESCC patients (Fig. 2).

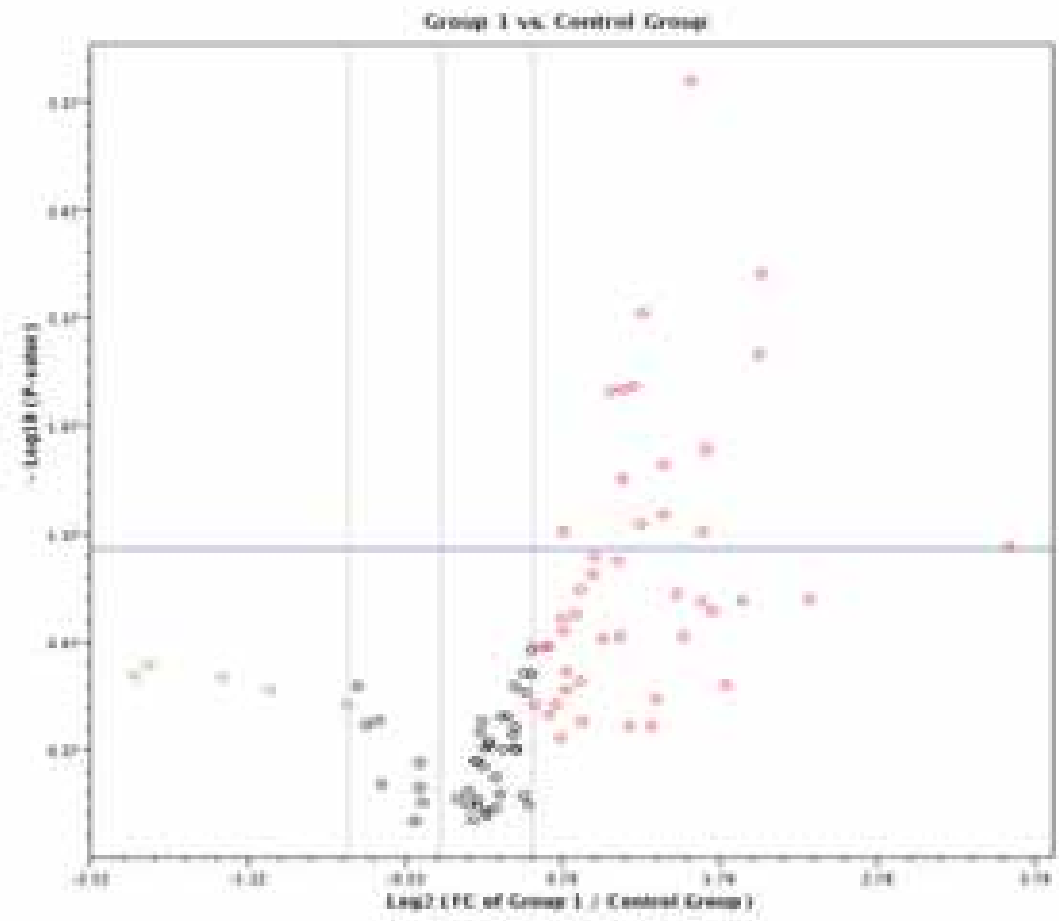


Fig. 1: The volcano plot displaying statistically significant (p-value ≤ 0.05) fold regulation in mRNA expression of genes coding chromatin modification enzymes. The plot displays statistical significance versus fold regulation on the y- and x-axes respectively. The boundaries or the fold regulation cutoff is 2 and a p-value cutoff value is 0.05. Red and blue dots represent genes upregulated and downregulated in tumor tissue compared to normal tissue respectively. Red dots above the blue line show statistically significant (p-value ≤ 0.05) up-regulated gene expression.

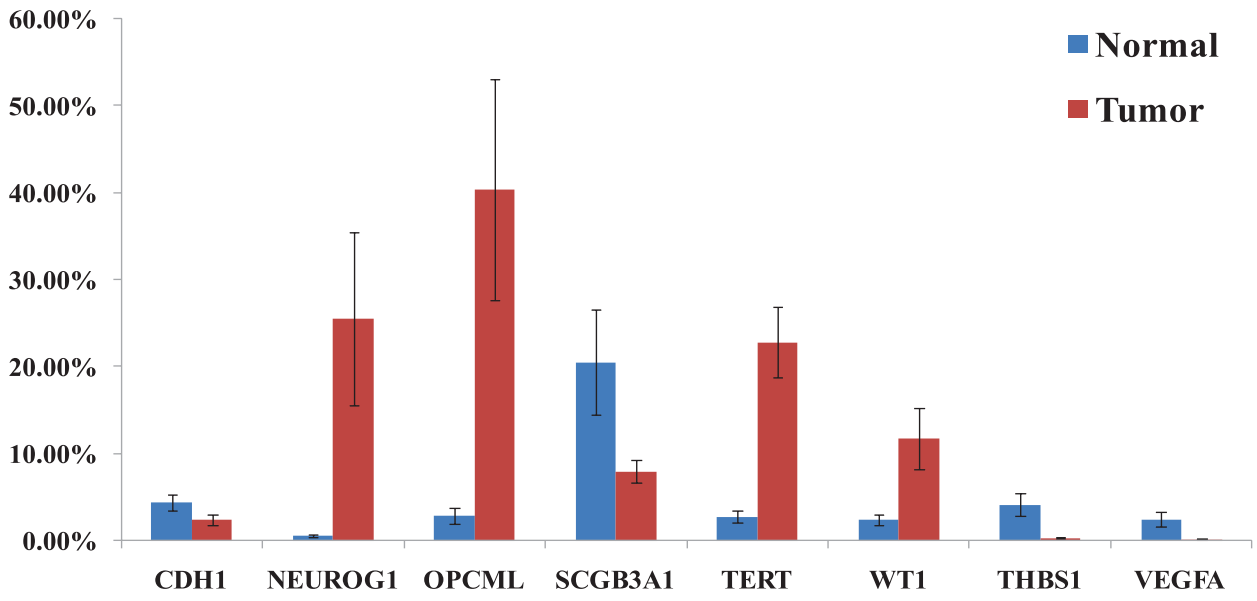


Fig. 2: The bar diagram showing significantly (p-value ≤ 0.05) differentially methylated tumor suppressor genes i.e. CDH1 (Cadherin 1, type 1, E-cadherin (epithelial), NEUROG1 (Neurogenin 1), OPCML (Opioid binding protein/cell adhesion molecule-like), SCGB3A1 (Secretoglobin, family 3A, member 1), TERT (Telomerase reverse transcriptase), WT1 (Wilms tumor 1), THBS1 (Thrombospondin 1) and VEGFA (Vascular endothelial growth factor A) between tumor and normal tissue. The X axis represents the name of genes and Y axis represents the mean percentage of methylation (Error bars show standard error of mean).

Further analysis and validation will be done to find out the significance of differences in chromatin modification enzymes and tumor suppressor genes promoter methylation between tumor and normal tissue samples of ESCC in high risk northeast Indian population.

6. Dynamic regulation of lymphocyte signaling of acute leukemia

Scientific Staff : Dr. Anand Kumar Verma, Dr. Sujala Kapur
In collaboration with : Dr. S.P. Kataria, Safdarjang Hospital, New Delhi
Duration : 2013-16

Aims, Objectives & Background:

Leukemia is a progressive, neoplastic disease of the hematopoietic system (myeloid or lymphoid) characterized by unregulated proliferation of uncommitted or partially committed stem cells. This heterogeneous group of neoplasms differs with respect to aggressiveness, cell of origin, clinical features, and response to therapy. Reactive oxygen species (ROS) is a signaling molecule that modulates the intracellular environment of cells. ROS level was found to be upregulated in CLL B-cells in an earlier study in our laboratory. Since, this molecule can be used as targeted therapy, the current study was designed to explore the potential of reactive oxygen species for therapy of acute leukemia.

The objective of the study is to investigate the role of cellular redox state in signaling of T-cells in acute leukemia. The aim is to find out if patents with AL can be treated with redox-mediated targeted therapy.

Work done during the year:

Samples were obtained from 67 patients with a confirmed diagnosis of acute leukemia being admitted to the Department of Hematology, Safdarjung Hospital, New Delhi. Diagnosis of acute leukemia was made on routinely stained bone marrow and peripheral blood smears and was evaluated according to the French-American-British (FAB) criteria. Peripheral blood samples was obtained from those patients having more than 80% blasts in their peripheral blood. Immunophenotyping was done by flow cytometery on Ficoll-Hypaque separated mononuclear cells from heparinised bone marrow aspirates or peripheral blood for confirmation of diagnosis (Fig. 1)

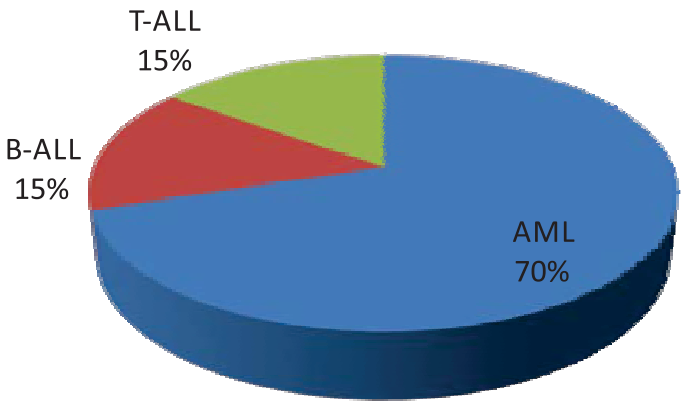


Fig. 1: Sample distribution of acute leukemia

For analysis of cellular redox state, PBMC from patient's samples and age and sex matched controls were cultured in conditioned media for 96 hours and induced with optimized concentration of hydrogen peroxide. Three colour flowcytometry was used to analyse the expression of CD3 and reactive oxygen species in gated 7-AAD negative live T-cells.

The T-cells in samples of patients with T –ALL were highly redox sensitive in comparison to T-cells of samples with AML. Results are shown in Fig. 2.

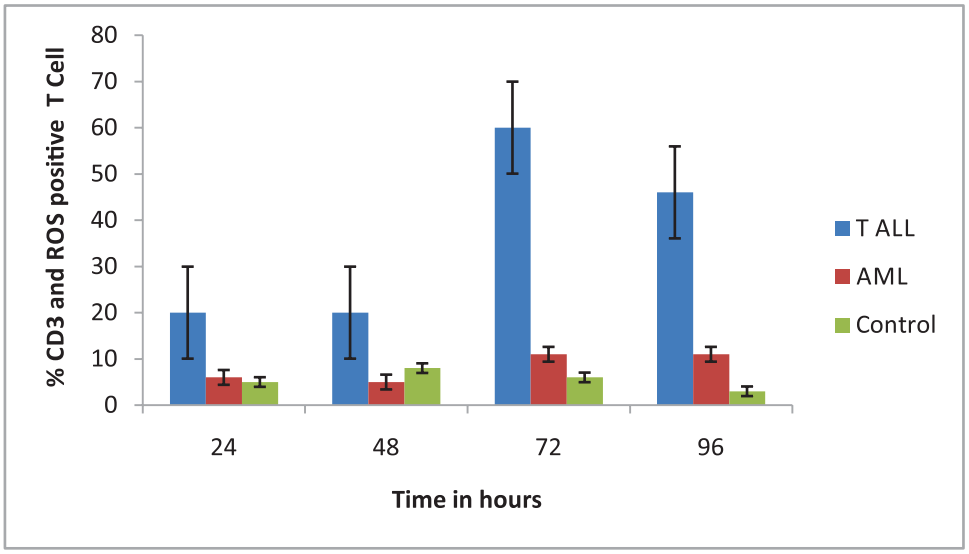


Fig. 2: Cellular redox state showing higher ROS level in CD3 positive cells in T-ALL samples as compared to AML samples.

Analysis of T-cell signalling molecules was done in PBMCs of 30 samples (15 T-ALL and 15 AML) and age-sex-matched controls. Samples were cultured in conditioned media for 96 hours and induced with optimized concentration of hydrogen peroxide. Expression of CD28, PD1 and CTLA4 was measured using three colour flowcytometry.

The T-cells of T –ALL samples were highly redox-sensitive but expression of CD28 showed no significant change during the 96 hour incubation period. However, expressions of PD1 and CTLA4 were found to be significantly modulated in T-ALL samples during the 96-hour incubation period. Expression of co-stimulatory molecule, CD28, in ROS +ve T-cells in acute leukemia (n=15) did not show significant kinetic change while expression of CTLA4 in ROS +ve T-cells (n=15) showed significant kinetic change in T-ALL and AML samples.

Redox-mediated interaction analysis between leukemic T-cells and NK cells will be done using CD56 antibody. This will be done by co-culturing T- and NK-cell subsets of patient samples. This may explain the role, if any, of NK cells in the pathogenesis of AL and its potential as a Redox-mediated targeted therapy.

7. Characterisation of host immune factors associated with progression of superficial TCC of bladder by microarray analysis

Scientific staff : Dr. Sunita Saxena, Dr. Usha Agrawal, Dr. Sujala Kapur, Dr. Saurabh Verma, Ms. Nitu Kumari

In collaboration with : Dr. N.K. Mohanty, Dr. Anup Kumar

Technical staff : Mr. Mohan Singh

Duration : 2009-12

Aims, Objectives & Background:

The morphologic spectrum of urinary bladder cancer is composed of PUNLMP (Papillary Urothelial Neoplasm of Unknown Malignant Potential), Non-muscle invasive Urothelial Cancer (NMIUC, previously known as Superficial TCC) and Muscle-Invasive Urothelial

Cancer (MIUC). NMIUC patients may progress to muscle invasive in 30% cases or recur in 70% cases. Considering that these patients are maintained with an immunotherapy regime of BCG to enhance their immune response to eliminate tumor cells it is found surprising that so many of these cancers recur. The objective of this study was to profile the local and systemic immune status of these patients to understand the reason for progression and recurrence. To achieve our objective, samples of tumor tissue, normal mucosa, blood and urine were collected from the patients enrolled in the study. Real-Time PCR was performed for profiling tumour cytokines/chemokines and NFκB pathway genes. Serum and urine cytokine concentrations for 27 analytes were performed by Multiplex assays.

Work done during the year:

Gene dysregulation in cytokine array

In both NMIUC and MIUC, an enhanced immune response was observed. In MIUC it was also seen that the patients had a significantly enhanced Th1 response (Fig. 1a)

Fig. 1a): Enhanced Th1 response in both NMIUC and MIUC patients with 8 significantly upregulated genes in MIUC and 2 in NMIUC compared to normal mucosa

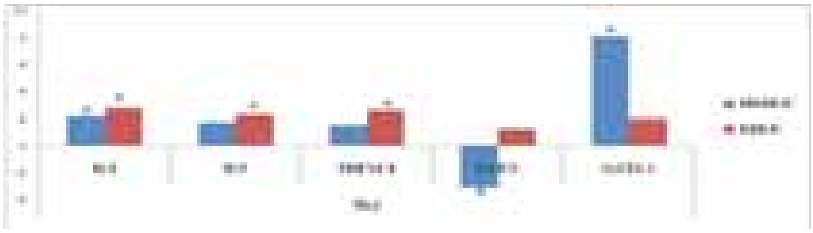


Fig. 1b): Enhanced Th2 response in both NMIUC and MIUC patients with 3 significantly upregulated genes in MIUC and 2 in NMIUC compared to normal mucosa. CCR3, a marker of invasion is found upregulated in MIUC compared to NMIUC ($p<0.05$)

Gene dysregulation in NFκB pathway

Gene expression of 84 genes of NFκB pathway showed dysregulation of 7 genes in urothelial cancer. Of these, 5 were found upregulated and 2 were downregulated.

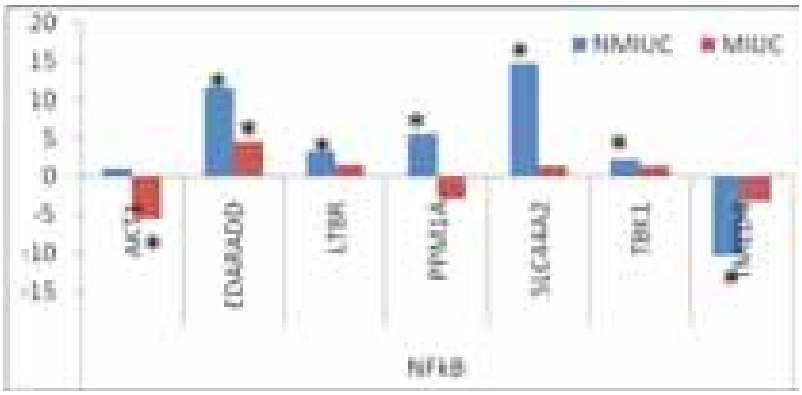


Fig. 2: NFκB gene dysregulation in Urothelial Cancers

Candidate Gene Analysis

RT-PCR by Taqman chemistry was done for IFN-gamma, IL-2, IL-4 and IL-10.

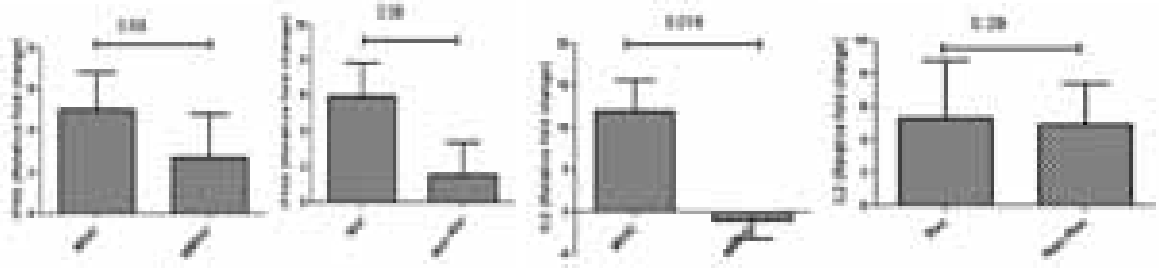


Fig. 3: IFN-G and IL-2 was upregulated in both MIUC and recurrent cases of Urothelial Cancers

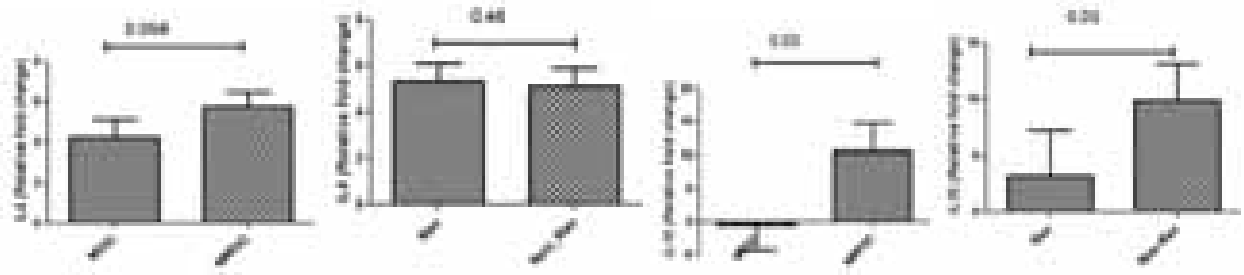


Fig. 4: IL-4 was upregulated in Urothelial Cancers but IL10 was significantly downregulated in both MIUC and recurrent cases of Urothelial Cancers

Cytokine assay by multiplex

Cytokine profile in urine

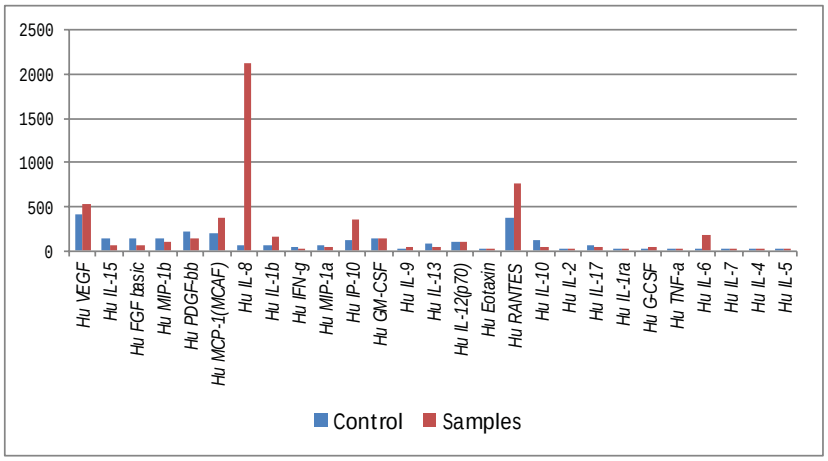


Fig. 5: Urinary cytokine concentrations in Urothelial Cancer Patients and Controls

Urine samples from patients showed significant increase in concentration of IL-1b and IL-9 and decrease in concentration of Eotaxin (CCL11) and IL-2 in patients. Other cytokines which were increased included VEGF, IL-8, IP-10, RANTES and IL-6 (Fig. 5).

Cytokine profile in serum

Serum of patients of urothelial cancer showed significant decrease in concentration of IL-1b, IL-9, Eotaxin (CCL11) and IL-2. IP-10 was found to be significantly increased in patients serum (Fig. 6).

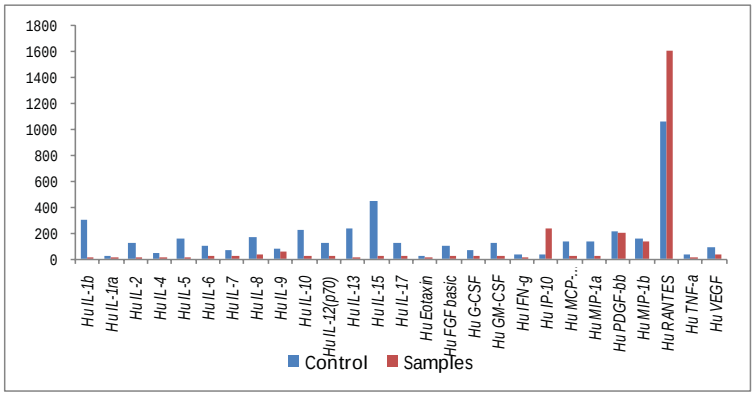


Fig. 6: Serum cytokine concentrations in Urothelial Cancer Patients and Controls

Immunohistochemistry on Tissue Microarray

Immunohistochemistry was performed for TBX21, GATA3, PRKCE and SRC (Fig. 7) and revealed significantly increased expression in *MIUC* for TBX21 (78.6% in *MIUC* and 0.0% in *NMIUC*) and in NMIUC for PRKCE (88.4% in *NMIUC* and 57.1% in *MIUC*) indicating the role of these markers in invasion.

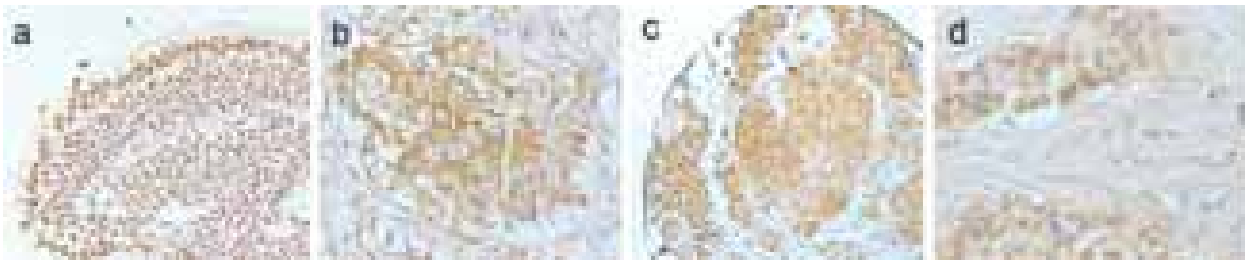


Fig. 7: Immunohistochemistry showing a) GATA 3 expression in non-muscle invasive bladder cancer b) TBX21 in invasive bladder cancer c) Cytoplasmic expression of SRC in invasive bladder cancer (d) cytoplasmic PRKCE expression in *MIUC*.

PATHOLOGY OF INFECTIOUS DISEASES

PATHOLOGY OF INFECTIOUS DISEASES

Leishmaniasis

1. Drug resistance in visceral leishmaniasis

Scientific staff : Dr. Poonam Salotra, Dr. Ruchi Singh, Ms. Vasundhra Bhandari, Mr. Deepak Kumar Deep, Mr. Uday Kishore P., Ms. Aditya Verma

In collaboration with : Dr. N. S. Negi, Dr. V. Ramesh

Duration : 2010-2014

Aims, Objectives & Background:

A. Miltefosine (MIL) resistance in visceral leishmaniasis:

Widespread resistance against Sodium antimony gluconate (SAG) has resulted in introduction of Miltefosine (MIL) as the first line drug in parts of Bihar, however, long half-life treatment poses threat of development of resistance. Reports of relapses following MIL treatment have surfaced already. Hence, it is essential to monitor the treatment efficacy and understand the mechanism of resistance towards MIL for effective control of VL. We earlier reported that expression pattern of LdMT/LdRos3 genes therefore, does not appear to be suitable marker for monitoring drug susceptibility in clinical *Leishmania* isolates. During the period under study, we analysed SNP's in MIL resistant and sensitive parasite and evaluated mRNA expression level of selected genes in clinical isolates of *L. donovani*.

Work done during the year

1. Analysis of SNP in LdMT and LdRos genes:

Both LdMT and LdRos genes were amplified from wild type parental *L.donovani* lines (MIL-S 1, MIL-S 2 and MIL-S 3) and MIL resistant lines (MIL-R1, MIL-R2 and MIL-R3) and sequenced. PCR amplification for LdMT was accomplished with the set of six primers

(Ldmt F1-R2, Ldmt F3-R4, Ldmt F5-R6) in three distinct fragments of size 1260 bp, 1400bp and 1193bp respectively. LdRos was amplified as full ORF of length 1098bp with a pair of primers i.e LdRos F1-R2. Amplicons were further sequenced on Automated Sequencer ABI 3730 and analyzed. Sequence analysis of LdMT and LdRos genes revealed the previously reported single nucleotide polymorphism, C1259→A resulting in substitution of Thr 420→Asn in the MIL resistant cell lines. Additionally a novel SNP, T 527→ A resulting in substitution of Val 176→ Asp and in LdMT gene of resistant cell lines was observed. However, no point mutations were detected in case of LdRos.

2. Expression analysis of selected genes in clinical isolates:

Based on our microarray results we selected genes which are involved in metabolic pathways and are highly modulated in experimental MIL resistant parasites compared to wild type *L. donovani* isolates. These genes like Tryparedoxin peroxidise (TRYP), and Trypanothione synthetase (TSH), are involved in increased antioxidant defence mechanism via elevated trypanothione metabolism and reduced oxidative phosphorylation, Lipase and Fatty acid elongase (FAE) which are known to be responsible for free fatty acid metabolism for generation of alternate energy source in MIL resistance parasites during drug pressure, and thus enhances the antioxidant defence mechanism in MIL-R, contributing to resistance.

We evaluated mRNA expression level of the above genes in pre- and post-treatment clinical isolates of VL, and relapse isolates of both VL and PKDL group by Real time PCR. We could not find any correlation with their mRNA expression level with that of microarray results.

3. Cloning and over-expression of selected genes:

Mitochondrial outer membrane protein, Tob55 (LinJ.29.1940) and Lipase precursor like protein (Lin.J.31.0870) were selected for cloning and over-expression studies. The open reading frame (ORF) of both Tob55 and Lipase precursor like protein were obtained from TriTryp DB genome database and their corresponding primers were designed by using primer 3 programming software.

Primers used for sequencing and overexpression studies:

1. Tob55ORF F- 5' ACTAGTATGACCGACACTACGCAACAGACGG 3'

2. Tob55ORFR-5'ACTAGT**CTACGCGTAGTCCGGCACGTCGTACGGGTA**GAACGAGGAACTGAATGACCAGACC 3'

3. Lipase ORF F- 5'ACTAGT ATGCGTCGCGTTCAGTCATGGACGTG 3'

4. Lipase ORF R-5'ACTAGT**CTACGCGTAGTCCGGCACGTCGTACGGGTA**AGCCCACGAAAGAACATTGCGAG 3'. The sequence underlined denotes the Spe1 site and sequence in reverse primer in bold represents HA tag.

The desired genes were PCR amplified from genomic DNA isolated from MIL sensitive and resistant *Leishmania* parasite using gene specific forward and reverse primer with HA tag. The amplicon size obtained for Tob55 and Lipase precursor like protein were of 1.4kb and 740bp respectively (Fig. 1).

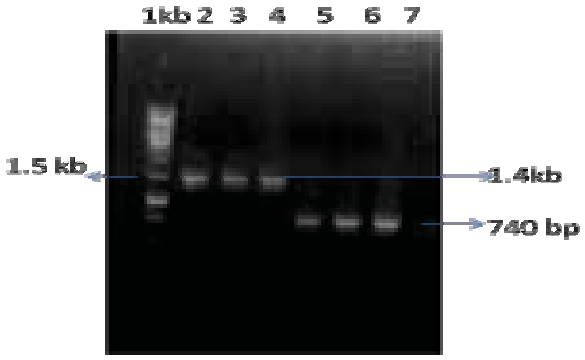


Fig. 1: PCR amplification of Tob55 and Lipase precursor like protein. Lane1 denotes 1kb ladder, lane 2 and 3 represents Tob 55 amplicon from wild type sensitive *L. donovani*, lane 4 represents Tob55 amplicon from LdM30 MIL resistant parasites. Lane 5 and 6 represents lipase precursor amplicon from wild type *L. donovani* and lane 7 represents from LdM30 MIL resistant parasites.

Cloning and sequence analysis:

The PCR products were gel purified and ligated into p-GEMT easy vector and transformed into *E. coli* DH5α strain. Selected plasmids were sequenced and comparison was performed

using Clustal W alignment program. Tob55 gene sequence of both sensitive and resistant *L. donovani* parasites showed 98% and 100% similarity with Tob55 gene sequence of *L. infantum* (Figure 2A). Lipase precursor like protein gene sequence from both sensitive and resistant *L. donovani* parasites showed 99% similarity with gene sequence of *L. infantum* (Figure 2B). Synonymous mutation seen at 46th position where valine is replaced by alanine, which does not seem to affect the protein properties.

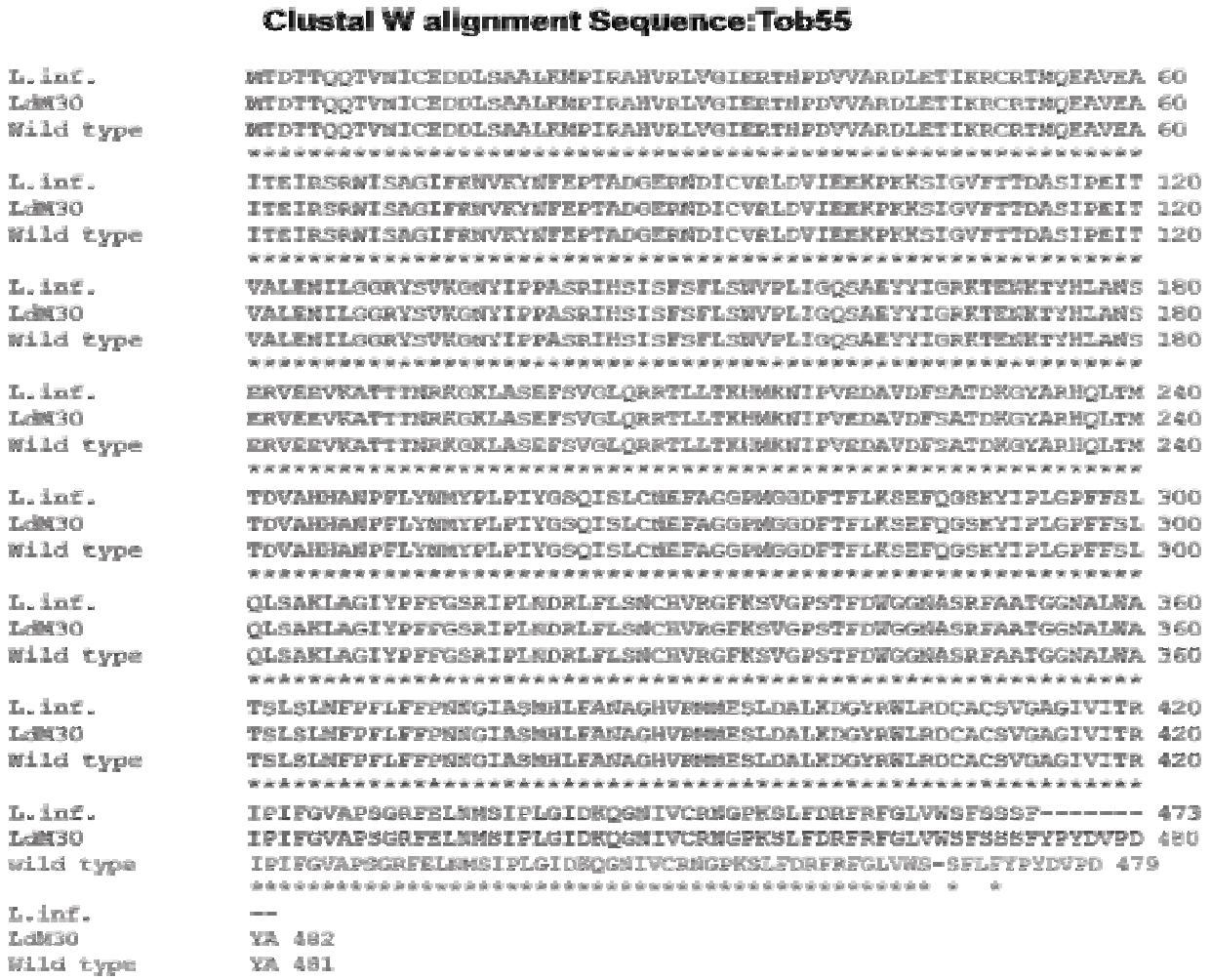


Fig. 2A: Alignment score of wild type *L. donovani* (sensitive) and LdM30 (resistant) showing 98% and 100% sequence similarity respectively with *L. infantum* Tob55 sequence.

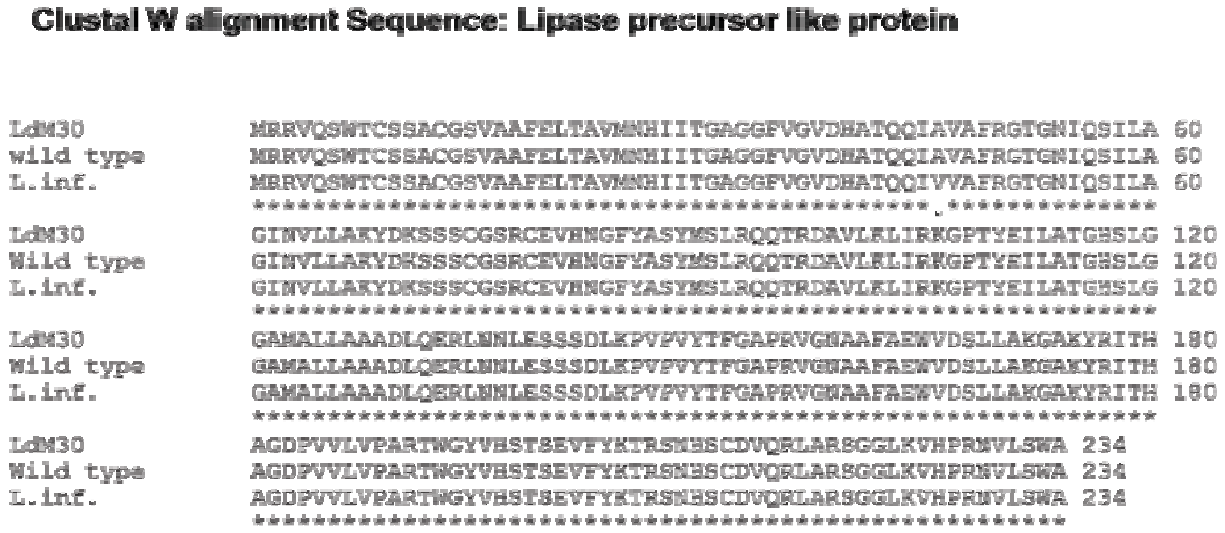


Fig. 2B: Lipase precursor sequence from both wild type *L. donovani* (sensitive) and LdM30 (resistant) showed 99 percent similarity with *L. infantum* lipase precursor sequence.

Subcloning into pKSNEO expression vector:

Further, Lipase precursor like protein was subcloned into pKSNEO expression vector and transformed into *E. coli* DH5 α strain. Recombinant clones were selected on LB ampicillin plate. Recombinant clones were screened by PCR with gene specific primer (Fig. 3A). A few recombinant transformant clones were grown in LB broth for plasmid preparation, which were further checked for right size of insert by restriction digestion with *Spe1* (Fig. 3B) giving fallout of 740 bp. Further, orientation was confirmed by restriction digestion with Nco1 and Not1.

Screening of pKSNEO Lipase positive clone

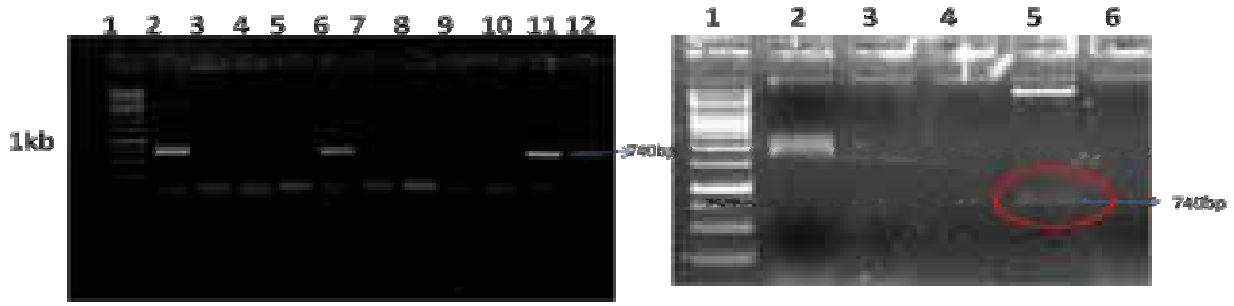


Fig. 3: A. Lane 1 shows 1kb ladder. Screening of recombinant clones pKSNEO Lipase by colony PCR. B: Lane1 shows 1kb ladder.Spe1 digestion of PCR positive pKSNEO Lipase clones.

Transfection into *L. donovani* :

pKSNEO Lipase clone with right orientation was transfected into sensitive *L. donovani* parasites by electroporation. Transfected parasites are being adapted on G418 selection medium for further analysis.

Future Plan:

Genomic microarray study will be performed in clinical isolates comprising pre-treatment, post-treatment, and relapse group. Based on microarray results a comparative transcriptomic profiling will be done between experimental MIL resistance parasites and clinically relapse isolates to identifying the genes associated with miltefosine resistance mechanism in clinical isolates. The transfected parasites over-expressing the desired gene will be confirmed by Western blotting using anti-HA antibody and will be assessed for growth, differentiation and drug susceptibility.

B. Paromomycin (PMM) resistance in visceral leishmaniasis:

Paromomycin (PMM) is a promising new antileishmanial agent registered for visceral leishmaniasis (VL) treatment in 2006, which is also effective in combination therapy. Hence, it is essential to understand the mechanism of resistance towards PMM for effective control of VL. During the period under study, we have utilized lab generated PMM-R strain to analyse the expression of ATP binding cassette (ABC) transporter genes. Further, the

tolerance of PMM resistant parasite towards host leishmanicidal/defence mechanisms including nitrosative, oxidative and complement mediated stresses was investigated.

1. Expression analysis of genes implicated in drug resistance:

In view of the role of ABC gene family in resistance of *Leishmania*, here we analyzed the expression of MDR1 and MRPA genes, to evaluate their role in PMM resistance. Marked increase in MDR1 (6.83±3.01 fold) and MRPA (11.47±0.22 fold) expression level were observed in PMM-R in comparison with PMM-S parasite (Fig. 4). Protein phosphatase 2A, was also found to be 4.47±0.71 fold up regulated in PMM-R parasite which has a suggestive role in phosphorylation of the transporter genes and thereby activating the expression of these transporters.

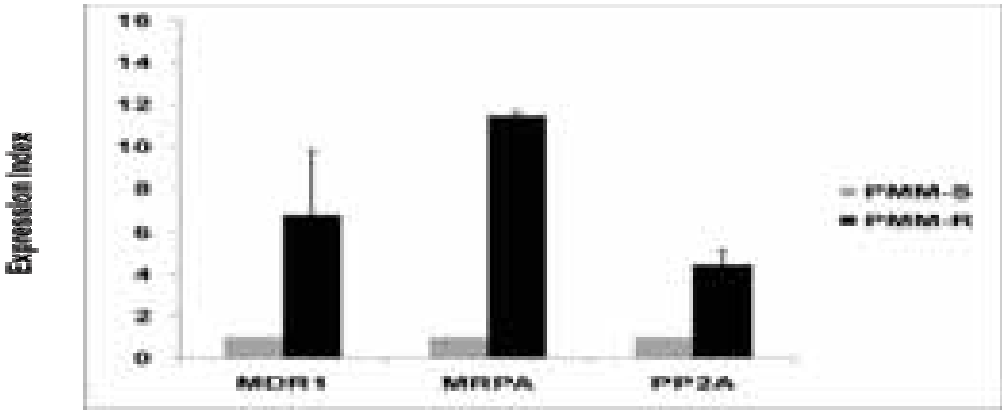


Fig. 4: **Expression of MDR1, MRPA and PP2A in PMM-R isolate.** Real-time PCR expression analysis of *L. donovani* ABC transporter (MDR1 & MRPA) and protein phosphatase 2A gene (PP2A) was performed using GAPDH and CBS as internal control. Graph shows the expression index, defined as the ratio of gene expression relative to that of PMM-S. Data represent mean±SD of the results of three independent experiments.

2. Innate susceptibility against host leishmanicidal activity:

PMM-R parasites exhibit increased tolerance to nitrosative stress at both promastigote and amastigote stages:

We compared the response of the PMM-R and PMM-S strains to nitrosative and oxidative stress. PMM-R promastigotes were significantly more tolerant to SIN-1 ($NO+O_2$ donor) and

SNAP (NO donor) as compared to PMM-S. The resistant parasite was twice more tolerant (P=0.045) to NO+O_2 stress with mean IC_{50} of 646.14±36.57µM, compared to PMM-S strain (mean IC_{50}±SD= 328±4.24µM, Fig. 5A). The PMM-R parasite exhibited significant decrease in susceptibility (9 fold, P=0.0048) against NO stress in comparison to PMM-S parasite, with respective mean IC_{50}±SD as 476.58±1.06µM and 52.95±3.48µM (Fig. 5B). In contrast, the susceptibility of PMM-R and PMM-S promastigotes towards H_2O_2 was similar with mean IC_{50}, 176.03±12.27µM and 158.57±9.10µM respectively (Fig. 5C).

Further, response of PMM-R to nitrosative stress (NO) was evaluated at amastigote stage, the PMM-R parasite showed significantly low susceptibility (P=0.0028) towards nitrosative stress (NO) at amastigote stage compared to PMM-S with mean IC_{50}±SD, 88.12±5.17µM and 31.55±5.35µM respectively (Fig. 5D).

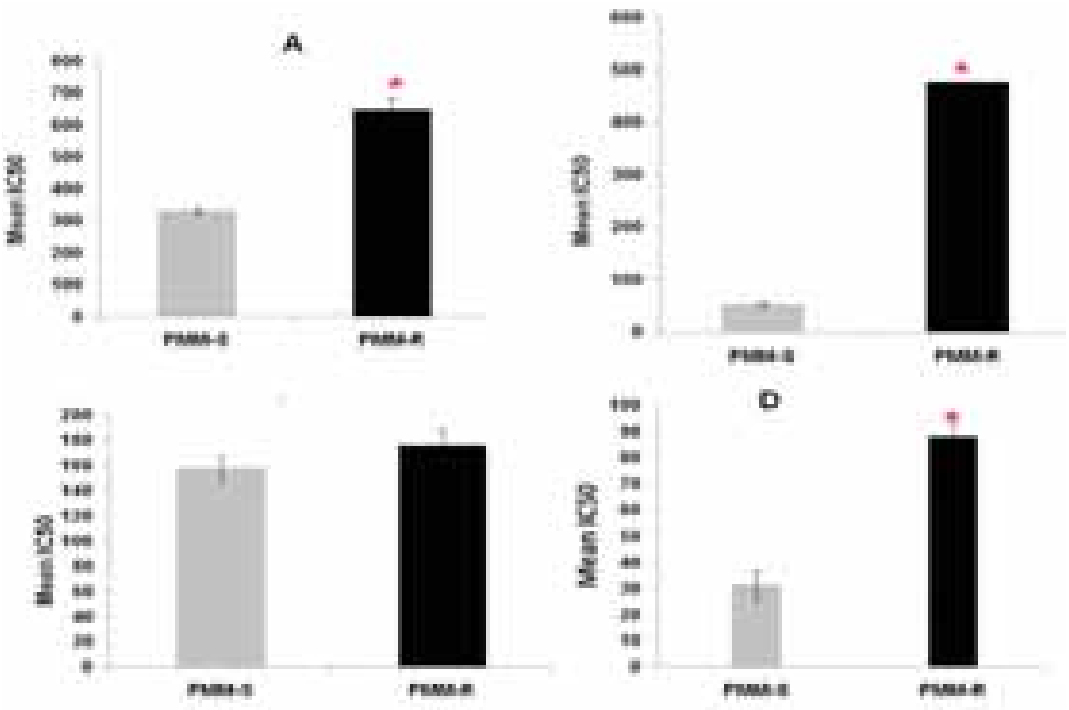


Fig. 5: ***In vitro* susceptibility of PMM-R isolate towards nitrosative and oxidative stress.** Susceptibility of PMM-R and PMM-S was determined towards A) 3-morpholinosydnonimine (SIN-1), B) S-nitroso-N-acetyl-DL-penicillamine (SNAP) and C) Hydrogen peroxide (H_2O_2) at promastigote stage using resazurin assay. D) Susceptibility oat intracellular amastigote stage was determined after incubation with different concentrations of SNAP using peritoneal macrophages. Each bar represents mean IC_{50}± SD of two independent experiments and the asterisk mark (*) represents P<0.05.

Increased tolerance of PMM-R to IFN-Ý/LPS mediated leishmanicidal activity:

Mean % infectivity of PMM-S and PMM-R isolates was determined in macrophages treated with different concentrations of IFN-Ý/ LPS (U/ml IFN γ: ng/ml LPS, 0.1, 1, 5, 10, 50). We observed significantly ($P<0.05$) higher parasite burden in macrophages infected with PMM-R isolate at concentration above 5U/ml IFN: 5ng/ml LPS compared to PMM-S parasite, indicative of resistance of PMM-R isolate to macrophage killing mechanisms (Fig. 6).

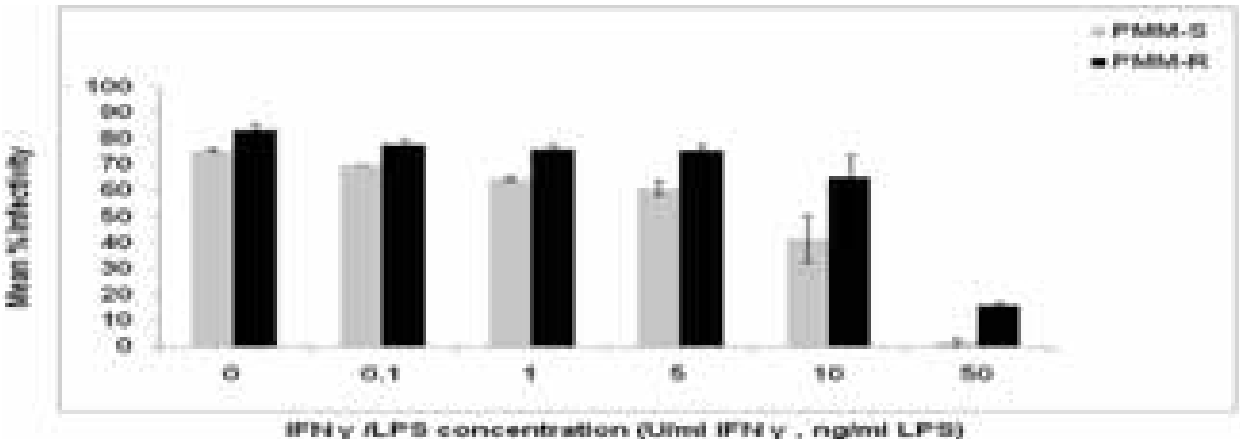


Fig. 6: Parasite burden of PMM-R in macrophages treated with IFN-Ý/LPS. Mean % infectivity was calculated by infecting peritoneal macrophages with PMM-R and PMM-S promastigotes and treating them with different concentrations of IFN-Ý/LPS or untreated (control). After 48h, the percentage of cells infected was determined. The graph represents mean% infectivity ± SD of two independent experiments in triplicate.

Increased resistance of PMM-R parasite to complement mediated stress:

The PMM-R isolate showed a significantly high ($P=0.005$) tolerance to complement mediated lysis (mean $IC_{50}=4.02\pm0.26\%$) when compared to PMM-S (mean $IC_{50}=2.08\pm0.16\%$) indicating a higher survival capability since complement lysis is one of the first immune mechanisms encountered by promastigotes upon inoculation into the vertebrate host, thus contributing to the fitness of PMM-R parasite (Fig. 7).

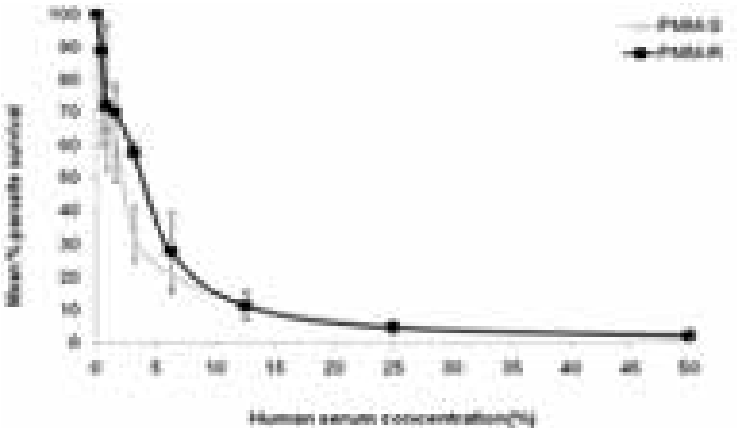


Fig. 7: Resistance to complement mediated lysis by PMM-R parasites. Parasites were incubated with fresh human serum and percentage parasite survival was measured at different serum concentrations. The assay was performed thrice in triplicate. Values given are mean percentage survival ± SD.

Increased host IL-10 levels elicited by PMM-R:

IL-10 plays an important role in promoting parasite survival in the host. We assessed IL-10 level in supernatants of macrophages infected with PMM-R and PMM-S isolates and found significantly high IL-10 production (P=0.03) in the host cells, in response to PMM-R as compared to PMM-S parasite (Fig. 8).

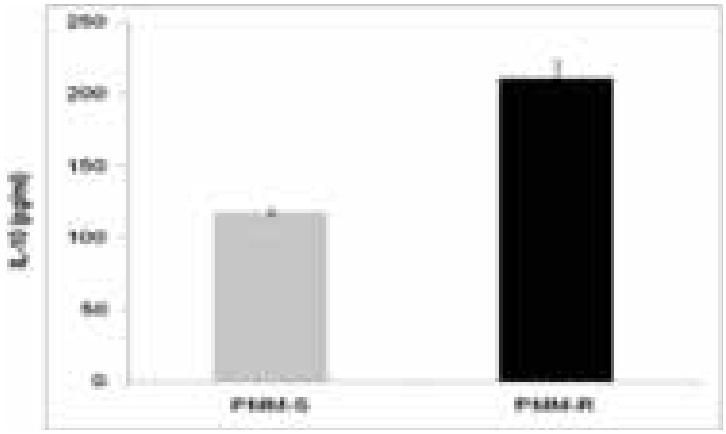


Fig. 8: Estimation of IL-10 levels elicited by PMM-R infected macrophages. IL-10 levels in the supernatant of macrophages infected with PMM-R or PMM-S parasites were quantified by Elisa. The graph represents mean ± SD of IL-10 produced in three independent experiments, each in triplicate.

Future Plan:

Comparative transcriptome profiling of PMM resistant *L.donovani* isolate using genomic microarray technology to identify genes showing altered expression in PMM-R isolate followed by functional characterization of the selected gene (s).

2. Studies on immunological responses to recombinant PSA (Parasite Surface Antigen) - A potential vaccine candidate

Scientific staff : Dr. Poonam Salotra, Mr. Himanshu Kaushal
In collaboration with : Dr. V. Ramesh, Dr. N. S. Negi, SJH, New Delhi
Duration : 2010-13

Aims, Objectives & Background:

Different *Leishmania* antigen preparations have been studied as vaccine candidates with different degrees of success, mainly in mice models. Recently, an effective canine vaccine, CaniLeish was develpoed in France. It was based on LiESAp, a crude excreted- secreted antigen obtained from promastigote culture supernatant of *Leishmania infantum* and formulated with muramyl dipeptide. Native soluble *L. infantum* Parasite Surface Antigen (nsLiPSA) has been identified as the active constituent of LiESAp that reproduces the observed protective immunity when used as a vaccine in dogs. The PSA is a naturally excreted secreted protein belonging to the family of Promastigote Surface Antigens and has been produced in a non-pathogenic *L. tarentolae* recombinant expression system at our collaborator's lab (Institut pour la Recherche et le Développement, Montpellier, France). As the nsLiPSA is present in all *Leishmania* species and showed 100% homology with PSA of *L. donovani*, therefore, we investigated immune responses to PSA in comparison with total soluble Leishmania antigen (TSLA), in order to evaluate its potential as immunoprophyactic antigen for human leishmaniasis.

Last year, we investigated *Leishmania*-specific cell mediated immune responses in term of their lymphoproliferative potential and the type of cytokines they generated upon

stimulation with PSA and TSLA *in vitro*. This year, we investigated *Leishmania*-specific cell mediated cytotoxicity based on the measure of granzyme B, upon stimulation of PBMCs with TSLA and PSA *in vitro*. In addition, we characterised cell mediated immunity based on the phenotype and proportion of the different peripheral blood lymphoid subsets in patients with active VL, PKDL and compared with individuals from healed VL and healthy controls.

Work done during the year:

Granzyme B analysis: in response to TSLA and PSA stimulation:

Granzyme B, a serine proteinase produced by the cytotoxic lymphocytes are able to induce rapid cellular death of the target by apoptosis. Here, granzyme B was measured in culture supernatant upon TSLA and PSA stimulation of PBMCs *in vitro*. Upon TSLA stimulation, 8/16 (50%) of PKDL individuals showed the measured values above cut-off, with group mean as (Mean±SD 254.26±361.05, $p < 0.05$) and was found significantly high compared to naïve group (Mean±SD 10.97±16.08). The granzyme B level in healed VL, with group mean±SD as 1453.67±1406.8, was found significantly high ($p < 0.001$) compared to naive group; with 11/12 (92%) individuals showed values above cut off. For active VL group, only 30% (3/10) VL individuals had granzyme B level above cut off, with group mean±SD as 56.53±68.95 and was found slightly high but comparable to naïve group (fig 1). Upon PSA stimulation, granzyme B production were found comparable among the different groups. However, the highest granzyme B level was observed in healed VL group (mean 84±156) followed by PKDL group (mean 76±131), naive group (mean 24± 43) and least in VL group (mean 2.7± 5.4) (Fig.9).

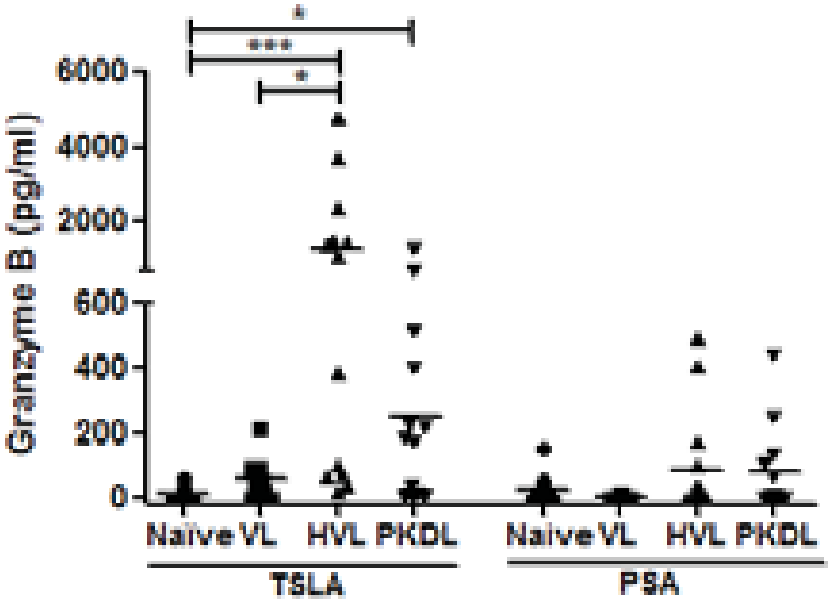


Fig. 9: *Leishmania*-specific cell mediated cytotoxic response in VL, PKDL, healed VL and healthy controls. PBMCs were isolated and incubated with TSLA (10µg/ml) and PSA (10µg/ml) for 120 hrs. Granzyme B level was analyzed in the supernatant of PKDL (n=16), VL (n=10), healed VL (n=12) and healthy individuals (n=16) upon TSLA and PSA stimulation, using CBA. Horizontal lines indicate mean values. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Correlation of granzyme B level with stimulation index:

The secretion of granzyme B upon TSLA stimulation was analysed in PKDL, active VL, healed VL and healthy controls with their respective stimulation index. In healed VL group, the level of granzyme B was found strongly correlated with their stimulation index with correlation coefficient r = 0.836 (p = 0.0007) (Fig. 10) whereas no correlation was observed in active VL, PKDL and healthy controls groups. This result provides evidence that *Leishmania*-specific cell mediated cytotoxicity is part of the acquired immune response developed in pre-exposed individuals with *Leishmania* parasite.

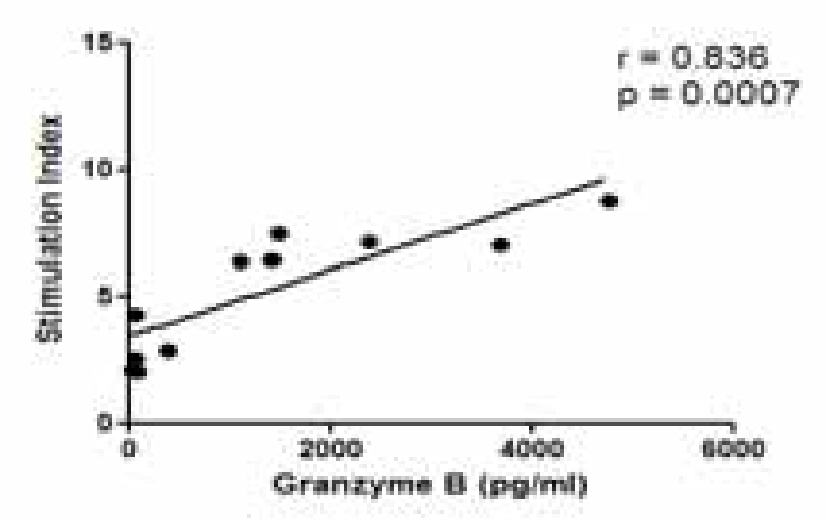


Fig. 10: Comparative assessment of the level of granzyme B and stimulation index upon TSLA stimulation of PBMCs of healed VL (n = 12) group. The levels of granzyme B in culture supernatant upon TSLA stimulation were measured by CBA and stimulation index was determined by ELISA. Diagonal line represents the best fit line.

Characterisation of cell mediated immunity based on the phenotype and proportion of the different peripheral blood lymphoid subsets:

Immunophenotyping of patients of active VL, PKDL and individuals of healed and healthy controls were done to identify T-cells ($CD3^+$), helper T cells ($CD3^+CD4^+$), cytotoxic T cells ($CD3^+CD8^+$), NK cells ($CD3^-CD16^+CD56^+$) and B cells ($CD3^-$ $CD19^+$). In patients with active VL, NK cells (Mean ± SD, 6.00±3.76) were significantly lower compared to healthy controls (mean ± SD, 13.48±5.24, $p < 0.001$) and PKDL (mean ± SD, 12.43±6.92, $p < 0.05$) which were significantly raised after post VL treatment in healed VL (mean ± SD, 12.13±4.14, $p < 0.05$). The percentage of $CD3^+$ T cells were significantly low in active VL (mean ± SD, 43.33±18.13, $P< 0.05$) compared to healed VL (mean ± SD, 60.74±9.31) and PKDL (mean ± SD, 62.16±9.82). Similarly, the percentage of $CD4^+$ cells of total $CD3^+$ cells were also found significantly low in active VL (mean ± SD, 37.16±8.69, $p < 0.01$) compared to healed VL (mean ± SD, 54.59±10.82), PKDL (mean ± SD, 53.29±9.75) and healthy controls (mean ± SD, 51.89±6.42) whereas $CD8^+$ cells of total $CD3^+$ cells were found significantly high in active VL group (mean ± SD, 52.79±7.43, $p < 0.01$) compared to healed VL (mean

± SD, 36.45±9.79) and healthy control (mean ± SD, 37.67±5.06). The percentage of B lymphocytes of VL, cured VL, and PKDL were comparable to healthy controls. The $CD4^+$/ $CD8^+$ T cell ratio was found significantly low for VL (mean ± SD, 0.69±0.20, $p < 0.001$) compared to healed VL (mean ± SD, 1.61±0.81), PKDL (mean ± SD, 1.48±0.63) and healthy controls (mean ± SD, 1.42±0.36) (Fig. 11), Table 1.

Table 1: Cell surface marker analysis in patients with active VL, PKDL, healed VL and healthy control

Cell surface marker analysis				
Surface marker	Naïve (n=22)	Active VL (n=16)	Healed VL (n=20)	PKDL (n=20)
% of gated lymphocytes				
CD3+	53.68±10.67	43.53±18.13	60.74±9.31	62.16±9.82
CD45+	15.8±4.3	10.37±7.78	15.66±6.41	15.22±7.67
% of gated CD45+ lymphocytes				
CD3-CD19+	12.21±3.8	11.28±4.83	12.68±5.75	13.26±5.54
CD3-CD16+CD56+	13.48±5.2	6.06±3.76	12.13±4.14	12.43±6.92
% of gated CD3+ lymphocytes				
CD4+	51.89±6.42	37.16±8.69	54.59±10.82	53.28±9.74
CD8+	37.67±5.06	52.79±7.43	36.43±9.79	39.31±8.69
CD4+/CD8+	1.42±0.36	0.69±0.20	1.61±0.81	1.48±0.63

Note: Data are Mean±SD. Whole blood was stained with surface-marker antibodies, and cells were acquired on a flow cytometer. Lymphocytes were gated on the basis of forward and side scatter.

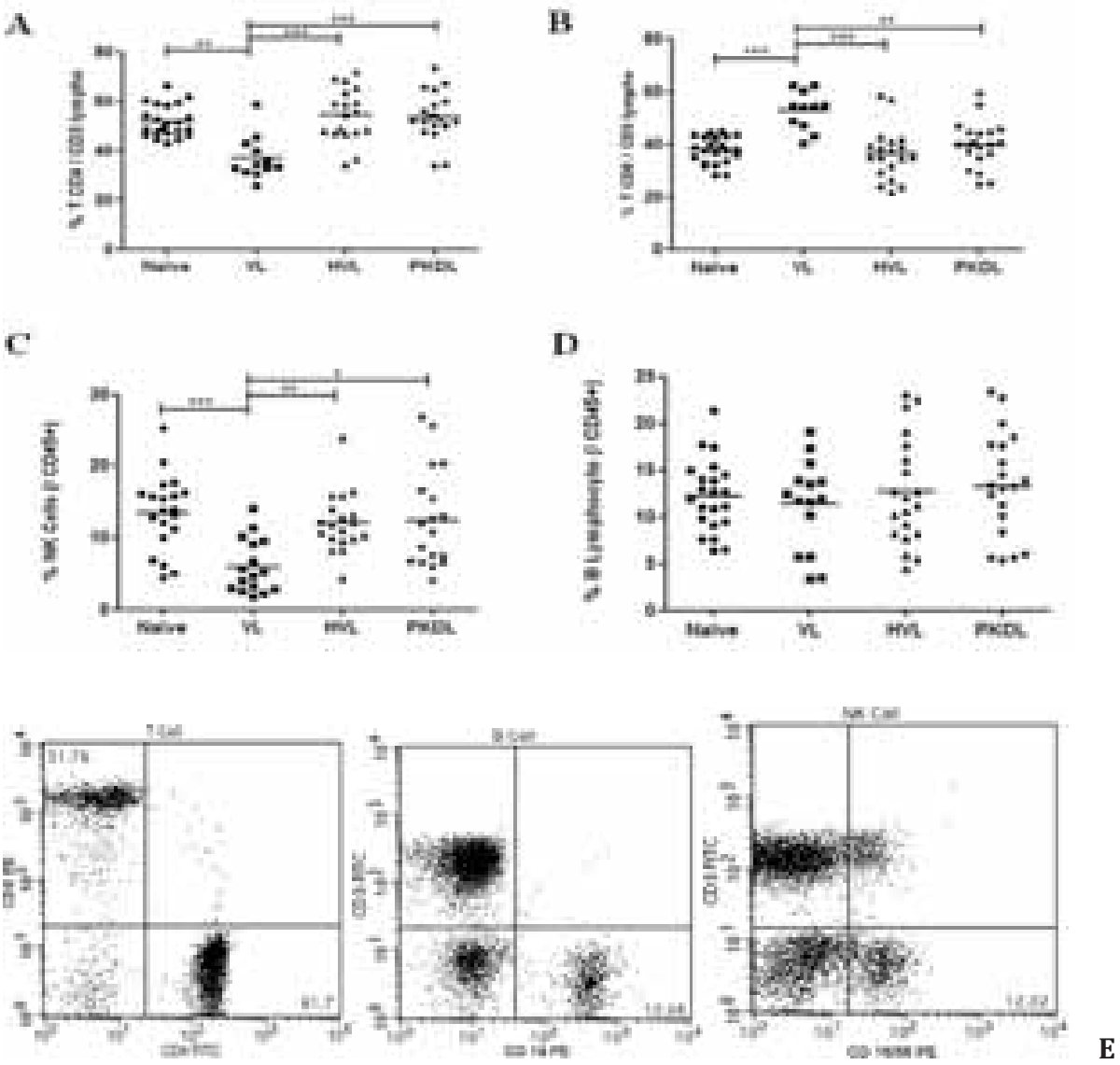


Fig. 11: Phenotype and proportion of the different peripheral blood lymphoid subsets in active VL (n=16), PKDL (n=20), healed VL (n=20) and healthy volunteers (n=22). PBMCs were surface-stained with Fluorochrome-conjugated antibodies to CD3, CD4, CD8, CD19, CD56, and CD16 along with appropriate isotype controls and assessed on the gated lymphocytes fraction on BD FACSCalibur flow cytometer, using BD CellQuest Pro software. Percentage of (A) T helper ($CD3^+CD4^+$), (B) T cytotoxic ($CD3^+CD8^+$) was gated on total $CD3^+$ cells whereas (C) NK ($CD45^+CD16^+/56^+$) and (D) B cells ($CD45^+CD19^+$) was gated on total $CD45^+$ cells. (E) Data showing one representative of a healthy volunteer. Data were analyzed between groups by the nonparametric Kruskal-Wallis test followed by the post hoc Dunn multiple comparison test. $p < 0.05$ is considered statistically significant. *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3. Development of live attenuated vaccine candidates for leishmaniasis

Scientific staff : Dr. Poonam Salotra, Mr. Kumar Avishek, Mr. Uday Kishore P., Ms. Aditya Verma

In collaboration with : Dr. Angamuthu Selvapandiyan, Dr. Hira Nakhasi, Dr. V. Ramesh, Dr. N. S. Negi

Duration : 2011-14

Aims, Objectives & Background:

Currently, only treatment option available for leishmaniasis is chemotherapeutic which is costly, limited and associated with high relapse and resistance rates. Despite substantial effort there is no licensed vaccine against human leishmaniasis. Parasite persistence may be important for effective protective response and could be achieved by immunization with live attenuated parasite strain with known irreversible gene defect. Live attenuated *Leishmania* parasite, generated by deletion of Centrin 1 and p27 gene have been found to be safe and protective in mice, hamster and dog model. This study aims to evaluate immune responses generated by Centrin knock out (KO) and p27 KO live attenuated *Leishmania* parasite in comparison to the wild type in human PBMCs. Study also aims to generate new live attenuated *Leishmania* parasite by targeted gene deletion of amastigote specific genes.

Work done during the year:

A. Evaluation of immune responses generated by Centrin KO and p27 KO in human PBMCs:

Cytokine ELISA was performed in the supernatant of parasite infected PBMCs of Healed visceral leishmaniasis (HVL) cases (n=6), Post kala-azar dermal leishmaniasis (PKDL) cases (n=6) and healthy individuals (n=6). Evaluation of Th1, Th2, and Th17 responses was done in culture supernatant of PBMCs from these 3 groups of subjects. Total eight cytokines, representative of Th1/Th2/Th17 response (IL-2, IL-4, IL-6, IL-10, IL-12, IL-

17, IFN-γ and TNF-α) were studied. Mean cytokine concentration ± SEM (pg/ml) was calculated for each cytokine. Statistical significance was determined by Mann-Whitney U test and *P*- values <0.05 were considered significant.

Th1 cytokines response:

Th1 response provides protection and prevents disease progression. Th1 response was evaluated by estimating four cytokines (IL-2, IL-12, TNF-α and IFN-γ) that favor Th1 response.

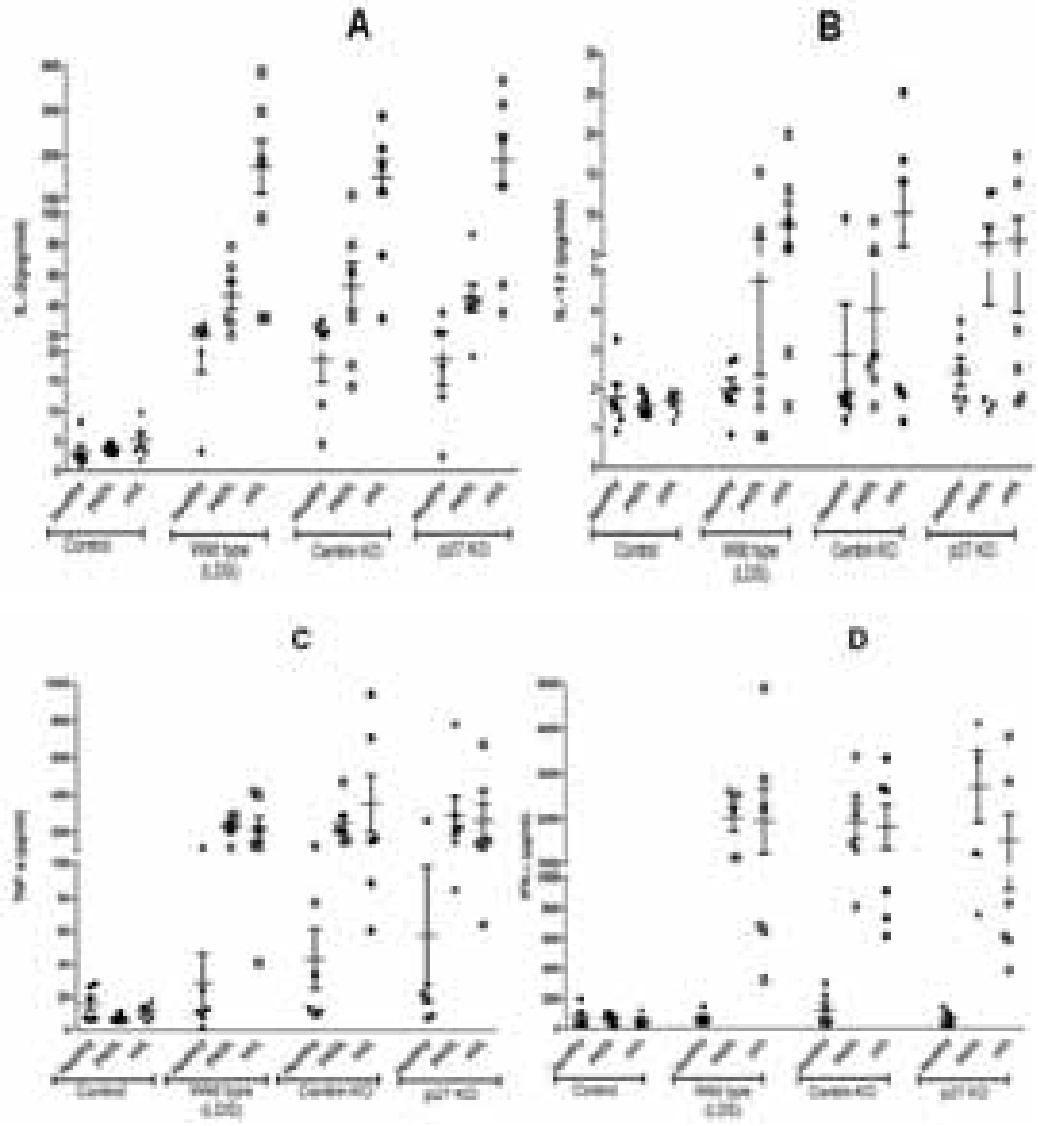


Fig.12: Th1 cytokines level in supernatant of peripheral blood mononuclear cells (PBMCs) from Healthy (n=6), PKDL (n=6) and HVL(n=6) patients in response to Wild type, Centrin KO and p27 KO parasite. (A) IL-2, (B) IL-12, (C) TNF-alpha and (D) IFN-γ. Data are given in Mean±SEM (pg/ml).

Results showed an increase in levels of IL-2, TNF-α and IFN-γ production by PBMCs after infection with wild type (LDS), Centrin KO and p27 KO parasite in comparison to control uninfected PBMCs. In case of IL-2; healthy, PKDL and HVL, all three groups showed significant stimulation (Fig. 12A) in comparison to control uninfected cells after exposure with Centrin KO, p27 KO as well as for wild type. However, TNF-α and IFN-γ showed significant stimulation only in HVL and PKDL group (Fig. 12 C&D). Further, stimulation of IFN-γ was much higher compared to IL-2 and TNF-α. The results also showed that production of IL-12 was marginally but significantly induced only in the HVL case compared to other groups control only with wild type and Centrin KO (Fig. 12 B).

Th2 and Th17 cytokine response:

Th2 response helps in progression of leishmaniasis by deactivation of macrophage while Th17 response has been found to provide resistance to it. Three Th2 types (IL-4, IL-6, IL-10) and one Th17 type (IL-17) cytokines were studied.

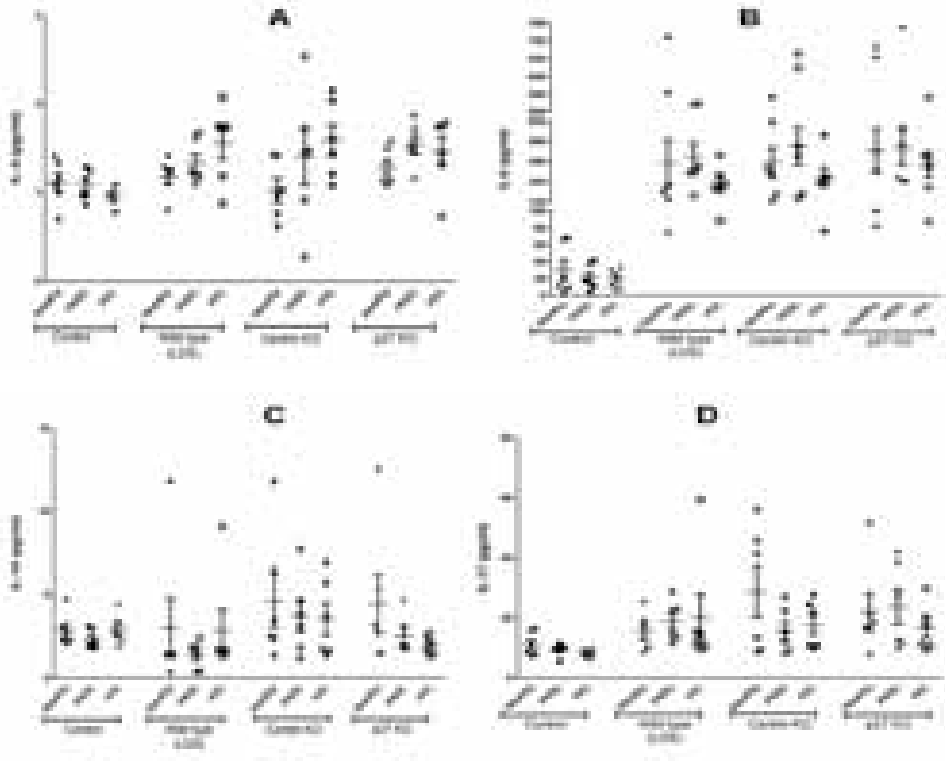


Fig. 13: Th2 and Th17 cytokine level in supernatants of peripheral blood mononuclear cells (PBMCs) from Healthy (n=6), PKDL (n=6) and HVL(n=6) patients in response to Wild type, Centrin KO and p 27 KO parasite. (A) IL-4, (B) IL-6, (C) IL-10 and (D) IL-17. Data are given in Mean±SEM (pg/ml).

Results showed no significant stimulation of IL-4 and IL-10 after infection with wild type, Centrin KO and p27 KO in any of the three groups. However, IL-6 showed significant stimulation after infection with wild type, centrin KO and p27 KO in all the three groups (Fig. 13). Further, the stimulation of IL-6 was comparable among wild type and knock out parasites. IL-17 also showed marginal but significant stimulation after infection. Wild type, Centrin KO and p27 KO showed comparable level of stimulation with no significant difference for each of the cytokines.

B. Development of new live attenuated vaccine candidates by deletion of amastigote specific genes:

In *Leishmania* many genes have been reported to be differentially expressed between promastigotes and axenic amastigotes stages. Based on the previous studies, A1 gene has been selected which is preferentially expressed at the amastigote stage of the parasite and appears to be a promising vaccine candidate. To understand the role of A1 gene in *Leishmania*, generation of gene knock out and over expression construct is ongoing.

(i) Construction of DNA for Targeted Gene Deletion of A1 gene:

5' and 3' flanking regions of A1 gene was PCR amplified by using genomic DNA of *L. donovani* and cloned into pGEMT – Easy TA cloning vector. Positive clones were screened by colony PCR and restriction digestion (Fig. 14). Clones found to be positive for 5' and 3' flanking regions of A1 were sequenced.

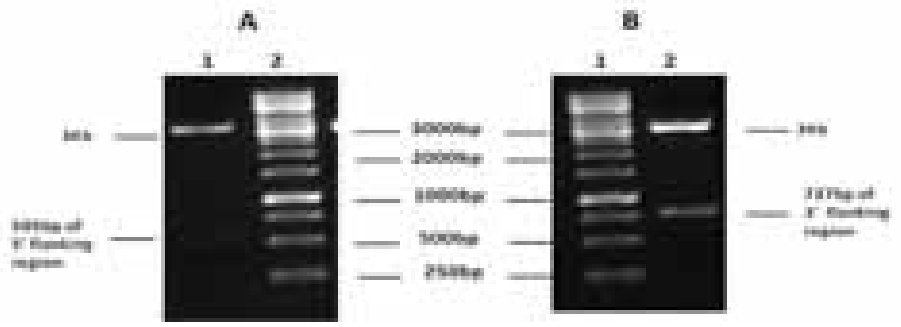


Fig. 14 (A): Bgl2-Sal1 digestion of pGEMT containing 5' flanking region insert; Lane 1: 3kb vector back bone and 505 bp of 5' flanking region; Lane 2: 1kb ladder. (B) SpeI- KpnI digestion of pGEMT containing 3' flanking region insert; Lane 1: 1kb ladder; Lane 2: 3kb vector back bone and 737 bp of 3' flanking region.

Sequence confirmed clones were digested to get 5' and 3' flanking region of A1 and for further cloning into A1 neo knock out vector (vector containing neomycin resistance gene and used to generate A1 knockout construct). Clones were confirmed for the presence of correct insert by restriction digestion (Fig. 15).

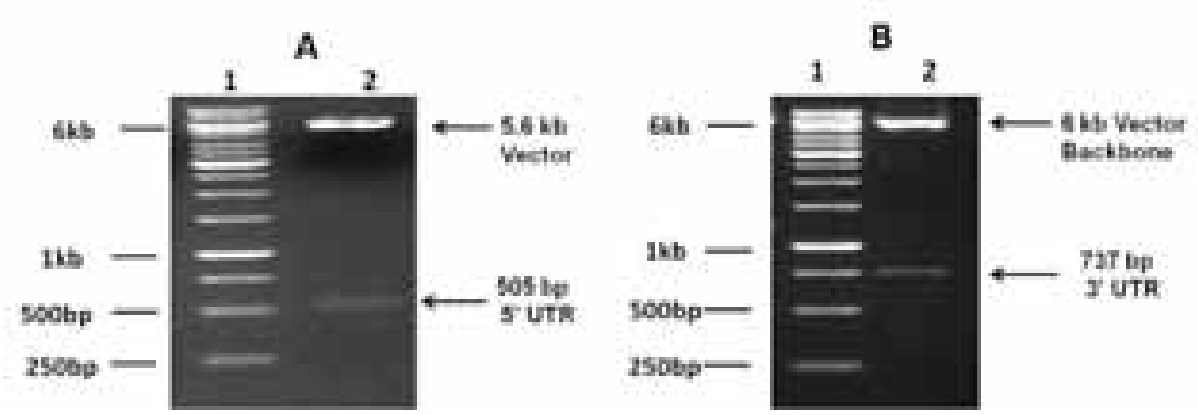


Fig.15: (A): *Swa1-Sal1* digestion of A1 neo KO vector containing 5' flanking region insert; Lane 1- 1 Kb ladder ; Lane 2- 5.6 kb vector back bone and 505 bp of 5' flanking region. (B): *SpeI-KpnI* digestion of A1 neo KO vector containing 3' flanking region insert; Lane1- 1 kb ladder, Lane 2- 6kb vctor back bone and 737 bp of 3' flanking region.

Recombinant A1 neo knockout vector, containing both 5' and 3' region was digested to get the fragment of neomycin flanked with the 5' and 3' flanking sequences of A1 (Fig16). This construct will be used for transfection to disrupt A1 gene in *L. donovani*.

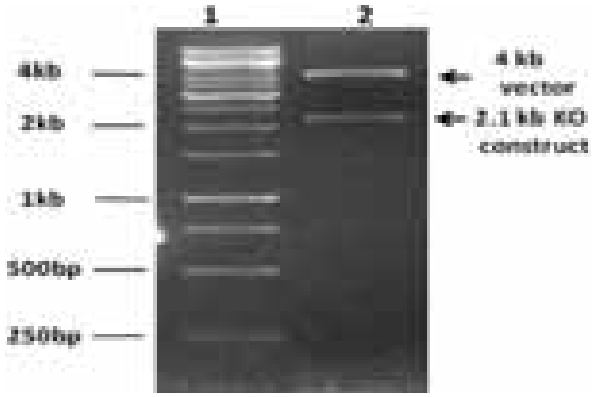


Fig.16: *SwaI-KpnI* digestion of A1 neo KO vector; Lane 1: 1 kb ladder; Lane 2: 4 kb vector backbone and 2.1 kb KO construct.

(ii) DNA construct for overexpression:

The DNA encoding full length of A1 gene (528bp) was PCR amplified from *L.donovani genomic* DNA and cloned into pGEMT – Easy TA cloning vector. Positive clones were screened by colony PCR and restriction digestion (8). Fidelity of the presence of right insert was confirmed by nucleotide sequencing. Sequence confirmed pGEMT clone was digested to release A1 insert and further cloned it into *Leishmania* expression plasmid pKSNeo (Figure 9).

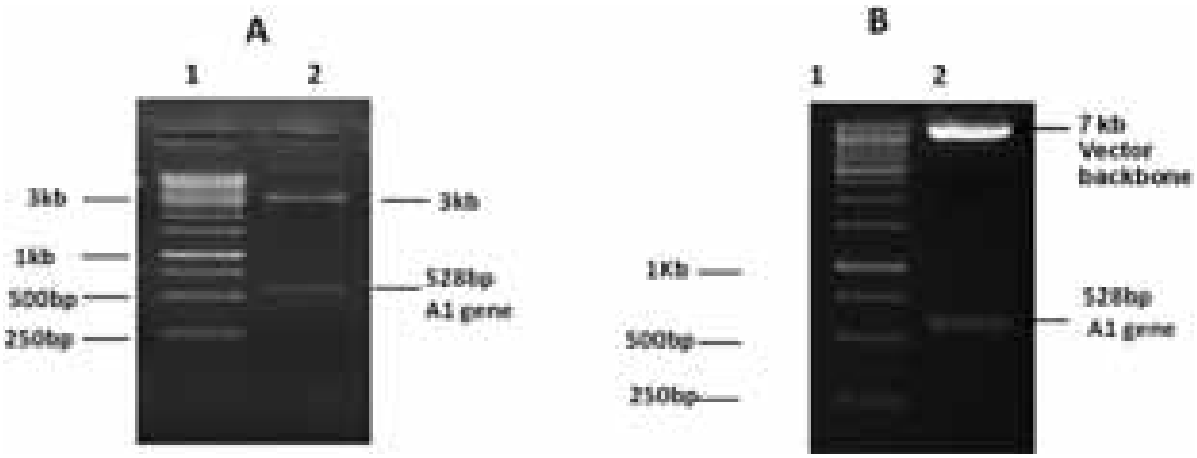


Fig.17: (A) *SpeI* digestion of pGEMT vector containing A1 insert; Lane 1: 1kb ladder; Lane 2: 3 kb vector back bone and 528 bp of A1 insert. (B) *SpeI* digestion of pKSNeo vector containing A1 insert; Lane 1: 1kb ladder; 7 kb vector back bone and 528 bp of A1 insert.

Future Plan:

Studies on T-cell Immune responses due to wild type, centrin KO and p27 KO parasites in the human cells will be extended to larger sample size in each of healthy, PKDL and HVL categories in order to obtain more meaningful data with statistical significance. Knock out and episomal expression of amastigote specific, A1 gene will be carried out to see effect of gene deletion and over-expression on survival, growth and phenotype of parasite.

4. Ubiquitin related modifier 1: A post-translational modification machinery *in Leishmania donovani*

Scientific staff : Dr. Poonam Salotra, Ms. Vanila Sharma
In collaboration with : Dr. Angamuthu Selvapandiyan
Duration : 2012-14

Aims, Objectives & Background:

Protein modifications by ubiquitin and ubiquitin like proteins is an intensely studied phenomenon in the search of new therapeutic targets. Apart from ubiquitin, many small ubiquitin like proteins called Ubls are also known. A diversity of functions is regulated by the Ubls in eukaryotic organisms. Inhibitors of the ubiquitin-proteasome pathway are either in clinical use or are being studied for their potential as anticancer drugs suggests that it may be important to study these pathways in human parasitic organisms. *Leishmania* during invasion of host cell undergoes oxidative stress and urmylation has been implicated to play potential role in oxidative-stress response. In this study, we aim to identify a unique *Leishmania* specific UBL-type modifier named Ubiquitin-related modifier 1 (LdUrm1) and its E1 like activating enzyme LdUba4 and identify target conjugates of LdUrm1 in *Leishmania donovani* by employing mass spectromery tools.

Work done during the year:

In the year under report, we have cloned, expressed and purified the proteins for both LdUrm1 and LdUba4. Polyclonal antibodies were raised against both the recombinant proteins in rabbit.

Identification and sequence analysis of a novel Ubiquitin-related modifier1, LdUrm1 and its activating enzyme LdUba4:

The genomic sequence encoding LdUrm1 was obtained by PCR (Fig. 18A) from *Leishmania* culture DNA using the primers LdUrm1F (5' AggatccGCATGCAGATGACGCACAGAAAAATC 3') and LdUrm1R (5' GctcgagGCCGCCGTGCAGAGTAGAT 3'). Forward primer contained the

restriction site for *BamHI* and reverse primer had *XhoI* restriction site. It was then subcloned into pGEMT vector (Promega). A similar approach was used to PCR amplify LdUba4 using the primers LdUba4 F with *NcoI* restriction site (5' AccatggGCATGCT CAACTCGGCCCTCTACG 3') and LdUba4 R with *XhoI* restriction site (5' GctcgagCGTCGTGGGTGGCG TCTCTTT 3') (Fig 18B) and to determine the sequence of LdUba4. Sequences of the genes were determined on automated sequencer ABI3770. Multiple sequence alignment for both LdUrm1 and LdUba4 are given in (Figs. 19-20).

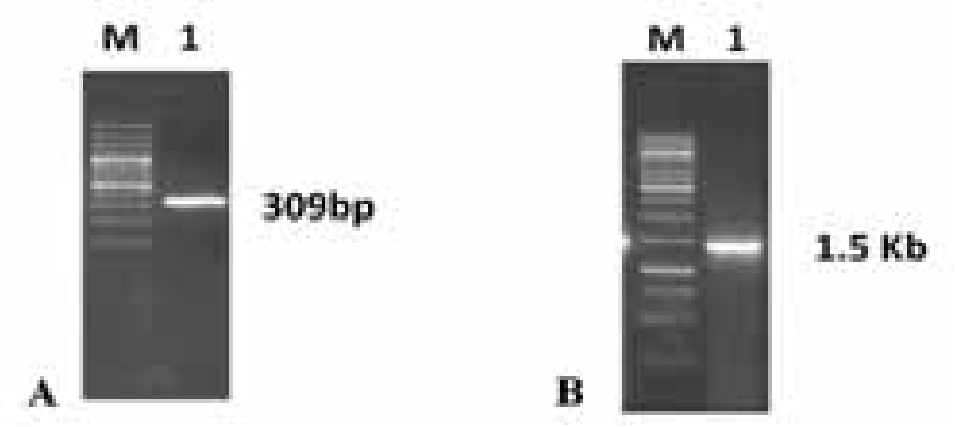


Fig. 18: **A**. Agarose gel picture showing PCR amplified band of 309bp corresponding to the size of LdUrm1 from *L. donovani* promastigote culture DNA (Lane 1); 100 bp DNA ladder (Lane M). **B**. Agarose gel picture showing PCR amplified band of approx. 1.5Kb corresponding to the size of LdUba4 from L. donovani promastigote culture DNA (Lane 1); 1Kb DNA ladder (Lane M).

Multiple sequence alignment of LdUrm1:

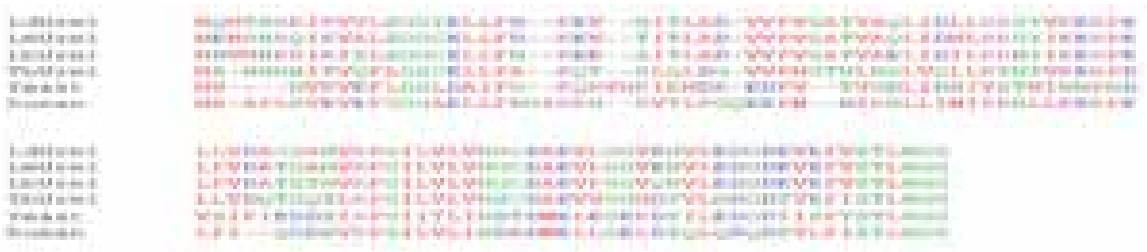


Fig. 19 : Multiple sequence alignment of LdUrm1 ORF sequences: *LdUrm1*, *L. donovani*; *LmUrm1*, *L. major* (LmjF.34.2830); *LbUrm1*, *L. braziliensis* (LbrM.20.2450); TbUrm1, *T. brucei* (Tb427.04.1830); yeast, *S. cereviseae* (NP_012258.3) and *Human;_H. sapiens* (NP_112176.1); and. The accession numbers for the *Leishmania* gene is obtained from GeneDB while the yeast and human sequence is available from GenBanK database.

Multiple sequence alignment of LdUba4

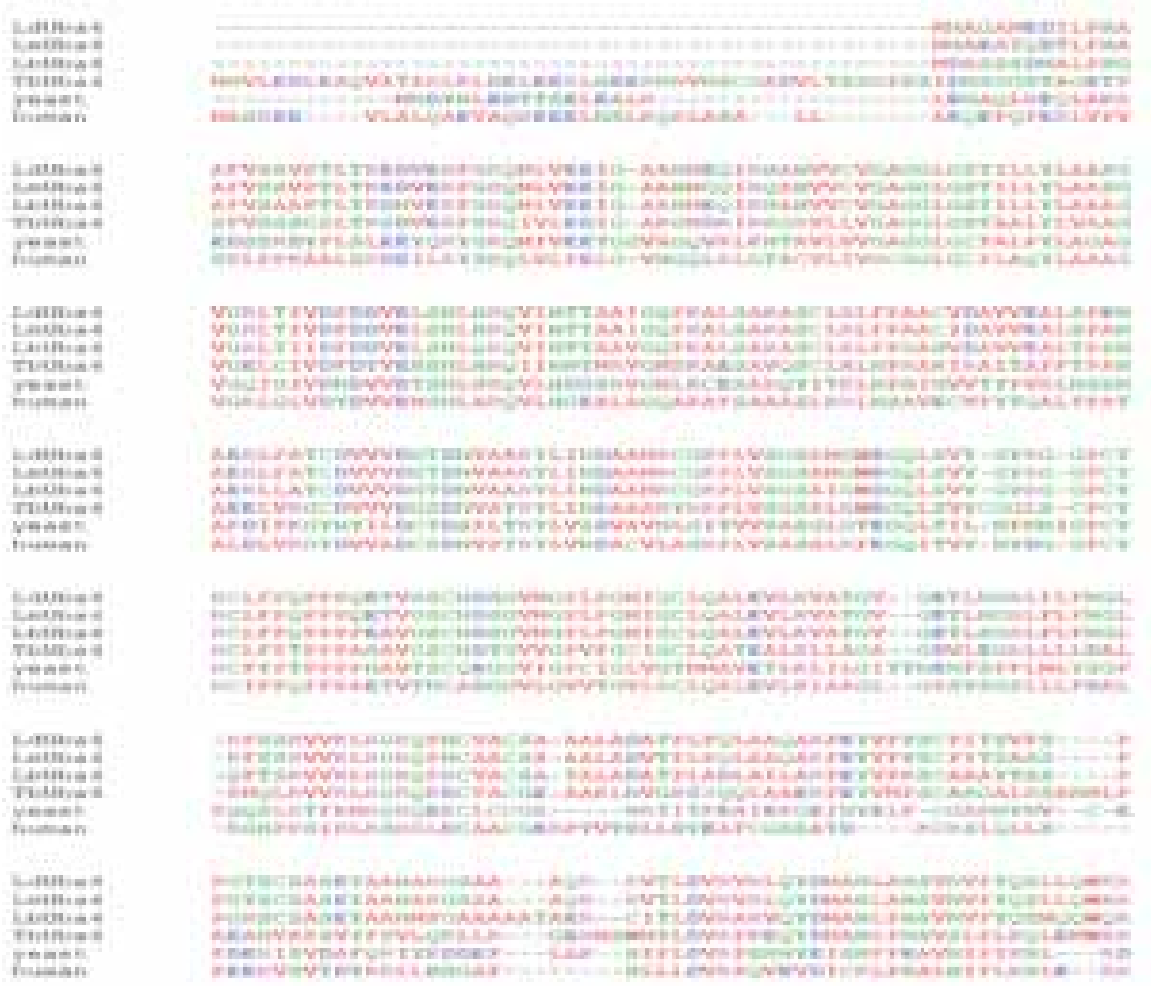


Fig. 20: Multiple sequence alignment of LdUba4 ORF sequences: LdUba4, L. donovani; LmUba4, L. major (LmjF.27.1670); LbUba4, L. braziliensis (LbrM.27.1800); TbUba4, T. brucei (Tb427tmp.22.0008); yeast, S. cereviseae (NP_011979.1) and Human; H. sapiens (NP_055299.1). The accession numbers for the Leishmania gene is obtained from GeneDB while the yeast and human sequence is available from GenBanK database.

Cloning, expression and purification of protein LdUrm1 and LdUba4:

(i) LdUrm1 protein expression in both pPROExhtc and pGEX4T : LdUrm1 gene as amplified above and cloned in pGEMT vector was restriction digested with *BamHI* and *XhoI* and subcloned into *BamHI/ XhoI* backbone of pPROExhtc a prokaryotic expression system containing C terminal 6X histidine tag and finally expressed in BL21 DE3 *E. coli*

strain using 1mM IPTG as inducer. Recombinant protein formed was purified using Ni-NTA metal affinity chromatography (Fig.21A). The protein thus obtained was quantified and 2mg/ml protein was used to raise polyclonal antibody against LdUrm1 in Rabbit (Merck Millipore). The *BamHI* and *XhoI* flanking insert was also subcloned into *BamHI / XhoI* backbone of pGEX4T Prokaryotic Expression System expressed in BL21 DE3 *E.coli* strain. Recombinant protein was purified using GST sepharose beads (Fig. 21B) as the protein formed contained an N terminal GST tag.

(ii) LdUrm1 protein expression in pET28a(+): LdUba4 gene as amplified above and cloned in pGEMT vector was restriction digested with *NcoI* and *XhoI* and subcloned into *NcoI/ XhoI* backbone of pET28a(+), a prokaryotic expression system containing C terminal 6X histidine tag and finally expressed in BL21 DE3 *E. coli* strain using 1mM IPTG as inducer (Figure 21C). The purified protein was then used to raise antibody.

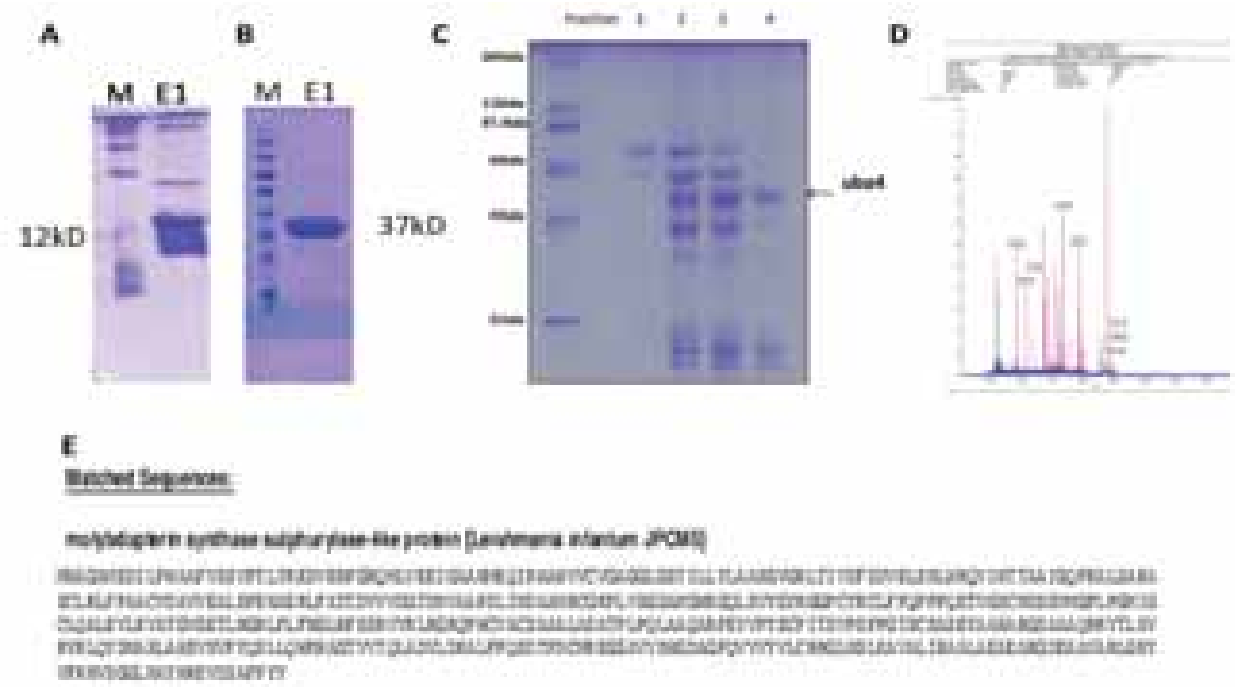


Fig. 21: A 12 kD recombinant protein for LdUrm1 containing C terminal 6X histidine tag expressed in Bl21 DE3 cells (**A**). A 37kD recombinant protein for LdUrm1 containing N-terminal GST tag (**B**). A 50 kD band corresponding to the size of LdUba4 (**C**) containing N terminal 6X histidine tag. Peak showing mass spectroscopic analysis for the protein LdUba4 and homologous protein sequence identified by MASCOT analysis (**D,E**).

Future Plan:

Studies would be carried to understand the mechanism of interaction between LdUrm1 and LdUba4 proteins in *Leishmania*. Also the proteinaceous targets of LdUrm1 in *Leishmania* would be identified by co-immunoprecipitation method using anti-LdUrm1 antibody followed by MS analysis of the adducts obtained on SDS PAGE. This would help us to identify the targets of this post translational machinery into *Leishmania* and an insight into pathogenesis of *Leishmania*.

5. Development of Loop-mediated Isothermal Amplification (LAMP) assay for diagnosis of *Leishmania* infection

Scientific staff : Dr. Poonam Salotra, Dr. Ruchi Singh, Mr. Sandeep Verma
Duration : 2012-14

Aims, Objectives & Background:

VL and PKDL diagnostic methods based on parasite detection (stained smears, culture or histopathology) are invasive and have poor sensitivity, while immunological methods (Direct Agglutination Test, enzyme-linked immunosorbent assay etc.) are not conclusive for PKDL because of persistence of anti-leishmanial antibodies after VL, and are not reliable in immune-compromised patients. Previously we reported utility of *L. donovani* specific loop mediated isothermal amplification (LAMP) assay based 4 primers as a diagnostic tool for VL and PKDL, and published our work. We have now established a new LAMP assay which detects *L. donovani*, *L. infantum*, *L. tropica* and *L. major* with variable sensitivity and higher sensitivity towards *L. donovani* and *L. tropica*. The new assay is based on 6 primers which impart increased sensitivity and specificity to the assay. Addition of two extra primers, known as loop-primers, accelerates the product formation, thereby shortening the required reaction time from 50 min (in published assay) to 30 min. We have evaluated the new assay with different *Leishmania* spp. parasite DNA.

Work done during the year

The new assay is based on 6 primers which impart increased sensitivity and specificity to the assay. Addition of two extra primers, known as loop-primers, accelerates the product formation, thereby shortening the required reaction time from 50 min (in published assay) to 30 min. We have evaluated the new assay with different *Leishmania* spp. parasite DNA. The assay was positive in *L. donovani*, *L. tropica* and *L. major* spp. using 10 ng DNA showing its specificity towards different species of *Leishmania* parasite (Fig. 22).

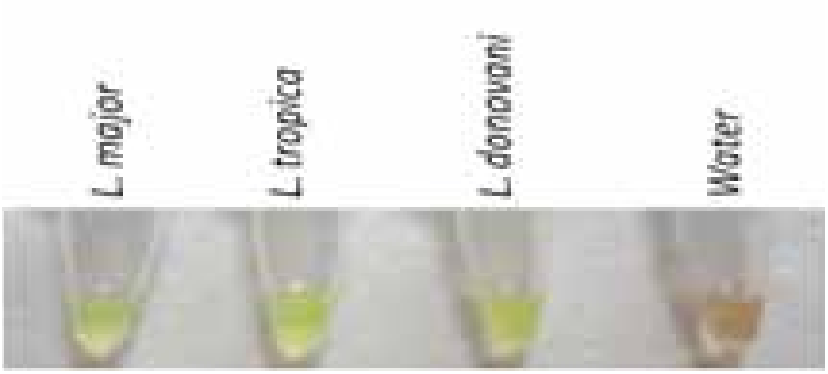


Fig. 22: Specificity of LAMP assay for different *Leishmania* spp. parasite DNA (10 ng). The assay was positive for *L. major, L. tropica* and *L. donovani* species of parasite.

Future Plan:

The sequence of LAMP products amplified from different species will be determined and diagnostic potential of new LAMP assay will be evaluated for diagnosis of KA, PKDL and CL samples.

CHLAMYDIASIS

1. Immunopathogenesis of reactive arthritis induced by chlamydia trachomatis

Scientific Staff : Dr. Sangita Rastogi, Mr. Praveen Kumar (SRF)

In collaboration with : Brig. (Dr.) DS Bhakuni, Army R & R hospital, New Delhi
Dr. VK Sharma, Dr. Geetika Khanna, CIO, VMMC & SJ hospital, New Delhi

Duration : 2010 - 14

Aims, Objectives & Background:

Reactive Arthritis (ReA), an inflammatory arthritic condition, is an aseptic inflammatory polyarthritis that usually follows non-gonococcal urethritis or infectious dysentery. The causative microorganisms of ReA include *Chlamydia trachomatis*, *Ureaplasma*, *Shigella*, *Salmonella*, *Yersinia* and *Campylobacter*. Among these, *C. trachomatis* is the most widely prevalent sexually transmitted pathogen and one of the most important causes of genitourinary induced-ReA (gReA) worldwide. However, this pathogen is poorly understood and underestimated in ReA due to initial asymptomatic infection and no specific diagnostic criteria. It has been reported that *the incidence of Chlamydia-induced ReA might even surpass that of rheumatoid arthritis, but awareness among health care professionals is low*. Furthermore, Undifferentiated Spondyloarthropathy (uSpA) is the most common presentation among various spondylarthritides and is considered as a *forme fruste* of ReA. Although high genital prevalence of *C. trachomatis* has been reported in India, yet there is a paucity of data on *C. trachomatis*-induced gReA/ uSpA.

C. trachomatis is present in the synovial tissue of patients with chronic disease in a persistent, rather than an actively growing form. Persistent infection with *C. trachomatis* provides a continuous source of bacterial components in the synovial compartment that stimulate the immune system, resulting in chronic inflammation and tissue damage in

ReA. The basic immune mechanism underlying ReA/ uSpA and specifically the joint injury that follows intra-articular *C. trachomatis* infection has not been fully elucidated and the Th1/ Th2 cytokine nexus during *C. trachomatis* infection in joint in patients with ReA/ uSpA is little understood. Data on IL-17 levels and IL-17+ T-cells is limited in ReA/ other forms of spondyloarthritis, and is still contradictory. Thus, persistence and the resulting pathogenesis of *C. trachomatis* in ReA/ uSpA patients needs to be explored for understanding the immunological mechanism whereby the bacteria escapes the immune system. It is hypothesized that cytokine imbalance plays a major role in the failure to eliminate the triggering bacteria and the microbial antigen, leading to the disease manifestations and chronicity. This research work initiated as an ICMR-SRF project aims to find the intra-articular prevalence of *C. trachomatis* in arthritic patients, *viz.:* ReA/ uSpA and also, to characterize the cytokine responses in these patients.

Work done during the year:

During the period under report enrollment of 27 age-matched arthritic patients, *viz.:* 4 with ReA, 8 with uSpA, 6 with Rheumatoid Arthritis (RA) and 9 with Osteoarthritis (OA) was done in consultation with the rheumatologist. Data is summarized in 80 arthritic patients (age range = 18 - 45 years) who were clinically diagnosed with gReA/ uSpA (n=50) and inflammatory/ non-inflammatory controls, *viz.:* RA/ OA (n=35). Both male (35)/ female (15) patients were included in the study. Patients with other arthropathies like preceding enteric infection/ inflammatory bowel disease/ psoriasis/ systemic lupus erythromatous/ ankylosing spondylitis were excluded. Also, patients found positive for other infections like tuberculosis/ viral infection were excluded. All patients in the study group were negative for rheumatoid factor. The disease duration in patients with ReA and uSpA was 15 $\pm$ 9.9 and 14.1 $\pm$ 9.8 months (mean $\pm$ SD), respectively. Majority of arthritic patients in the study group had oligoarthritis (68%) while those with RA were diagnosed with polyarthritis. 45.5% gReA/ uSpA patients had synovitis in their major joints (knee/ ankle) while 9% had enthesitis.

PCR assay was performed for the diagnosis of *C. trachomatis* in the synovial fluid (knee joint) of arthritic patients. Intra-articular *C. trachomatis* infection was found in 18% (9/50)

ReA/ uSpA patients by nested PCR (nPCR), while 14% (7/50) patients were positive by semi-nested PCR (snPCR). For comparison of these findings, sensitivity and specificity were calculated; considering nPCR as gold standard, snPCR was found to be 75% sensitive and 100% specific. Overall, the presence of *C. trachomatis* in the joint fluid of ReA/ uSpA patients was significant ('p' value <0.05) in comparison to controls (1/35; 2.8%).

Various studies have observed that the cytokine profile in patients with ReA/ uSpA does not show a clear Th1 or Th2 polarity. Hence, another objective of the study was to investigate the Th1/ Th2 cytokine pattern in the serum of *C. trachomatis*-induced ReA/ uSpA patients (Table I). In *C. trachomatis*-positive ReA/ uSpA patients, the range of IFN-gamma and IL-4 was 13.7 - 77 pg/ml and 3 - 33 pg/ml, respectively. In comparison to the *C. trachomatis*-negative controls, *viz.:* RA/ OA, *C. trachomatis*-positive ReA/ uSpA patients had significantly higher IFN-gamma ('p' value < 0.05) levels. IL-4 levels ('p' value = 0.02) were also found to be significantly upregulated in *C. trachomatis*-positive patients with ReA in comparison to *C. trachomatis*-negative OA patients. The study is being continued.

Table I: Serum cytokine concentrations in arthritic patients

Cytokine *(pg/ml)*	ReA *CT positive*	uSpA *CT positive*	RA *CT negative*	OA *CT negative*
IFN-gamma	60.6*	43.4*	21.0	11.2
Range	(20.8 - 77.0)	(13.7 - 77.0)	(9.0 - 40.5)	(9.0 - 21)
IL-4	28**	14.2	18.9	9.4
Range	(3.1 - 33.0)	(3.0 - 18.2)	(3.0 - 27.0)	(4.0 - 11.6)

Note: Differences between two groups were evaluated using *Mann-Whitney U test*.
'p' value < 0.05 was considered to be statistically significant.
* p < 0.05 ReA/ uSpA versus RA/ OA patients
** p = 0.02 ReA versus OA patients

Abbreviations: ReA: reactive arthritis; uSpA: undifferentiated spondyloarthropathy; RA: rheumatoid arthritis; OA: osteoarthritis; CT: *Chlamydia trachomatis*

TUBERCULOSIS

1. Understanding the underlying mechanism of macrophage immune modulation and identification of markers for TB treatment end point determination

Scientific Staff : Dr. Nasreen Z. Ehtesham, Mr. Naseeruddin, Mr. Ashraf Ashfaq
In collaboration with : Dr. Seyed E. Hasnain, IIT, Delhi
Duration : 2009-14

Aims, Objectives & Background:

Tuberculosis (TB), despite being completely curable, claims the lives of >0.4 million people in India every year. Every day, more than 20,000 people become infected with the TB bacillus and about 5000 develop the disease *(RNTCP report 2011)*. Current Anti-Tubercular Treatment (ATT), under the directly observed treatment, short-course (DOTS) regime has documented positive outcomes of successful treatment due to stringent treatment compliance. In addition to the focus on pathogen, there has been considerable interest about the host defenses in *M. tuberculosis* infection and the associated host-pathogen response. The innate immune system provides the first line of defense against invading pathogens and is critical for the production of elevated levels of cytokines in tuberculosis. The innate immune pathway is activated when certain antigens bind to specific receptors like toll-like receptors (TLRs).

Work done earlier:

We earlier showed, using FACS, that resistin stimulates TLR2 in THP1 cells. Resistin was also found to strongly inhibit Caspase 8 activation *via* TLR2. Resistin was found to stimulate Bcl2 expression, which is not TLR2 dependent, indicating the possibility of the cytoprotective role of resistin. Caspase 3/7 have also been found to be inhibited by Resistin.

Work done during the year:

Having established earlier that resistin level increases in patients with tuberculosis and decreases in patients as a function of treatment *(Ehtesham et al., 2011)*, we investigated the role of resistin in pathogen signalling. Different pro-and anti-inflammatory cytokines (IL-10, IL-6 and TNF-α) were scored using ELISA kits *(BD biosciences)*. Resistin treatment at concentrations of 5-50 ng induces IL-10 (Fig. 1A), TNF-α (Fig. 1B) and IL-6 (Fig. 1C). This reveals a complex network of signalling events in response to resistin.

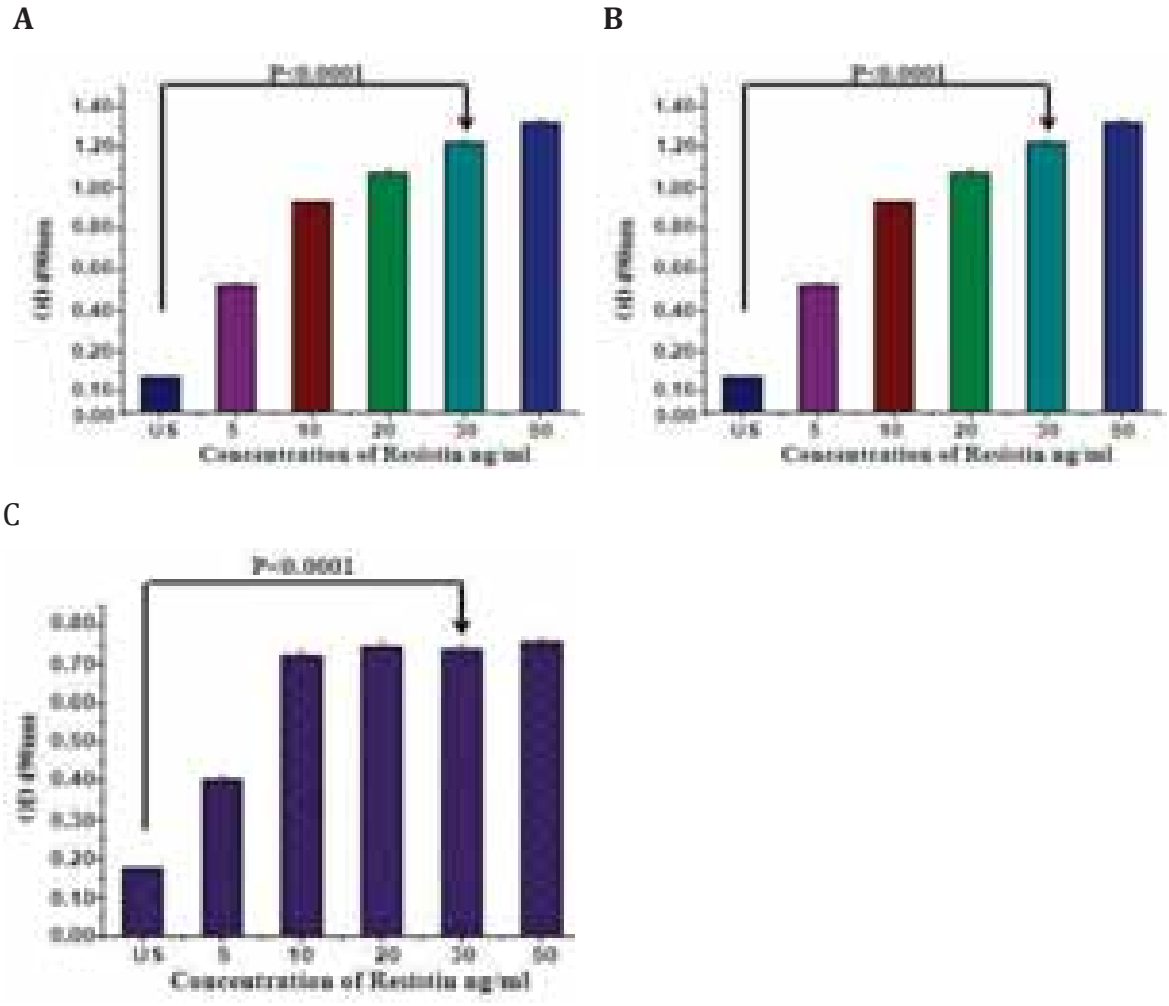


Fig. 1: Resistin induces the secretion of cytokines in THP-1 cells. PMA differentiated THP-1 cells were incubated for 48 hours in the absence (US) or in the presence of increasing concentration of human resistin. Levels of IL-10 (A), TNF-α (B), IL-6 (C) in the culture supernatant of THP-1 cells were then scored by sandwich ELISA.

Furthermore to check the interaction of TLR2 with resistin, computational docking was performed. *In silico* analysis using ClusPro/Hex showed large number of predictions for TLR2. Among these, the first ranked cluster centre shows highest number of possible binding interface for resistin-TLR2. Confidence level of acceptance in Ramchandran plot shows more than 90% of residues in the most favoured region. Further analysis based on hydrogen bond interaction, buried surface area and minimization energy score identified amino acid THR (amino acid residue 218) from resistin and ARG (amino acid residue 340) of TLR2 as possible binding residue. These interacting residues are from beta sheet region and are within 2.0 Å (0.50183Å) (Figs. 2A & 2B). This is the least hydrogen bond distance observed among all the other residues of interaction. It is thus theorized, that these interacting residues may play a role in intermolecular recognition of resistin and TLR2 receptor.

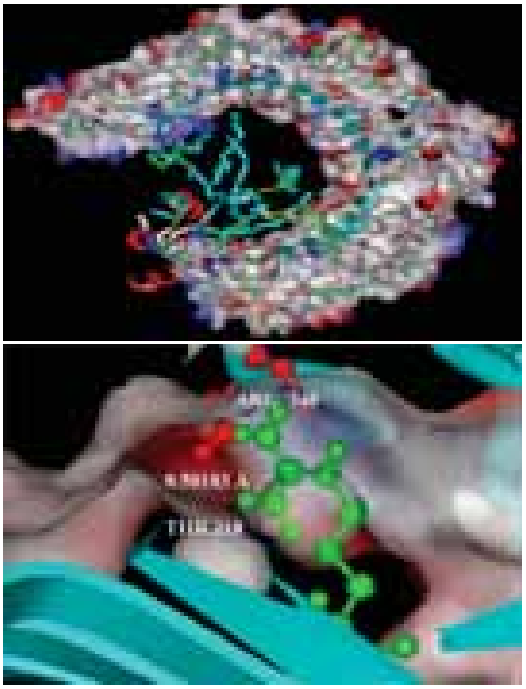


Fig. 2: **(A)** Superimposed model showing Resistin and TLR2 interaction. **(B)** Interacting residues of resistin and TLR 2

2. Understanding the structural flexibility and functional diversity of PE/PPE protein family of *Mycobacterium tuberculosis:* Identification and role of unstructured/disordered regions in this family using *in-silico* tools

Scientific Staff : Dr. Nasreen Z. Ehtesham, Mr. Javeed Ahmad (JRF)

In collaboration with : Dr. Seyed E. Hasnain, IIT, Delhi,
Dr. A Srinivasan, AIIMS, New Delhi

Duration : 2012-17

Aims, Objectives & Background:

The acid rich PE/PPE protein family is exclusive to *Mycobacterium* and particularly abundant in pathogenic strains. This group of proteins is potential source of antigenic variation and has critical roles in pathogenesis. Many PE/PPE proteins are shown to have membrane anchor regions and are surface localized. Few of these PE/PPE proteins are involved in host-pathogen interactions and can also function as immune modulators thus, modifying the host immune response. Members of PE/PPE protein family also have been linked to virulence. The molecular three-dimensional structure of a protein is vital to its function; therefore, understanding protein structure is a very important for assigning and understanding its function.

Work done during the year:

All the PE/PPE gene family of *Mycobacterium tuberculosis* were selected and included in this study. *In-silico* analyses for disordered region, protein binding sites in disordered region and protein-protein interaction for these proteins was carried out using RONN, ANCHOR and STRING, respectively.

Prediction of disordered region in PE/PPEs:

RONN analysis was used to predict the percent disordered structure in PE/PPE proteins.

The percentage of their disordered profile is presented in Table 1. As could be seen most of the proteins of PE, PPE and PE_PGRS family were partially disorderd (20-80%).

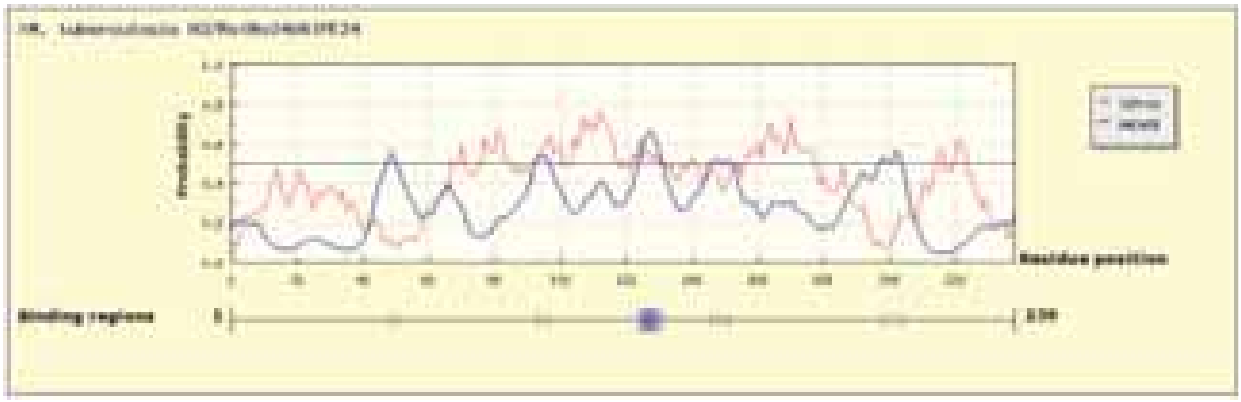
Table 1: Percent Disordered protein content in PE/PPE protein family

	Disordered (80-100%)	Partially Disordered (20-80%)	Ordered (0-20%)
PPE(69)	-	65	4
PE(35)	1	32	2
PE_PGRS(64)	31	31	3

Prediction of protein binding sites in disordered regions:

ANCHOR was used for predicting protein binding sites in disordered regions of PE/PPE protein family. These analyses showed the presence of putative protein binding sites in disordered regions.

Comparative ANCHOR analysis of PE/PPE family of H37Rv (Virulent strain) with that of H37Ra (avirulent strain) revealed loss and gain of protein binding sites in disordered regions. Comparative analysis of PE24 of H37Rv showed gain of some protein binding sites in disordered regions in PE24 of H37Ra strain as is evident from Fig. 1.



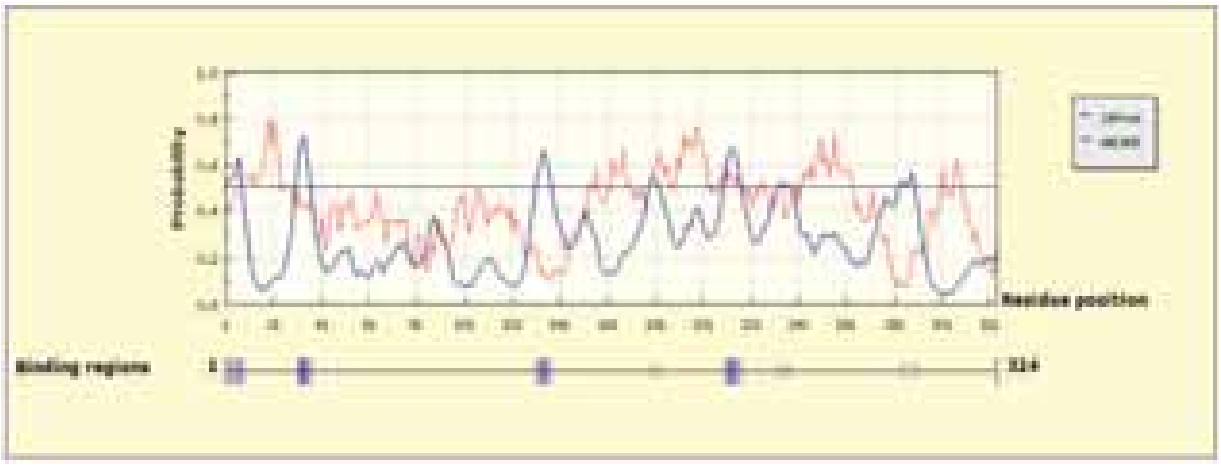


Fig. 1: Blue regions in the figure show protein binding sites in disordered regions

Further analysis of its secondary structure, antigenecity, hydrophobicity, domain analyses are underway.

LEPROSY

1. Clinicopathological determinants in leprosy type 1 reactions

Scientific staff : Dr. Avninder Singh, Ms. Ira Sharma, Dr. L.C. Singh

Technical staff : Mr. Ishwar Singh

In collaboration with : Dr. V. Ramesh, Department of Dermatology, Safdarjung Hospital, New Delhi
Dr. Neelam Sood, Department of Pathology, DDU Hospital, Delhi
Dr. Asha Kubba, Dermatopathologist, Delhi Dermpath Lab, Delhi

Duration : 2010-13

Aims, Objectives & Background:

1. To define and validate key clinical parameters for early diagnosis of leprosy reversal reaction and evaluate clinicopathological discordance
2. To define and validate standard diagnostic criteria for histopathological diagnosis of Type 1 Reaction with special emphasis on interobserver variability
3. To study immunohistochemical expression of i-NOS, nuclear factor-kappa B, interferon inducing protein IP-10/CXCL 10 and its chemokine receptor CXCR3 and to study their co-localization in lesional skin using confocal microscopy
4. To study messenger-RNA expression of interferon-inducible protein (CXCL10/IP-10), CXCR3 gene and inducible nitric oxide synthase (i-NOS) gene using real-time PCR

In continuation of the work done in preceding areas where each pathologist gave his histopathological findings of the biopsies from leprosy type 1 reaction, histopathological parameters seen by all the three pathologists and their degree of agreement was assessed by applying kappa analysis. The histopathologically confirmed cases were included for immunohistochemical validation and statistical significance was calculated.

Immunoflourescence and double labeling immunohistochemical staining was done for localization . Real time PCR was used for studying gene expression for CXCR3, CXL10 and i-NOS genes.and comparing the expression between leprosy type 1 reaction and non-reaction controls. Interobserver agreement for each pathologist was compared pair-wise using algorithm for a simple K-coefficient that is a measure of 1.0 agreement given by Cohen. Kappa statistics was applied and an overall good agreement was seen between the three pathologists P1 (κ=0.83), P2 (κ= 0.61), P3 (κ=0.62) in diagnosing a T1R as shown in Table.

Work done during the year:

Table: Strength of agreement between pathologists

No	Parameter	P1 vs P2 (κ)	P1 vs P3 (κ)	P2 vs P3 (κ)
1	Dermal edema	0.74	0.68	0.70
2	Intragranuloma edema	0.72	0.57	0.67
3	Intragranuloma lymph	0.53	0.34	0.60
4	Epidermal invasion	0.72	0.57	0.62
5	Large Giant cells	0.62	0.61	073
	Type 1 Reaction diagnosis	**0.83**	**0.61**	**0.62**

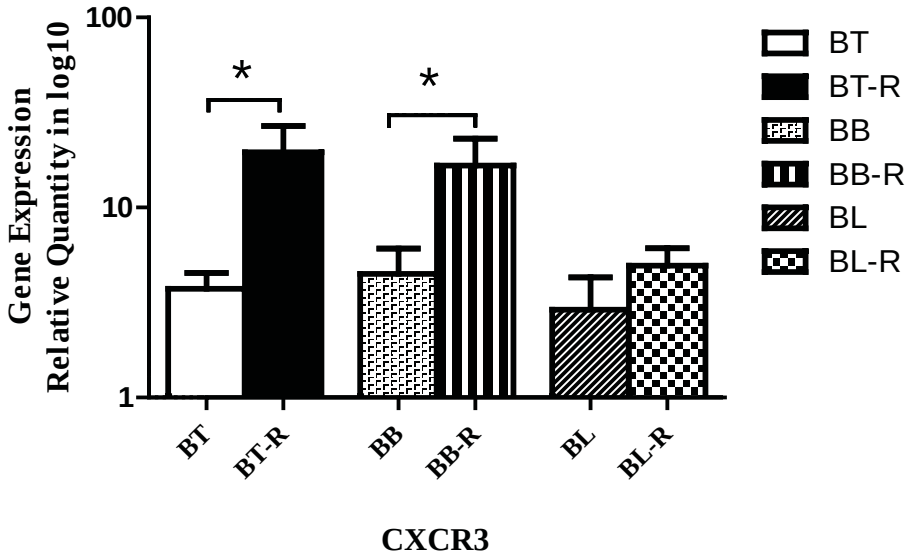
P1:pathologist 1, P2:pathologist 2, P3:pathologist 3

Real Time PCR Results: Relative quantification of gene expression was done using TaqMan chemistry. The data obtained was analysed using ABI PRISM® 7000 SDS Software v1.1. Normal skin was taken as calibrator and all samples were normalized to GAPDH *(endogenous control).*

Statistical test applied: Statistical analysis was performed using SPSS 17.0 software. All comparisons were made using non-parametric Mann-Whitney U test for unpaired data. A *p*- value of less than 0 .05 was considered as statistically significant.

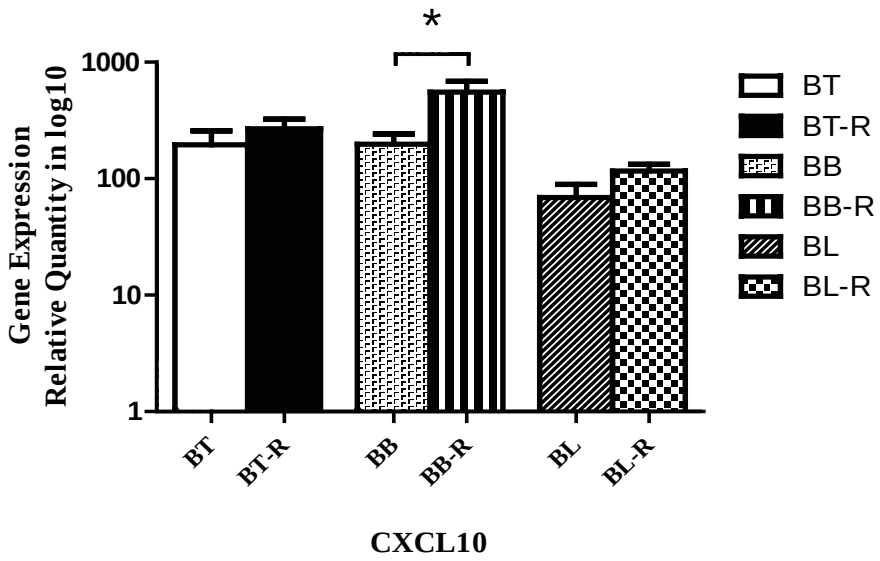
CXCR3 expression in BT, BT-R; BB, BB-R; BL, BL-R:

In gene expression study, it was found that CXCR3 expression was higher in type 1 reaction in comparison to non-reactional leprosy skin biopsies throughout the spectrum. However statistical significance was seen in BT-R and BB-R but not in BL-R. A graphical representation is as follows:



CXCL10 expression in BT, BT-R; BB, BB-R; BL, BL-R:

CXCL10 expression was consistently high in all the groups with mRNA level reaching >1000- fold in BB-R. However, statistically significant difference was observed only between BB and BB in type 1 reaction.



Inference from gene expression study: In gene expression study, it was found that expression of CXCR3 was elevated significantly ($p< 0.02$) in borderline tuberculoid leprosy in type 1 reversal reaction compared to non-reaction borderline tuberculoid leprosy while CXCL10 and iNOS expression showed no significant difference. Further in midborderline leprosy in type 1 reversal reaction, both CXCR3 and CXCL10 expressions were elevated when compared to non-reactional midborderline leprosy skin biopsies with statistical significance of $p<0.04$ and $p<0.02$ respectively. Thus, it can be inferred that CXCR3 is a more consistent marker in differentiating reversal reaction from non-reactional leprosy in comparison to other markers included in this study.

Validation by immunohistochemistry and immunofluorescence:

For studying immunohistochemical expression of i-NOS, NFkB, CXCL10 and CXCR3 in leprosy type 1 reaction biopsies *versus* non-reaction leprosy, we included 60 biopsies. BT Hansens (n=15) and BT in type 1 reaction BT-R (n=15), BB Hansens disease (n=10) and BB Hansens in type 1 reaction BB in type 1 reaction (n=10) and BL Hansens disease(n=5) and BL in type 1 reaction BL-R (n=5). The immunopositivity was scored as shown before in methodology. The results were analyzed by applying Wilcoxon Mann-Whitney test. The results showed that at protein level chemokine receptor CXCR3 was the most statistically

significant marker that was useful in differentiating both BT from BT in type 1 reaction ($p < 0.001$) and BB from BB in type 1 reaction ($p=0.001$). CXCL10 immunoexpression was statistically significant in differentiating BB Hansens' from BB in type 1 reaction ($p=0.002$) while it did not differentiate BT from BT in type 1 reaction ($p=0.403$). i-NOS immunoexpression was statistically significant in differentiating BB from BB in type 1 reaction ($p=0.023$) but not BT Hansens' disease from BT in type 1 reaction ($p=0.13$). NFκB was not statistically significant in any of the leprosy types. The iNOS immunoexpression was seen mostly in macrophages and epitheloid cell granuloma. The CXCR3 was present in the cell membrane of predominantly macrophages and some lymphocytes. CXCL10 was present in the cytoplasm of macrophages and lymphocytes with strong staining of eccrine glands and sebaceous glands that served as internal controls in the skin biopsy. The epidermal keratinocytes stained with anti-CXCL10 but not with CXCR3 antibodies.

Immunolocalization of CXCL10 and CXCR3 by double immunostaining and immunofluorescence staining showed that CXCL10 was localized in the cytoplasm of macrophages and epitheloid cells whereas CXCR3 was located on the cell membrane of histiocytes and occasionally on the lymphocyte cell membrane (Fig. 1)

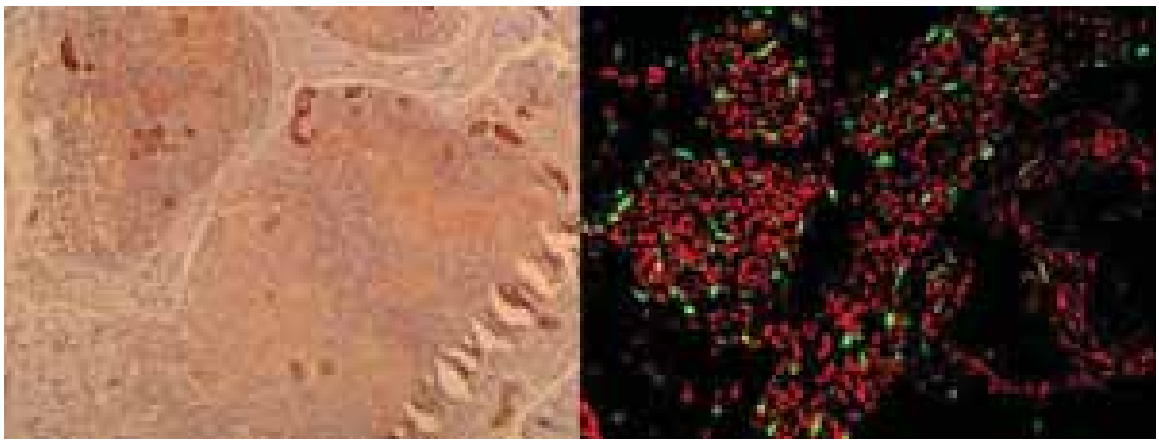


Fig. 1: Immunohistochemical expression of CXCL10 (left panel) and immuno fluorescent staining of CXCR3 localized to membranes of histiocytes (right panel)

Conclusions

To conclude, leprosy reversal type 1 reaction is underdiagnosed on histopathology compared to clinical diagnosis. There is a trend that shows increased expression of chemokine receptor CXCR3 mRNA in BT type 1 reaction as well as BB type 1 reaction. CXCL10/IP-10 mRNA shows statistically significant increase in BB type 1 reaction. Since BB Leprosy is the most unstable form in the leprosy spectrum, it is BB Hansens in type 1 reaction that shows the tendency to develop a type 1 reaction as shown by increased expression of CXCR3 both at m-RNA and protein levels. The increased expression of CXCL10 has been demonstrated in serum of patients from type 1 reaction by the other author. Though our study does show a trend of increased expression at mRNA and protein level in type 1 reactions, additional studies are required before CXCL10 and its receptor CXCR3 can be assumed to be predictors of an impending type 1 reaction. However, these findings may prove to be useful laboratory aids in the diagnosis of a type 1 reaction. Though there was limited follow-up available of these patients who after their acute symptoms (swelling or pain) subsided rarely returned to the dermatologist. It will therefore be worthwhile if more number of sequential biopsies can be studied before reaction and at the time of reaction with a long-term follow-up so as to know which were the patients that progressed to type 1 reaction. It can thus be concluded that elevation of CXCR3 /CXCL10 axis is a better marker to diagnose a type 1 reaction than iNOS as previously reported in literature.

ADULT STEM CELL BIOLOGY

ADULT STEM CELL BIOLOGY

1. A novel arithmetic approach for fool-proof production of growth arrest in 3T3 cells suitable for human epidermal culture

Scientific staff : Dr. L.K.Yerneni
Technical staff : Mr. Bijender Kumar, Mr. Rishi Man Chugh (SRF)
Duration : 2010-13

Aims, Objectives & Background:

The present investigation is on the use of SWISS 3T3 cells to develop a culture system for growing cultured epithelial autografts, by adopting a strategy similar to our earlier innovative process of growth arrest in NIH 3T3 feeder and to characterize the epidermal sheets produced thereof with objectives :

1. To find out correct exposure conditions for a fool-proof Mitomycin C induction of 3T3 feeder cells attenuation through arithmetic derivation of effective concentration based on cell number.
2. To verify cell proliferative influence of such fool-proof growth arrested 3T3 cells on human epidermal keratinocytes.
3. To characterize the human epidermal keratinocytes and epidermal sheets cultivated using such fool-proof growth arrested 3T3 cells.

Work done during the year:

Until previous year, the accomplished work involved, briefly, the attainment of optimal growth arrest employing arithmetically derived doses of mitomycin C (MC) on Swiss albino 3T3 cell line (ATCC Cat No.CCL-92) after establishing a validated 2-tier banking protocol; the derivation of volumetric doses was on the basis of a series of cell density variation experiments followed by volumetric titrations demonstrating their static, inhibitory and

toxic outcomes as indicated by the MC-treated cell extinctions, subsequently co-culture experiments were performed to evaluate the stimulatory potential of such varied produced feeders. It was observed that out of a myriad of concentration-dose combinations tested, doses of p[x], q [5x] and r [10x] or s [30x] μg/10^6 cells when combined with concentrations of B [X+1] and C [X+2] μg/ml produced significantly varied keratinocyte growth outcomes when compared within a given concentration, while 'r' and 's' caused a more or less same extent of keratinocyte stimulation. During the current year of reporting, additional growth studies were performed to identify optimal Kc: feeder ratio & comparing with the standard Gamma Irradiated 3T3, Colony Forming Efficiencies (CFE), chromosomal stability and production of prototype epidermal sheets and their characterization.

Methods:

Growth studies: Working bank Swiss 3T3 cells after 2 passages were seeded into T75 flasks at a density of 24,000 cells per cm^2 and exposed to a 2-hour pulse of MMC concentrations of A,B,C & D which were [X], [X+1], [X+2] and [2(X+2)] μg/ml, respectively. The median concentrations of 'B' & 'C' were further subdivided into 4 arithmetically derived doses per cell viz., p,q,r & s which were [x], [5x], [10x] & [30x] μg/10^6 cells, respectively, while minimal and higher concentrations were tested at single served as controls for comparison in addition to γ-irradiated (γ-Irr) cells (ATCC, 48X-IRR 3T3). At the end of exposure, the cells were re-plated at a density of 7.5 x 10^3 cells/cm^2 and co-cultured with 5 or 7.5 or 10 x 10^3 Kc/cm^2 using banked human epidermal keratinocytes (Kc) at passages 3-6. Growth curves were performed in 24-well plates with & differential cell counts were performed at days 3, 6, 9 and 12 in triplicate.

Colony Forming Efficiency (CFE) was performed with 250 Kc of 3rd passage and 15000 feeders of both 'B' and 'C' groups per well of 6-well plate on day 10 after staining with Rhodamine B and feeders from 'A-p' and 'D-p' and γ-Irr served for comparison. Subsequently, CFE was performed using 170 cells of 1st passage Kc on those feeders produced by the growth arrest identified as optimal which was obtained by MMC concentration of 'B' with a dose of 'r' while comparing with feeders produced by a sub-optimal dose and also 'γ-Irr' feeders. The colonies produced by 340 cells of 1st passage Kc per plate were further

subjected to growth area and/or colony area measurements by a semi-automated image analysis technique as previously reported *(A Kumar & LK Yerneni, Biologicals 37:55-60, 2009)*.

Chromosomal analysis was performed on Kc grown over the optimally growth arrested feeders (B-r) using a standard procedure of metaphase pooling with demicolcine, hypotonic treatment with 0.4% buffered KCl and metaphase spread preparation in Cornoy's fixative followed by Giemsa staining.

Prototype Epidermal sheets were grown in 6-well plates using 1st passage Kc using 400/800/1700 Kc per well and 10,000 feeders/cm^2 growth arrested by MMC concentration of 'B' with doses of 'p' & 'r'. Similarly sheets were grown using 'γ-Irr' feeders as well. The sheets were embedded in paraffin and processed for histology and immunfluorescent demonstration of epidermal markers.

Statistics: The data from growth studies were plotted as line diagrams for Kc & 3T3 with viable cell number on y-axis and time (days) on x-axis. The influence of different concentration-dose combinations on cell numbers of 3T3/keratinocytes in growth curves were statistically analyzed by ANOVA. The CFE and growth areas were subjected to Mann-Whitney Mann-Whitney and Student's t-Test, respectively.

Significant Results:

The growth stimulatory influence of feeders produced by medial toxic concentration of 'B' and dose-concentration combinations of MMC at 'p' & 'r' were verified by growth patterns of epidermal keratinocytes at varying feeder-keratinocyte ratios (Fig. 1). It was revealed that feeders treated with a concentration of 'B' μg/ml significantly increased growth of keratinocytes at an intermediate dose of 'r' μg/10^6 cells than by any other doses. Feeders generated by 'γ-Irr', used as controls for comparison, brought about significantly ($p<0.05$) lower growth than 'B-r' but equivalent to 'B-p' or 'B-s'.

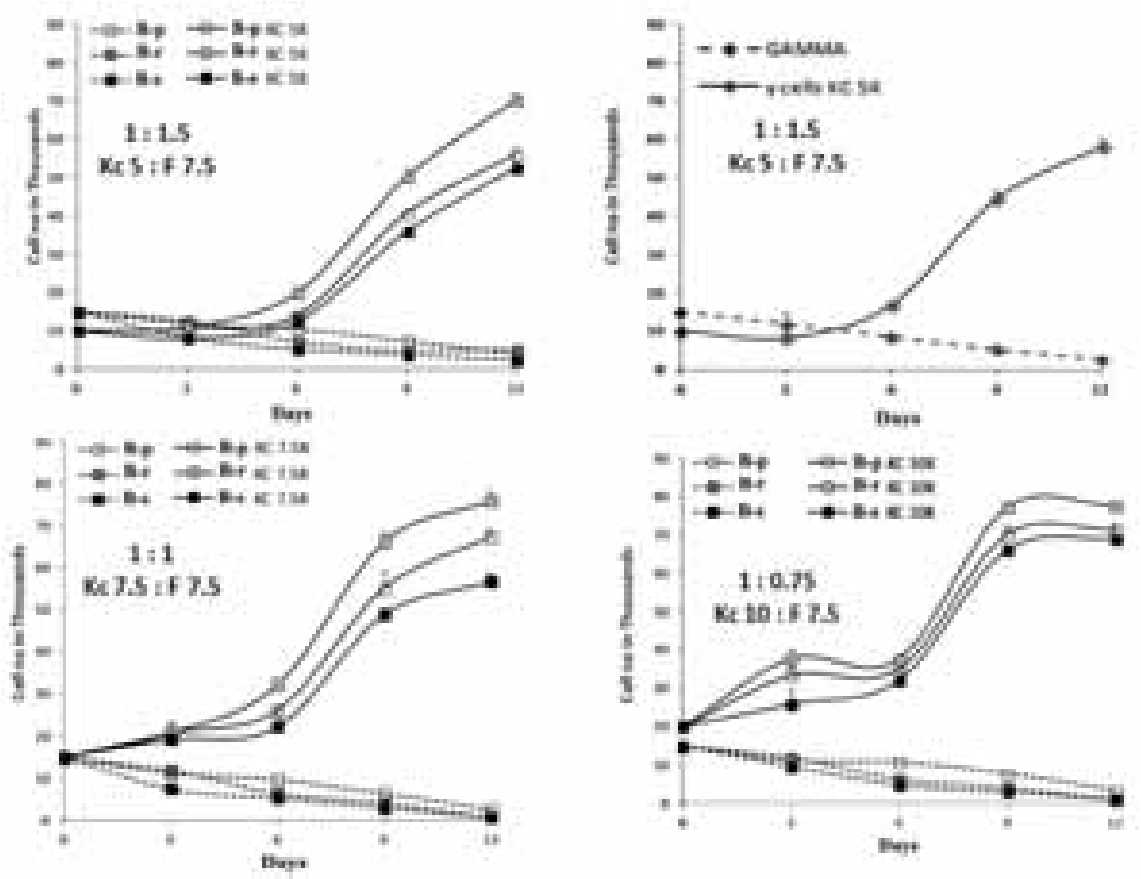


Fig. 1: *Differential stimulation of keratinocyte proliferation by feeder cells growth arrested by combinations of concentration and dose per cell of mitomycin C. The differential was significant (P<0.01 by ANOVA) at various keratinocyte (Kc) and feeder (F) ratios.*

While verifying the differences in dose titrations within the medial concentrations of 'B' and 'C', it was further revealed that the feeders treated with an intermediate MMC dose of 'r' μg/10^6 cells compared to a lower doses of 'p' or higher dose of 's' resulted in significantly increased keratinocyte colony forming efficiencies (Fig. 2). Further, feeders generated by 'γ-Irr' used as controls for comparison produced a lower CFE than even 'A-p' and 'D-p' of MMC indicating superiority of the technique employed.

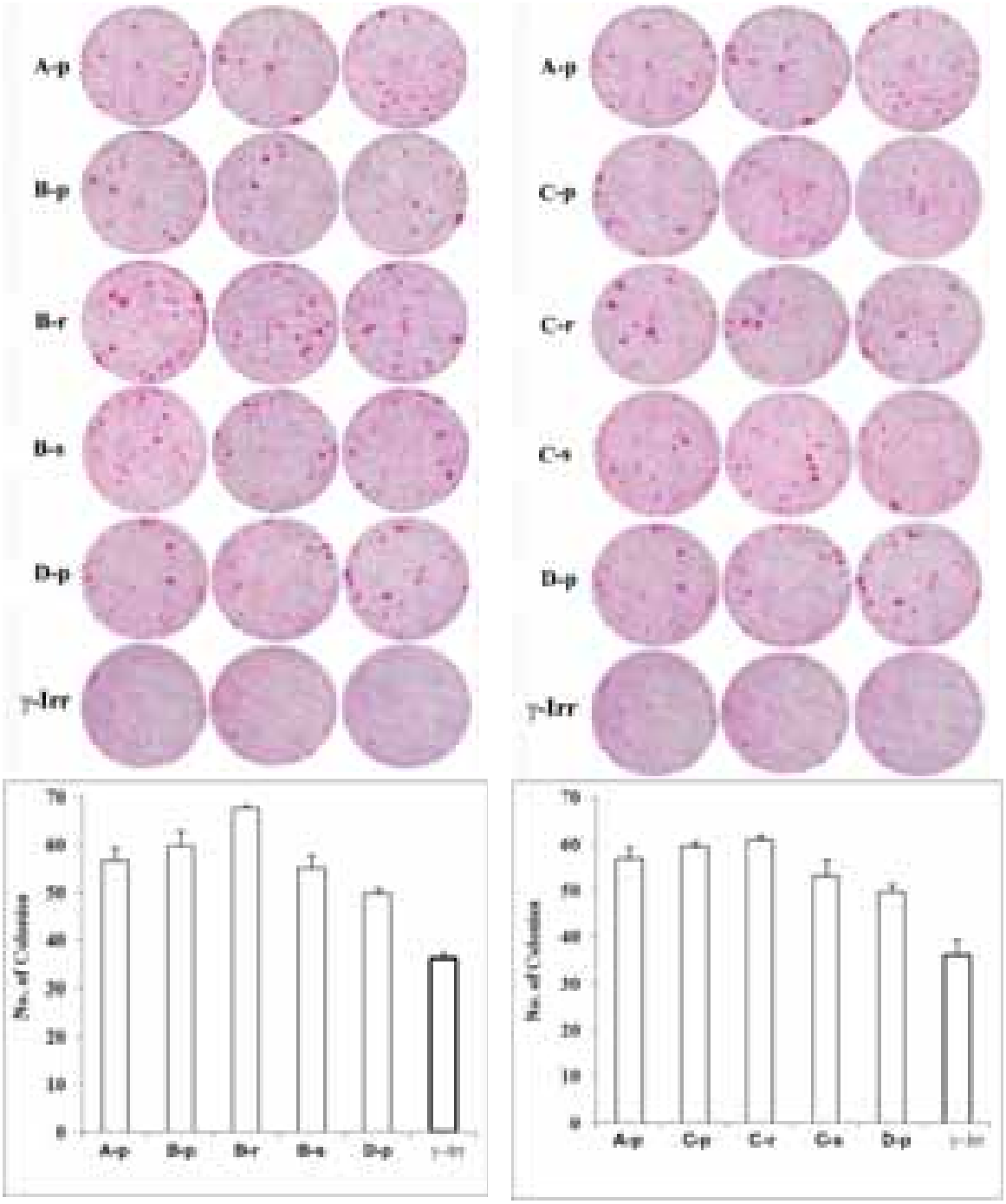


Fig. 2: *Colony forming efficiency assay of Keratinocytes in presence of feeder cells that were growth arrested by combination of concentrations and doses of mitomycin C (Triplicate dishes) and the corresponding graphical representation of colony number.*

Further, colony area/number assessment of CFE dishes following plating 170 Kc cells/ plate in presence of groups 'B-p', 'B-r' and 'γ-irr' feeders, revealed significantly (P<0.01) increased growth area in 'B-r' than by others (Fig. 3).

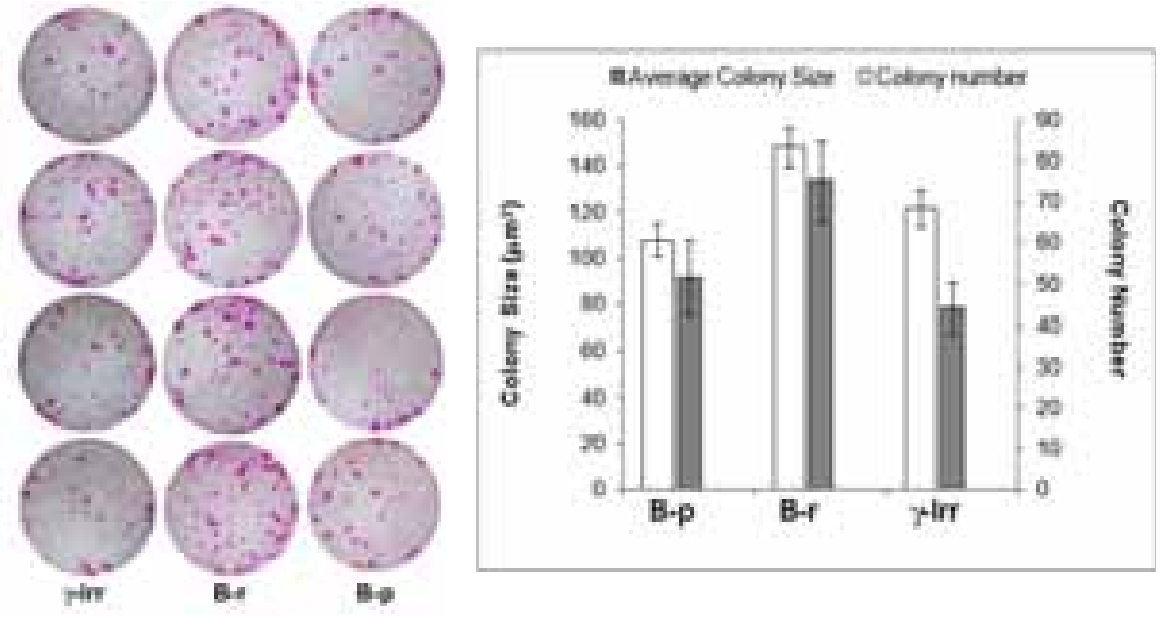


Fig. 3: *Comparative colony size and number (Quadruplicate dishes) of keratinocytes in dishes in presence of 'B-p', 'B-r' and γ-irradiated feeders.*

Additionally, growth area assessment undertaken by image analysis of CFE dishes following plating 340 Kc cells/plate in presence of groups 'B-p', 'B-r' and 'γ-Irr' feeders, revealed significantly (P<0.01) increased growth area in 'B-r' than by others (Fig. 4).

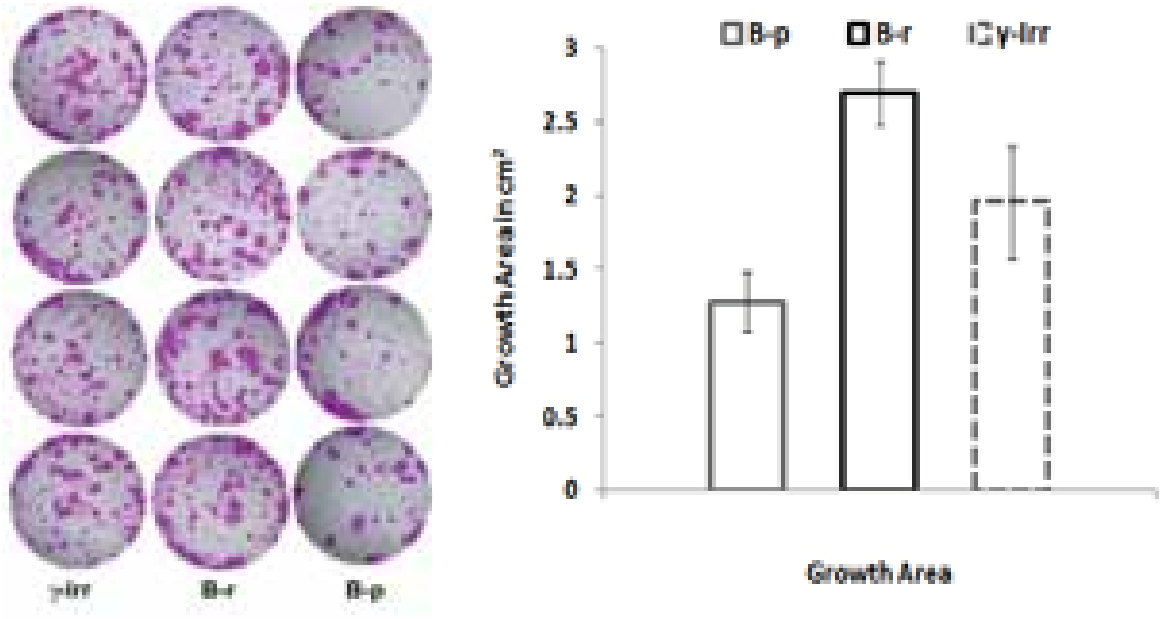


Fig. 4: *Comparative Growth area (Quadruplicate dishes) of keratinocytes in dishes in presence of 'B-p', 'B-r; and γ-irradiated feeders.*

Preliminary chromosomal analysis revealed normal chromosomal number in accordance with the normal male donor of keratinocytes (Fig. 5). G-banding work is ongoing.

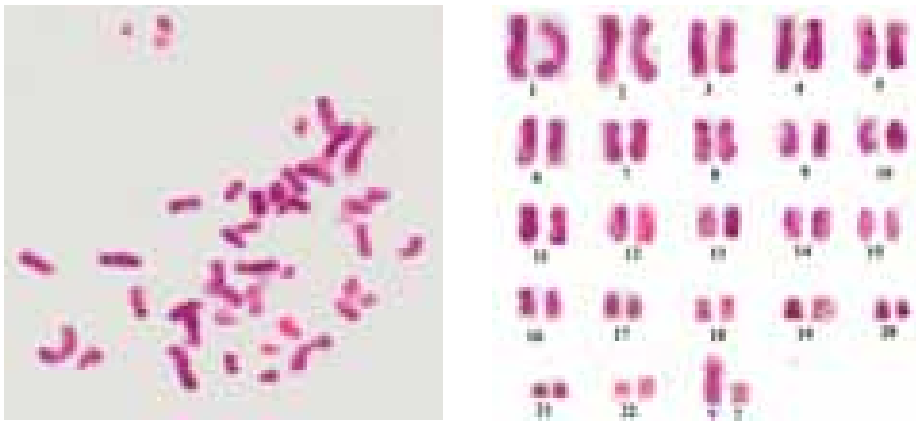


Fig. 5: *Normal male karyotype prepared from human epidermal keratinocytes grown in the presence of swiss 3T3 feeders growth arrested with a combination of Concentration 'B' and dose of 'r' of MMC.*

The epidermal sheet cultures in dishes plated with 800 cells became confluent in 8, 10 & 12 days, by feeders of 'B-r', 'γ-Irr' feeders and 'B-p' groups, respectively. The epidermal

sheets produced by plating 400 or 800 cells/dish were found to be histologically uniform but there were more layers along with an obvious basal cell compartment in the latter, while the sheets were less differentiated in dishes plated with 1700 cells (Figure 6).

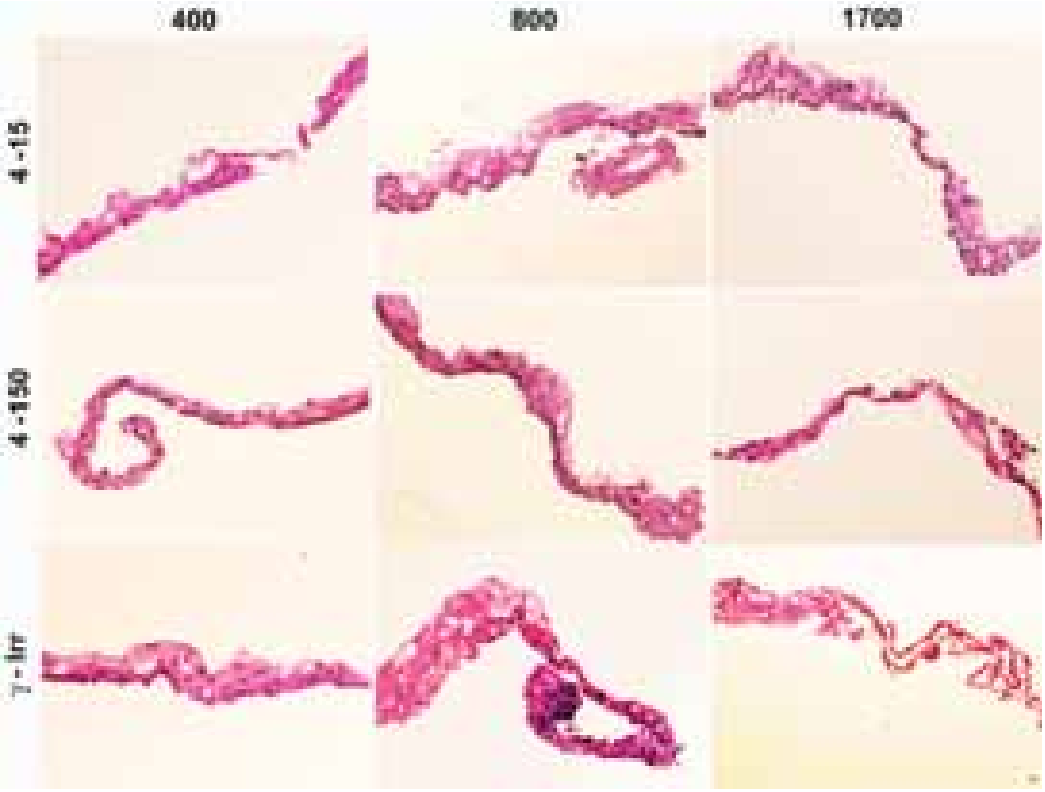


Fig. 6: *H&E stained paraffin sections of cultured epidermal sheets produced by plating 400, 800 and 1700 Kc /cm2 together with feeders from 'B-p', 'B-r; and 'γ-Irr' groups. (250 X)*

Comparative morphometry on the basis of distribution of various epidermal markers in epidermal sheets produced by the 3 feeder types in 800 cell-plating group is in progress. Staining for Ck 14 and Involucrin is complete so far in 'B-r' feeder group (Fig. 7).

Fig. 7A: Isolation of epidermal sheet. From Top:- Confluent culture at 0 minutes; 45 min; 75 min of Incubation in Dispase.

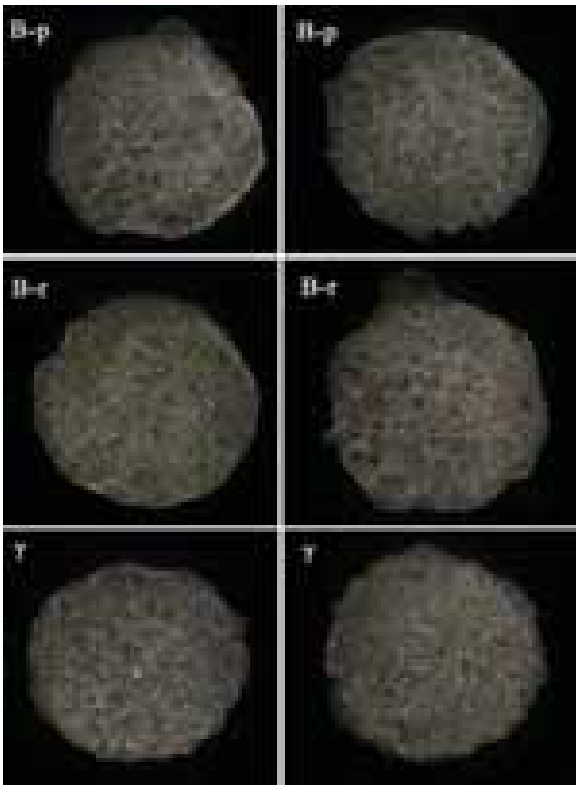


Fig. 7B: *Isolated Epidermal Sheets from B-p, B-r and γ-Irr groups. (3 X)*

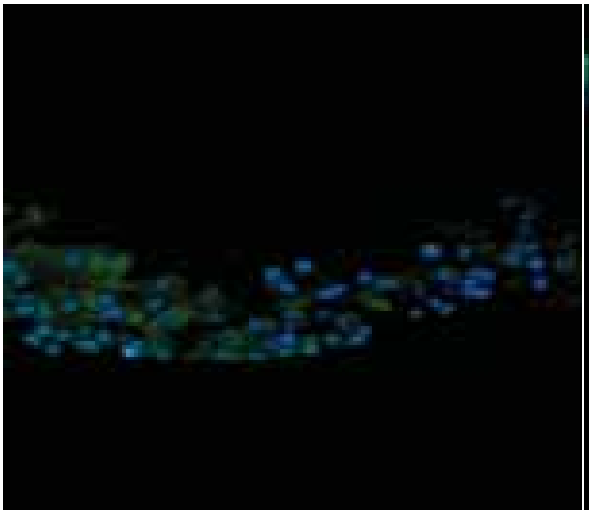


Fig. 7C: Immuno-fluorescent localization of cytokeratin 14 (basal and supra basal) in cultured epidermis (800 X)

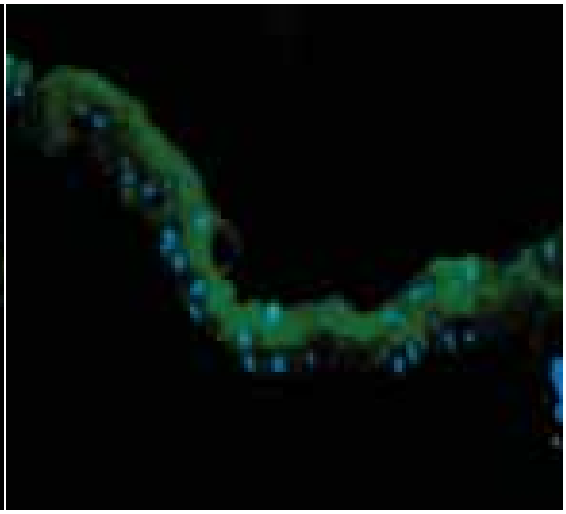


Fig. 7D: Immuno-fluorescent localization of supra basal Involucrin in cultured epidermis (800 X)

Conclusions:

It is thus surmised that the reported discrepancies on effectiveness of the two growth arrest protocols, viz., MMC or γ-irradiation, are in a way linked to the method of exposure of feeder cells to the agent of choice. The present approach identified a cost-effective and optimized growth of epidermal keratinocytes and sheets. Characterization of cultured epidermal sheets & demonstrating chromosomal stabilityby G-banding is currently ongoing to pave way for preparing towards clinical application of CEA in burns.

Future course of action:

The identified protocol needs to be practiced on a biopsy of burns patients enabling preparation of complete set of SOPs necessary before clinical application work is initiated.

2. Studies on epithelial-mesenchymal interactions using human epidermal keratinocyte stem cells and innovatively growth-arrested fibroblast feeders in 3-D collagen model

Scientific Staff : Dr. L. K. Yerneni (Scientist E)
Mr. Madhusudan Chaturvedi (SRF)

Duration : 2010-13

Aim, Objectives & Background:

In view of the hypothesized varied extent of growth arrest of 3T3 fibroblasts through manipulation of arithmetically derived doses of Mitomycin C (MMC) resulting in differential stimulation of epidermal keratinocyte proliferation, experiments are designed to understand the complex fibroblast-keratinocyte interactions. This research work, initiated as an ICMR-SRF project, has been aimed at assessing if there were any qualitative and quantitative differences in the morphogenesis of epidermis by the variedly generated feeders employing an *in vitro* 3-D skin model.

So far, Swiss 3T3 cells were differentially growth arrested employing a previously worked out MMC-protocol were used to construct 3-D collagen based Dermal Equivalents (DE) and their cell extinction rates in DE and routine 2-D culture, were estimated. The minimal, medial and maximal concentrations of MMC treatments resulted in significantly varied feeder cell extinctions synonymous to what was observed in 2-D experiments.

Objectives:

1. To verify if the differentially growth arrested fibroblast (mesenchymal) feeders through employment of numerical doses of MMC as per the innovative technique, bring about varied growth extinction curves in an organotypic dermal-equivalent culture system.
2. To compare such varied mesenchymal outcomes with the growth of epidermis over the dermal-equivalent consisting of variedly growth arrested 3T3 fibroblasts produced experimentally by employing various numerical doses of MMC.
3. To characterize such variedly produced whole skin-equivalents to establish differential mesenchymal - epithelial interactions that supposedly result through manipulation of MMC dosing.

Work done during the year:

The basic technique of constructing whole skin model consisting of 3T3 fibroblasts in Type-I collagen gel as DE and an epithelialization of the same using human epidermal keratinocytes followed by complete stratification of the epidermis at air-liquid interface are accomplished. Comparative morphometric evaluation of various stratified mature epithelia produced by varied combinations of submerged culture time periods and periods of growth at Air-Liquid Interface (ALI) was performed using normally proliferating 3T3 cells and keratinocytes. It was indicated that well developed basal and supra-basal compartments required 2-5 days of submerged condition and formation of partial stratification was obtained following incubation to a further 4-5 days under ALI (Fig. 1). When the epidermis was grown in the presence of those feeders that were growth arrested by an MMC concentration-dose combination of 'B-r', the epidermal structure

produced Rete-ridge like structures (Fig. 2) and stratification was distinct with acellular stratum corneum occupying the topmost position which further became more distinct after 10 days of ALI. Similar studies on other variedly growth arrested feeder cells and morphometric distribution of various layers following immuno-fluorescent staining of various epidermal markers are in progress.

Conclusions:

Production of skin-equivalent model with desirable extent of differentiation is achieved.

Future course of action:

The fully stratified 3-D skin model will be employed for the projected study on the role of differentially attenuated feeders towards healing of experimentally produced wounds in such *in vitro* models as proposed in the future project entitled *"Studies on wound-healing using human epidermal keratinocytes and innovatively growth-arrested fibroblast feeders in 3-D collagen 'Burn Wound' model".*

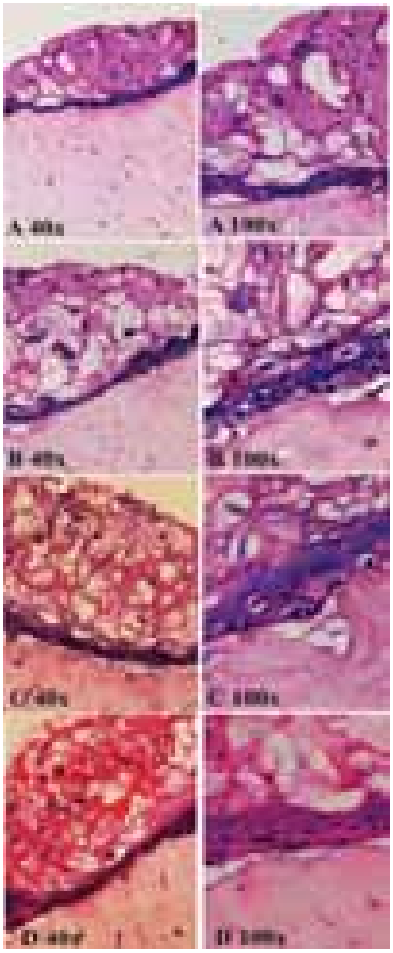


***Fig. 1**: Organotypic whole Skin model prepared by seeding human epidermal keratinocytes over dermal equivalent containing un-attenuated Swiss 3T3 cells in collagen type I. Cultures were incubated for various time points in submerged and Air-Liquid interface (ALI) conditions:-*

A = 2 days submerge+6 days ALI
B= 3 days submerge+5 days ALI
C= 4 days submerge+4 days ALI
A = 5 days submerge+3 days ALI

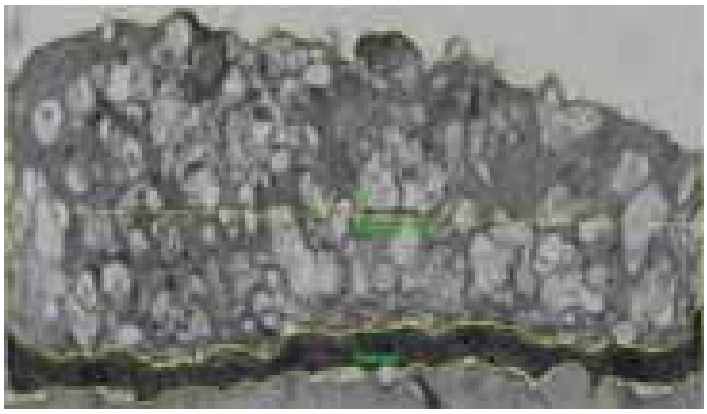


***Fig:** Line markings drawn using image pro-express analysis software for undertaking morphometric measurements of desired areas.*

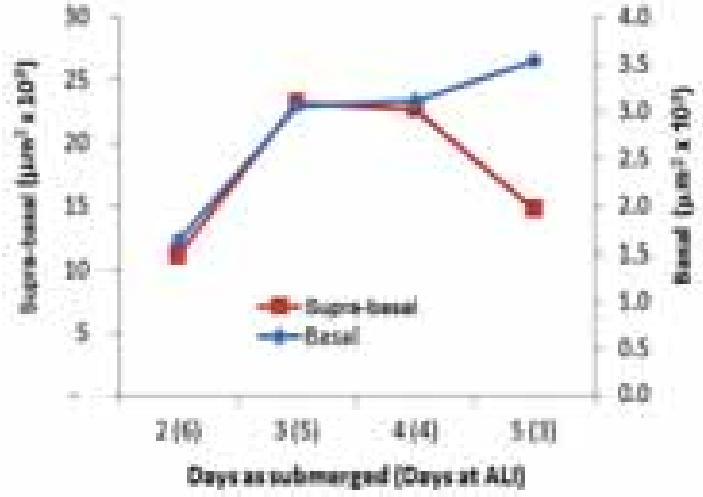


***Fig:** Basal and partially cornified supra-basal area measurements under the above growth conditions.*
2 (6) days = 2 days submerge+6 days ALI
3 (5) days = 3 days submerge+5 days ALI
4 (4) days = 4 days submerge+4 days ALI
5 (3) days A= 5 days submerge+3 days ALI
Open numbers refer to days of incubation in submerged condition and numbers in parenthesis refer to days of incubation under ALI.

Fig. 2: *Structures of regenerative epidermis in human skin equivalent caused by keratinocytes in a culture with (B, C & D) or without (A) mesenchymal support i.e. Swiss 3T3 (A). Absence of mesenchymal support brought about formation of a thin and immature epidermis without epidermal ridge-like structures (B). Skin Equivalent containing normal Swiss 3T3 cells as mesenchymal elements in Dermal Equivalent, when submerged and exposed to ALI for 5 days and 3 days, respectively, produced a distinct basal living compartment with a relatively immature epidermis as visualized by the presence of nucleated cells in supra-basal layers (C). Skin Equivalent containing 3T3 cells growth arrested with 'B-r' Mitomycin C in the dermal compartment cultured under submerged condition and exposed to ALI for 4 day each, respectively, produced a more or less equivalent basal compartment but presented epidermal Rete ridge-like structures (arrow heads) and also exhibited more mature epidermis as visualized by the well developed stratum corneum (Str Corn). The stratum corneum gave a more stratified look when the same SE cultures were maintained at ALI for 10 days (D).*

ENVIRONMENTAL BIOLOGY

EVNIRONMENTAL BIOLOGY

1. Human environmental biomonitoring of Polynuclear Aromatic Hydrocarbons (PAHs) in urban megalopolis of NCR Delhi and investigate the association between PAH exposure and intrauterine growth restriction

Scientific Staff : Dr. Arun Kumar Jain, Dr. Nida Akhtar

In collaboration with : Dr. Shashi Prateek, Deptt. of Obstetrics & Gynaecology,
Dr. K.C. Aggarwal, Deptt of Pediatrics,
Dr. Harish Chellani, Deptt of Pediatrics, Safdarjang Hospital, New Delhi

Duration : 2012-2014

Aims, Objectives & Background:

PAHs are one of the potent and most widespread organic atmospheric pollutants formed by incomplete combustion of carbon-containing fuels such as wood, coal, diesel, fat, tobacco, and incense. Due to highest density of automobiles along with high density of population in Delhi, the risk associated with the human exposure to atmospheric PAHs is also the highest. PAHs are known to be mutagenic and include some of strongest known carcinogens *(IARC, 1984)*. They are also associated with acute and chronic health problems and therefore may have a potential health impact on human *(Fang et al 2004)*. High concentrations of PAHs have been measured in smoke from solid-fuel stoves, burning wood, coal and dried cattle manure and kerosene stoves *(Saksena et al., 1996)* used as the primary cooking device by urban slum residents. Cooking of food (grilling, roasting, frying) at high temperature are other major sources generating PAHs *(Guillen et al., 1997; Phillips, 1999)*. They can also be emitted from various combustion sources such as agricultural waste burning, diesel and gasoline engine exhaust, tobacco smoke, waste incineration facilities and asphalt roads *(Zielinska et al. 2004)*. Other sources of PAHs in environment include lubricating oils, atmospheric depositions, power plants, domestic heating systems and various industrial activities.

PAH exposures due to environmental air pollution are reported to be associated with reduced fetal growth and various other morbidities in human pregnancy *(Perera et al., 1999, Salafia, 1999, Bernstein et al., 2000, Cnattingius, 2004)*. Low birth weight leads to an impaired growth of the infant with its attendant risks of a higher mortality rate, increased morbidity *(Ashworth, 1998)*, impaired mental development *(Grantham- McGregor, 1998)*, and the risk of chronic adult disease *(Barker, 1998)*. Exposure to PAHs during pregnancy due to place of residence, road traffic, dietary and cooking habits may cause IUGR with delivery of low birth weight babies and increase of woman's risk of giving birth to children with congenital anomalies, such as limb defects, nervous system, musculoskeletal or cardiovascular defects, oral clefts, etc. The adverse reproductive effects that are non-fatal produce future risks for the individual and for the next generation. Therefore, this study has been designed to examine the association between IUGR and PAH exposure in expectant women. The aim of this study is to generate basic data about the extent of PAH exposure in pregnant women residing in and around Delhi and likelihood of PAH exposure associated with IUGR.

Work done during the year:

Pregnant women admitted for delivery in labour rooms of Safdarjung Hospital, New Delhi were enrolled for the study. During the year under report, 25 IUGR and 30 AGA (control) cases were recruited for this study.

Samples of placenta, cord blood, maternal blood and urine were collected and processed for PAHs exposure analysis. Details of residential history (location and duration of residence), respiratory symptoms (cough, defaulting breathing, wheeze, probable asthma, etc.), home characteristics including heating and cooking sources and ventilation, and dietary habits regarding PAH-containing foods (i.e., fried, boiled, barbecued meat, etc.) were recorded in the proforma questionnaire at the time of collection of samples alongwith informed consent. The questionnaire also addressed the psychosocial environment and typical daily activities, including usual routes and methods of travel outside the current area of residence during the past year, exposure of fire places, socioeconomic information related to income and education.

Most common sub-clinical symptom was cough which was reported by more than 80% of cases. Similarly, weakness and fatigue were the other major problems faced by IUGR subjects. The data showed low maternal weight and BMI in IUGR deliveries as compared to AGA deliveries. The weight of placenta collected from IUGR subjects ranged from 300-450 grams. However, 400-670 grams weight was recorded in AGA cases. The weight of baby ranged from 2.2-2.6 kg in IUGR delivered subjects and 3.2-3.8 kg in AGA subjects. The haemoglobin content varied from 7.0–12 gm/dl in IUGR cases and 9.5–12.5 gm/dl in control cases.

Polynuclear aromatic hydrocarbons analysis by HPLC:

According to USEPA priority list, the following PAHs are considered as a group in this profile: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k] fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo-[a,h]anthracene, and benzo[ghi]perylene, respectively. Initially attempts were made to optimize HPLC conditions for achieving best chromatographic separation of mixed PAHs. For this purpose several gradient elution methods using acetonitrile/water (in different proportions) and different flow rates of mobile phase from 0.8 to 1.5 ml/minute were evaluated with individual standards as well as a mixture of standards. The best separation of mixed 16 PAHs was achieved by using gradient elution conditions.

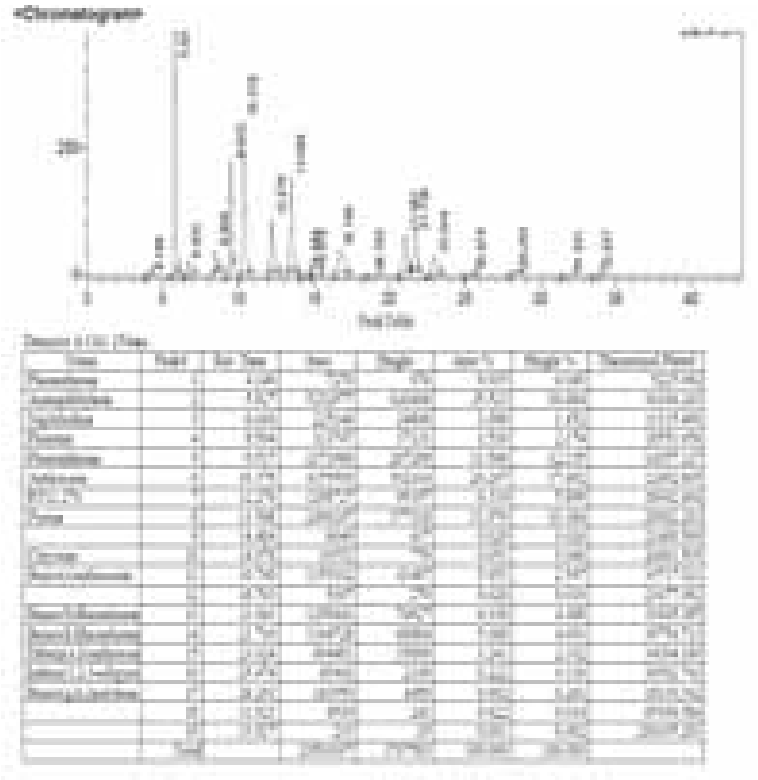


Fig. 1: Chromatogram showing the peaks of different polynuclear aromatic hydrocarbons

Detection of PAHs:

After optimization of the analytical conditions, the extracts obtained from placental tissue and blood as per the standardized protocol were analyzed for presence of the PAHs residues by HPLC using PDA Detector. The pollutants present in the samples were identified by comparing the retention time of the peaks observed in the sample chromatogram with the retention times recorded for the PAHs standard analysed with the same HPLC analytical conditions. Analysis of the HPLC chromatograms obtained from these extracts showed presence of one or more peaks. Some of these peaks could be recognised based on the RT of the standards. The PAHs recognized included naphthlene, pyrene, acenaphthylene, phenanthrene, chrysene, benzo(a)anthracene, dibenz(a,h)anthracene, indeno [1,2,3-cd] pyrene, etc. However, several of the peaks could not be recognized. Attempts will be made to recognize these peaks with fresh and more comprehensive array of persistent organic pollutants standards. The study is being continued.

ACADEMIC ACTIVITIES

MAJOR ACTIVITIES AT NATIONAL INSTITUTE OF PATHOLOGY (2012-13)

1. Organized **Interactive Radio Counseling** session on "*How to prepare for the DNB Examinations*" on 12th April 2012 broadcast on Radio Gyanvani at 5:00 PM.

2. **Quarterly IAPM Delhi Chapter Meet** was organized by NIP-SJ hospital on 27th April 2012.

3. **12th Smt. Pushpa Sriramachari Foundation Day Oration** titled, "*Respiratory Effects of Bhopal Gas Disaster*" was delivered by **Dr. VK Vijayan**, Advisor to DG, ICMR for BMHRC and NIREH, Bhopal and Former Director, VPCI, University of Delhi at NIP on 15th May 2012. Chief Guest: Dr. VM Katoch, Secretary, Department of Health Research & Director-General (ICMR).

4. On the eve of **15th August 2012** and **26th January 2013**, flag hoisting was done at NIP (ICMR).

5. Organized "**Workshop on Scholarly Publishing for Scientists and Medical Professionals**" on 21st September 2012 in collaboration with ICMR.

6. **Guest lecture** entitled "*Fighting cancer– A non-toxic approach*" was delivered by **Dr. Robert W Gorter,** M.D. Ph.D., Founder Director of Medical Centre, Germany, at NIP on 24th September 2012.

7. **ICMR and NIP Training Programme on "Hybridoma" & Technology"** given to **Accurex, Mumbai** at Tissue Culture Laboratory, NIP from 24th September-5th October 2012.

8. Workshop on **"Research Methodologies for Medical Scientists"** was conducted from 9th-12th October 2012 at NIP.

9. Dr. Sunita Saxena (NIP) and Prof. Raisuddin (Jamia Hamdard) signed a **Memorandum of Understanding between NIP and Jamia Hamdard University, New Delhi** for initiating Ph.D. on 19th October 2012.

10. Organized **Scientific Advisory Committee meeting** at NIP on 16th November 2012.

EXTRAMURAL PROJECTS 2012-13

NEW PROJECTS

1. **Study on micro-RNA signatures associated with Breast Cancer Stem like Cells (CSCs) and their role in drug response.**
   Dr. Sunita Saxena, Dr. BSA Raju- ICMR (2013-15)

2. **Understanding the role of micronutrients in *Mycobacterium tuberculosis* infection using guinea pig as a model.**
   Dr. Nasreen Z. Ehtesham- ICMR (2013-17)

3. **Second phase of biomedical informatics centers of ICMR.**
   Dr. Sujala Kapur, Dr. AK Jain- ICMR (2013-17)

4. **Understanding the role of androgen receptor signalling in breast cancer.**
   Dr. Sunita Saxena, Dr. Jatin Mehta- ICMR (2013-15)

5. **Molecular mechanism of drug resistance in Acute Myeloid Leukemia (AML): Role of ATP-Binding Cassette (ABC) transporters.**
   Dr. Sujala Kapur, Dr. Pradeep Chauhan- ICMR (2013-15)

ONGOING PROJECTS

1. **Characterization of host immune factors associated with progression of superficial TCC of bladder by microarray analysis.**
   Dr. Sunita Saxena, Dr. Usha Agrawal- ICMR (2009-12)

2. **Epigenetic studies in esophageal cancer in high risk region of north-east India.**
   Dr. Sunita Saxena, Dr. Sujala Kapur- Twining Program of DBT (2011-14)

3. **Genome wide analysis of genetic alterations in patients with esophageal cancer from north-east India using single nucleotide polymorphism array.**
   Dr. Sujala Kapur, Dr. Sunita Saxena- ICMR (2011-14)

4. **Immunogenetic profile of nasopharyngeal cancer.**
   Dr. Sujala Kapur, Dr. Sunita Saxena- DBT (2010-13)

5. **Study on the effect of siRNA mediated androgen receptor gene silencing on androgen signaling pathway in breast carcinoma.**
   Dr. Anurupa Chakraborty- Post-Doctoral Fellowship, ICMR (2010-12)

6. **Study on effects of anti-cancer drugs on breast cancer stem cells.**
   Mrs. Shanti Lata- ICMR-SRF (2011-13)

7. **Virtual centre of excellence on multidisciplinary approaches aimed at interventions against *Mycobacterium tuberculosis*.**
   Dr. Nasreen Z. Ehtesham - DBT (2008-13)

8. **Detection of *Chlamydia trachomatis* in synovial samples from patients with undifferentiated spondyloarthropathy/ reactive arthritis.**
   Dr. Sangita Rastogi- ICMR-SRF (2010-14)

9. **Development of new live attenuated vaccine candidates for kala-azar.**
   Dr. Poonam Salotra- DBT (2011-14)

10. **Protective immunogenicity of centrin KO live attenuated *Leishmania* parasite in the animal models and in the human cells.**
    Dr. Poonam Salotra- DBT (2011-14)

11. **New tools for monitoring drug resistance and treatment response in visceral leishmaniasis in the Indian subcontinent.**
    Dr. Poonam Salotra- European Commission (2009-13)

12. **Pre-clinical studies of a PSA-based human vaccine candidate targeting visceral, cutaneous and muco-cutaneous leishmaniasis and development of the associated procedures for further clinical trials.**
    Dr. Poonam Salotra- European Commission (2009-12)

13. **A novel arithmetic approach for fool-proof production of growth arrest in 3T3 cells suitable for human epidermal culture.**
    Dr. LK Yerneni- ICMR (2010-13)

14. **Studies on epithelial-mesenchymal interactions using human epidermal keratinocyte stem cells and innovatively growth-arrested fibroblast feeders in 3-D collagen model.**
    Dr. LK Yerneni- ICMR-SRF (2010-13)

15. **Clinicopathological determinants in leprosy type 1 reactions.**
    Dr. Avninder Pal Singh- ICMR Leprosy Task Force project (2010-13)

COMPLETED PROJECTS

1. **Study on gene expression and hypermethylation profiles in early onset breast cancer.**
   Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. BSA Raju- DBT (2008-12)

2. **Transcriptome profiling for identification and characterization of miltefosine resistance associated genes of *Leishmania donovani*.**
   Dr. Poonam Salotra- ICMR (2009-12)

3. **Transcriptome and proteome analyses of ALR2 and its involvement in the pathogenesis of diabetic retinopathy.**
   Dr. Nasreen Z. Ehtesham- DST (2009-12)

4. **Anti-inflammatory potential of n-3 polyunsaturated fatty acids in experimental ulcerative colitis: Biochemical and molecular mechanisms.**
   Dr. Nasreen Z. Ehtesham- DBT (2009-12)

5. **Characterisation of host immune factors associated with progression of Superficial TCC of bladder by microarray analysis**
   Dr. Usha Agrawal ICMR (2009-2012)

INTRAMURAL PROJECTS 2012-13

1. Dynamic regulation of lymphocyte signaling of acute leukemia

2. Drug Resistance in Visceral Leishmaniasis

3. Ubiquitin related modifier 1: a post-translational modification machinery in *Leishmania donovani*

4. Development of Loop-mediated isothermal amplification (LAMP) assay for diagnosis of *Leishmania* infection

5. Immunopathogenesis of reactive arthritis induced by *Chlamydia trachomatis.*

6. Understanding the Underlying Mechanism of Macrophage Immune Modulation and Identification of Markers for TB treatment end point determination

7. Understanding the structural flexibility and functional diversity of PE/PPE protein family of *Mycobacterium tuberculosis :* identification and role of unstructured/ disordered regions in this family using *in-silico* tools

8. Human Environmental Biomonitoring of Polynuclear aromatic hydrocarbons (PAHs) in urban megalopolis of NCR Delhi and investigate the association between PAH exposure and intrauterine growth restriction

PUBLICATIONS

1. **Bansal A**, Soni A, Rao P, **Singh LC,** Mishra A, Mohanty NK, **Saxena S**. Implication of DNA repair genes in prostate carcinogenesis in Indian men. ***Indian J Med Res,*** 136: 622-632, 2012.

2. **Bansal A,** Bhatnagar A, **Saxena S.** Metastasizing granular cell ameloblastoma. ***J Oral Maxillofac Pathol,*** 16: 122-4, 2012.

3. Sony A, **Bansal A,** Mishra AK, Batra J, **Singh LC,** Chakraborty A, Yadav DS, Mohanty NK, Saxena S. Association of androgen receptor, prostate specific antigen and CYP19 gene polymorphisms to prostate carcinoma and benign prostatic hyperplasia in north Indian population. ***Genetic Testing and Molecular Biomarker***, 16(8): 835-840, 2012.

4. Chauhan PS, Ihsan R, Mishra AK, Yadav DS, Saluja S, Mittal V, **Saxena S, Kapur S.** High order interactions of xenobiotic metabolizing genes and p53 codon 72 polymorphisms in acute leukemia. ***Environmental and Molecular Mutagenesis,*** 53(8): 619-30, 2012.

5. Kaushal M, Mishra AK, Sharma J, Zomawia E, Kataki A, **Kapur S, Saxena S.** Genomic alterations in breast cancer patients in betel quid and non-betel quid chewers. ***PLoS One,*** 7(8): e43789, 2012.

6. Mishra AK, **Agrawal U,** Negi S, **Bansal A,** Mohil R, Chintamani C, Bhatnagar A, Bhatnagar D, **Saxena S.** Expression of androgen receptor in breast cancer and its correlation with other steroid receptors and growth factors. ***Indian J Med Res,*** 135(6): 843-52 (2012).

7. Chauhan PS, Bhushan B, **Singh LC,** Kumar AK, Saluja S, Mittal V, Gupta DK, **Kapur S.** Expression of genes related to multiple drug resistance and apoptosis in acute leukemia: Response to induction chemotherapy. ***Exp Mol Pathol,*** 92(1): 44-9, 2012.

8. **Singh LC,** Chakraborty A, **Kapur S, Saxena S.** Study on predictive role of AR and EGFR family genes with response to neo-adjuvant chemotherpy in locally advanced breast cancer in Indian women. ***Med Oncol,*** 29(2): 539-46, 2012.

9. Nyati KK, Prasad KN, Kharwar NK, Soni P, Husain N, Agrawal V, **Jain AK.** Immunopathology and Th1/ Th2 immune response of *Campylobacter jejuni* induced paralysis resembling Guillain-Barré syndrome in chicken. ***Medical Microbiology and Immunology,*** 201: 177-87, 2012.

10. Tomar R, Mishra AK, Mohanty NK, **Jain AK.** Altered expression of succinic dehydrogenase in asthenozoospermia infertile male. ***Am J Reprod Immunol,*** 68: 486-90, 2012.

11. Kohli S, Singh Y, Sharma K, Mittal A, **Ehtesham NZ,** Hasnain SE. Comparative genomic and proteomic analyses of PE/ PPE multigene family of *Mycobacterium tuberculosis* H37Rv and H37Ra reveal novel and interesting differences with implications in virulence. ***Nucleic Acids Res,*** 40: 7113-7122 (2012).

12. Tyagi A, Kumar U, Reddy S, Santosh VS, Mohammed SB, **Ehtesham NZ,** Ibrahim A. Attenuation of colonic inflammation by partial replacement of dietary linoleic acid with α-linolenic acid in a rat model of inflammatory bowel disease. ***Br J Nut,*** 108: 1612-1622, 2012.

13. Sachdeva R, Bhardwaj N, Huhtaniemi I, **Agrawal U,** Jain SK, *et al*. Transgenesis-mediated reproductive dysfunction and tumorigenesis: Effects of immunological neutralization. ***PLoS One,*** 7(11): e51125. doi:10.1371/journal.pone.0051125, 2012.

14. Jairajpuri ZS, **Agrawal U.** Tumor associated macrophages- Friends turned foe? A case report and review of literature. ***Bangladesh Journal of Medical Science,*** 11(2): 139-142, 2012.

15. Agrawal BK, Deswal V, **Agrawal U.** "Aluminium phosphide" toxicity is not due to "aluminium". ***Am J Emerg Med,*** 30(7): 1306. doi: 10.1016/j.ajem.2012.04.026, 2012.

16. Kumar P, Bhakuni DS, Mullick G, Kartik S, **Rastogi S.** Diagnosis of *Chlamydia trachomatis* in joint fluid of chronic reactive arthritis and undifferentiated spondyloarthropathy patients. ***Indian Journal of Rheumatology,*** 7: S1-S2, 2012.

17. Kumar P, Khanna G, Batra S, Sharma VK, **Rastogi S.** A pilot study for detection of intra-articular chromosomal and extra-chromosomal genes of *Chlamydia trachomatis* among genitourinary reactive arthritis patients in India. ***Int Res J Medical Sci,*** 1: 16-20, 2013.

18. Gannavaram S, Connelly PS, Daniels MP, Duncan R, **Salotra P,** Nakhasi HL. Deletion of mitochondrial associated ubiquitin fold modifier protein Ufm1 in *Leishmania donovani* results in loss of β-oxidation of fatty acids and blocks cell division in the amastigote stage. ***Mol Microbiol,*** 86(1): 187-98, 2012.

19. Subba Raju BV, Gurumurthy S, Kuhls K, Bhandari V, Schnonian G, **Salotra P.** Genetic typing reveals monomorphism between antimony sensitive and resistant *Leishmania donovani* isolates from visceral leishmaniasis or post-kala-azar dermal leishmaniasis cases in India. ***Parasitol Res,*** 111(4): 1559-68, 2012.

20. Katara GK, Ansari NA, Singh A, Ramesh V, **Salotra P.** Evidence for involvement of Th-17 type responses in Post-Kala-Aazar Dermal Leishmaniasis (PKDL). ***PLoS Negl Trop Dis,*** 6(6): e1703, 2012.

21. Bhandari V, Kulshrestha A, Deep DK, Stark O, Prajapati VK, Ramesh V, Sundar S, Schonian G, Dujardin JC, **Salotra P.** Drug susceptibility in *Leishmania* isolates following miltefosine treatment in cases of visceral leishmaniasis and post kala-azar dermal leishmaniasis. ***PLoS Negl Trop Dis,*** 6(5): e1657, 2012.

22. Hendrickx S, Inocêncio da Luz RA, Bhandari V, Kuypers K, Shaw CD, Lonchamp J, **Salotra P,** Carter K, Sundar S, Rijal S, Dujardin JC, Cos P, Maes L. Experimental induction of paromomycin resistance in antimony-resistant strains of *L. donovani*: Outcome dependent on *in vitro* selection protocol. ***PLoS Negl Trop Dis,*** 6(5): e1664, 2012.

23. Kumar D, Singh R, Bhandari V, Kulshrestha A, Negi NS, **Salotra P.** Biomarkers of antimony resistance: Need for expression analysis of multiple genes to distinguish resistance phenotype in clinical isolates of *Leishmania donovani*. ***Parasitol Res,*** 111(1): 223-30, 2012.

24. Srividya G, Kulshrestha A, Singh R, **Salotra P.** Diagnosis of visceral leishmaniasis: Developments over the last decade. ***Parasitol Res,*** 110(3): 1065-78, 2012.

25. Selvapandiyan A, Dey R, Gannavaram S, Lakhal-Naouar I, Duncan R, **Salotra P,** Nakhasi HL. Immunity to visceral leishmaniasis using genetically defined live-attenuated parasites. ***J Trop Med,*** 2012: 631460, 2012.

26. Verma S, Bhandari V, Avishek K, Ramesh V, **Salotra P**. Reliable diagnosis of post-kala-azar dermal leishmaniasis (PKDL) using slit aspirate specimen to avoid invasive sampling procedures. ***Trop Med Int Health***. 18(3):268-75, 2013.

27. Kulshrestha A, Bhandari V, Mukhopadhyay R, Ramesh V, Sundar S, Maes L, Dujardin JC, Roy S, **Salotra P**. Validation of a simple resazurin-based promastigote assay for the routine monitoring of miltefosine susceptibility in clinical isolates of *Leishmania donovani*. ***Parasitol Res***. 112(2):825-8, 2013.

28. **Singh A,** Sawhney M, Das S. Granular cell tumor of skin diagnosed on fine needle aspiration cytology. ***Ind J Dermatol,*** 57: 33-331, 2012.

29. Sipayya V, Sharma I, Sharma KC, **Singh A.** Immunohistochemical expression of IDH1 in gliomas: A tissue microarray based approach. ***J Cancer Ther***, 8: 71-74, 2012.

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33. Puri P, Ramesh V, **Singh A,** Muralidhar S, Capoor MR. Facial eruption in a human immunodeficiency virus (HIV) seropositive patient: *Penicillium marneiffei* as a manifestation of immune restoration disease. ***Int J Dermatol,*** 51: 777-779, 2012.

34. **Singh A,** Mishra AK, Ylaya K, Hewitt SM, Sharma KC, **Saxena S.** Wilms tumor-1, claudin-1 and ezrin are useful immunohistochemical markers that help to distinguish schwannoma from fibroblastic meningioma. ***Pathol Oncol Research,*** 18: 383-389, 2012.

35. Kathuria S, Ramesh V, **Singh A.** Pentazocine induced ulceration of the buttocks. ***Ind J Dermatol Venereol Leprol,*** 78: 521-3, 2012.

36. Mallya V, **Singh A,** Sharma KC. Clear cell meningioma of the cauda equina in an adult. ***Ind J Pathol Microbiol,*** 55: 262-4, 2012.

37. **Gautam P,** Nair SC, Gupta MK, Sharma R, Polisetty RV, Uppin MS, Sundaram C, Puligopu AK, Ankathi P, Purohit AK, Chandak GR, Harsha HC, Sirdeshmukh R. Proteins with altered levels in plasma from glioblastoma patients as revealed by iTRAQ-based quantitative proteomic analysis. ***PLoS One,*** 7(9): e46153, 2012.

38. Dolly M, **Gautam P**, Sundaram CS, Sirdeshmukh R. Expanded protein expression profile of human placenta using two-dimensional gel electrophoresis ***Placenta.*** 34(2): 193-6, 2013.

Accepted

1. Ihsan R, Chauhan PS, MishraAK, **Singh LC,** Sharma JD, Zomawia E, Verma Y, **Kapur S, Saxena S.** Investigation on copy number polymorphism of GSTM1 and GSTT11 in susceptibility to lung cancer in a high-risk population from north-east India. ***Ind J Med Res***, 2013.

2. Kumar P, Khanna G, Batra S, Sharma VK, **Rastogi S.** Diagnosis of circulatory antibodies to *Chlamydia trachomatis* among asymptomatic undifferentiated spondyloarthropathy patients in India. ***Internet Journal of Rheumatology & Clinical Immunology***, 2013.

3. Kumar P, Bhakuni DS, **Rastogi S.** Detection of *Chlamydia trachomatis*: A causative pathogen of reactive arthritis/ undifferentiated spondyloarthropathy ***Journal of Infection in Developing Countries***, 2013.

MONOGRAPHS/BOOKS:

1. Study on cox-2 Expression in Breast & Prostate Cancer patients: Flow Cytometric and IF analysis of cox-2 expression. **S. Varma** and Anwesha Mallik. Lambert Academic Publications, Germany, 2012.

SCIENTIFIC ACTIVITIES

DR. SUNITA SAXENA

1. Dr. Sunita Saxena was appointed examiner for the practical examination of National Board of Examination held at St. John's Medical College, Bangalore from 10th – 13th April 2012.

2. Dr. Sunita Saxena was invited to attend meeting on Task Force on Leprosy held on 26th April 2012 at ICMR, New Delhi.

3. Dr. Sunita Saxena was invited to attend review meeting of Dr. Aruna Singh's Technology held on 3rd May 2012 at ICMR New Delhi.

4. Dr. Sunita Saxena was invited to participate as an expert in the interview of faculty for six AIIMS being set up under PMSSY held at National Institute of Health and Family Welfare, New Delhi during 3rd – 5th May 2012.

5. Dr. Sunita Saxena was invited to attend viva of Ms. Abha Soni held at Guru Gobind IP University, Dwarka on 10th May 2012.

6. Dr. Sunita Saxena was invited to attend Condemnation Committee meeting held on 14th May 2012 at ICMR, New Delhi.

7. Dr. Sunita Saxena attended 12th Smt. Pushpa Sriramachari Foundation Day Oration by Dr. V.K. Vijayan, Advisor to Director-General, ICMR at National Institute of Pathology, New Delhi on 15th May 2012.

8. Dr. Sunita Saxena was invited to attend Project Review Committee meeting for Cellular and Molecular Biology/Genomics held at ICMR, New Delhi on 28th May 2012.

9. Dr. Sunita Saxena was invited to attend and deliver guest lecture on "Integrated analysis of cancer genome : Approach to understand cancer biology and identification of biomarkers" in Kolkata Annual Research and Medical International Congress organized by Indian Medical Students' Association held at Conclave Verde Vista, Kolkata during 2nd – 5th June 2012.

10. Dr. Sunita saxena attended 4th Meeting of the Data Safety Monitoring Board (DSMB) on "Curcumin clinical Trial in CaCx Cancer" held at DBT, New Delhi on 22nd June 2012.

11. Dr. Sunita Saxena attended Licensing agreement executed on 28th June 2012 between National Institute of Pathology and M/s Accurax Company, Mumbai on Monoclonal antibody based technology for detection of *Chlamydia trachomatis* developed by Dr. Aruna Singh.

12. Dr. Sunita Saxena attended meeting for multi institutes project held at National Institute of Pathology on 19th July, 2012.

13. Dr. Sunita Saxena attended meeting on Time Line for the 4th year of National Retionoblastoma Registry held at National Institute of Pathology, New Delhi on 24th July 2012.

14. Dr. Sunita Saxena attended and chaired the meeting "Dr. V. Ramalingaswamy Oration" and Hepatopathology Update 2012" held at Institute of Liver and Biliary Sciences, New Delhi on 8th August 2012.

15. Dr. Sunita Saxena attended guest lecture by Dr. Robert W. Garter, Founder and Director of Medical Centre, Cologene on 22nd August 2012 at National Institute of Pathology, New Delhi.

16. Dr. Sunita Saxena attended Delhi Chapter Quarterly meet held at R&R Hospital, New Delhi on 25th August 2012.

17. Dr. Sunita Saxena Chaired Screening Committee for screening of application for the post of Scientist C held at ICMR, New Delhi on 17th September, 2012.

18. Dr. Sunita Saxena inaugurated Half Day Author Workshop held at National Institute of Pathology on 21st September 2012.

19. Dr. Sunita Saxena was appointed examiner for the practical examination of National Board of Examination held at Gandhi Medical College, Hyderabad. from 24th – 27th September 2012.

20. Dr. Sunita Saxena was appointed as an Appraiser to conduct appraisal of ESI Hospital, Basai Darapur, New Delhi for DNB Trainees at ESI Hospital on 30th September 2012.

21. Dr. Sunita Saxena was invited to attend pre Scientific Advisory Committee meeting of National JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra held on 1st October 2012.

22. Dr. Sunita Saxena attended 6th PI meeting of the Naional Retinoblastoma Registry held at National Institute of Pathology on 3rd October 2012.

23. Dr. Sunita Saxena was invited to attend 85th Governing Body meeting of the ICMR held at Nirman Bhawan, New Delhi on 10th October, 2012

24. Dr. Sunita Saxena attended Pre Scientific Advisory Committee meeting on Tumour Biology of National Institute of Pathology, New Delhi held on 17th October, 2012.

25. Dr. Sunita Saxena attended Pre Scientific Advisory Committee meeting on Infectious Diseases of National Institute of Pathology, New Delhi held on 18th October 2012.

26. Dr. Sunita Saxena attended singing of Memorandum of Understanding between National Institute of Pathology and Jamia Hamdard University, New Delhi on 19th Ocotober, 2012

27. Dr. Sunita Saxena attended and presented paper on "Molecular pathology of breast cancer stem cell in early onset breast cancer" in 3rd International Conference on Stem Cells and Cancer (ICSCC-2012) : Proliferation, Differentiation and Apoptosis held at Dr. Ram Manohar Lohiya Hospital, New Delhi during 27th – 30th October 2012.

28. Dr. Sunita Saxena was invited to attend the Selection Committee meeting for the selection of Emeritus Medical Scientist held at ICMR, New Delhi on 29th October 2012.

29. Dr. Sunita Saxena attended IAPM Delhi Chapter meeting of Executive Committee held at MAMC, New Delhi on 30th October 2012.

30. Dr. Sunita Saxena was invited personally to attend and interact with the Directors on individual basis to get an update of the progress for the ongoing translational research projects held at ICMR, New Delhi on 14th November 2012.

31. Dr. Sunita Saxena was invited to attend 86th Governing Body meeting of the ICMR held at Nirman Bhawan, New Delhi on 15th November 2012.

32. Dr. Sunita Saxena attended Scientific Advisory Committee meeting of the National Institute of Pathology held on 16th November 2012.

33. Dr. Sunita Saxena attended and presented the work in 2nd global Cancer Genomics Consortium held at Tata Memorial Centre, Mumbai during 19th - 20th November 2012.

34. Dr. Sunita Saxena attended Selection Committee meeting for the selection of Consultant for ICMR Deemed University held at National Institute of Pathology on 14th December 2012.

35. Dr. Sunita Saxena was invited to attend Technical Committee meeting of ICMR held on 3rd January 2013 at ICMR, New Delhi.

36. Dr. Sunita Saxena was invited to attend Technical Committee meeting of ICMR held on 7th January, 2013 at ICMR New Delhi.

37. Dr. Sunita Saxena was invited to attend Selection Committee meeting for Scientist 'C' post held on 13th January, 2013 at Central JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra.

38. Dr. Sunita Saxena invited to attend the meeting of Scientific Advisory Committee of National Institute for Research in Environmental Health, Bhopal on 23rd January 2013

39. Dr. Sunita Saxena was invited to attend as panelist on Panel discussion titled "Ethical, legal, social economic & technical issues in genetic analysis and road map for establishing Cancer Genetics Unit in India" held on 25th January 2013 organized by Tata Memorial Centre, ACTREC, Mumbai.

40. Dr. Sunita Saxena was invited to attend Condemnation Board meeting of ICMR held on 31st January 2013 at ICMR, New Delhi.

41. Dr. Sunita Saxena was invited to attend meeting of the Bio – Safety Committee held on 5th February 2013 at Institute of Molecular Medicine, Okhla, New Delhi.

42. Dr. Sunita Saxena was invited to attend 1st Scientific Review Board (SRB) meeting of APAC Biotech Pvt. Ltd., Gurgaon held on 9th February 2013 at Crowne Plaza Hotel, Gurgaon.

43. Dr. Sunita Saxena was invited to attend Pre- SAC meeting of Institute of Cytology & Preventive Oncology, Noida held on 11th February 2013.

44. Dr. Sunita Saxena was invited to attend meeting for formal discussion on independent evaluation of performance of ICMR regarding the activities to be carried over to XII Plan on 15th February 2013 held in ICMR, New Delhi.

45. Dr. Sunita Saxena was invited to deliver lecture on "Integrated genomic approaches to identify molecular signature of esophageal cancer in north-east India" in 32nd Annual Convention of Indian Association for Cancer Research on "Emerging Trends in Cancer Research : Road to Prevention & cure" & International Symposium on Infection & Cancer held during 13th-16th February 2013 organized by Dr. B.R. Ambedkar Centre for Biomedical Research, University of Delhi.

46. Dr. Sunita Saxena was invited to deliver guest lecture in depth interaction and brain storming session on "Understanding of Biological complexities through integrated biology" organized by M/s Agilent Technologies, Gurgaon on 18th February 2013.

47. Dr. Sunita Saxena attended Project Review Committee Meeting for 'Cellular and Molecular Biology and Genomics' held on 27th February 2013 at ICMR New Delhi.

48. Dr. Sunita Saxena was invited to attend Project Review Committee meeting on Oncology held during 5th – 7th March 2013 at ICMR, New Delhi.

49. Dr. Sunita Saxena was invited as speaker in National Conference on "Women's Health and Nutrition – A Community Based Approach" and delivered lecture on "Status of breast cancer in Indian women : Risk and awareness" on 8th March 2013 at Vigyan Bhavan, New Delhi.

50. Dr. Sunita Saxena was invited to participate in the function on "CHIKITSHA VIGYAN SAMBANDHI LOKPRIYA HINDI PUSTAKO KE LIYE DEVARSHIK (2010-11) ICMR PURASKAR" held on 22nd March 2013 at ICMR, New Delhi.

DR SUJALA KAPUR

1. Dr. Sujala Kapur was invited speaker on "Recent Updates in Diagnostic Pathology: Molecular Diagnosis of Hematologic Cancers", Government *Medical College*, Amritsar, 01 October 2012.

2. Educational Telecast on "Overview of DNB Exam preparation for Pathology"at IGNOU, New Delhi, 12 April 2012. http://www.natboard.edu.in

3. Dr Sujala Kapur attended UICC Conference in Montreal, Canada, World Cancer Congress on the prevention and early detection of cancers (including tobacco control) on 27th to 31st August 2012. Travel Grant obtained from 2012 World Cancer Congress

4. Dr. Sujala Kapur attended Project Review Committee Meetings in Gastroenterology, Division of NCD, ICMR, New Delhi

DR. POONAM SALOTRA

1. Dr. Poonam Salotra participated in "Molecular Diagnostics Challenges Vis-à-vis Growth Potential" meeting held at Indian Habitat Centre, New Delhi, on 8th June 2012.

2. Dr. Poonam Salotra participated in Post Kala Azar Dermal *Leishmaniasis* meeting held at Hotel Taj Ambassador, Sujan Singh Park, New Delhi, on 27th - 29th June 2012.

3. Dr Salotra participated in the Steering Committee Meeting of *Leishmania* Kala Drug Project at Montpellier on 18th June 2012.

4. Dr Salotra participated in the Steering Committee Meeting of *Leishmania* Rapsodi Project at France from 20th -22nd June 2012.

5. Dr. Salotra participated in WHO informal consultative meeting on preparation for PKDL case management and control guidelines, 2nd -3rd July 2012, Kolkata, India.

6. Dr. Poonam Salotra participated in meetings held at INSA, New Delhi, on 8th and 17th August 2012.

7. Dr. Poonam Salotra participated in INDO-US Vaccine action programme twenty five Year celebration held at ICGEB, Aruna Asaf Ali Marg, New Delhi, on 3rd September 2012.

8. Dr Salotra participated in the Gordan Research Conference, USA from 29th July - 3rd August 2012.

9. Dr Salotra participated in the Steering Committee Meeting of Leishmania Kala-Drug Project at Kathmandu, Nepal in 23rd to 27th September 2012.

10. Dr. Salotra participated in the Steering Committee Meeting on PKDL organised by DNDI and PATH at American Society of Tropical Medicine and Hygiene at USA from 7th to 15th November 2012.

11. Examiner and Co-ordinator for Pre-PhD course work and PhD qualifying examination at National Institute of Pathology for BITS, Pilani and Symbiosis University programme.

12. Co-ordinator of weekly Journal Club at NIP.

13. Reviewer for several projects submitted for funding to ICMR, CSIR, DBT and DST.

DR. NASREEN Z. EHTESHAM

1. Dr. Nasreen Z. Ehtesham attended an International meeting "Bioworld 2012" organized by IIT Delhi from Dec 10-12, 2012.

2. Dr. Ehtesham participated in Indo US symposium on "Human Immunity and Infectious Diseases, Sept. 4-5, 2012.

3. Dr. Ehtesham attended as the member of PRC meeting of Obesity and Diabetes, NCD, ICMR.

4. Dr. Ehteshamwas invited to attend the Pre-SAC meeting of National JALMA Institute of Leprosy and other Mycobacterial Diseases, Agra.

5. Faculty for Pre PhD course for students of BITS, Pilani and Symbiosis, Pune.

6. Exxternal examiner for PhD thesis, BITS, Pilani.

7. Dr. Ehtesham delivered an Invited lecture at DRILS, May 8, 2012.

8. Reviewer for manuscript submitted to J of BioSciences, Current Biology and Cytokines.

DR. SANGITA RASTOGI

1. Attended Seminar on '*Health research in context of social needs*' at INSA, New Delhi on 24th April 2012.

2. Participated in '*Workshop on Flow Cytometry*' at MAMC, New Delhi on 12th October 2012.

3. Convened Institutional Animal Ethics Committee Meeting at NIP (ICMR) on 21st November 2012.

4. Attended Seminar on '*Optimization Techniques for IHC & WB*' at ICGEB, New Delhi on 29th November 2012.

5. Member, Selection Committee for selection of JRF for DBT Consortium project titled, '*Epidemiology, Pathogenomics and System Biology of A. flavus Infections in India- An Integrative Approach*' at Department of Microbiology, VMMC & SJ hospital, New Delhi on 21st December 2012.

6. Assisted in compilation of Annual Report and Highlights documents of NIP (ICMR), New Delhi (2012).

7. Evaluated Ph.D. (Zoology) thesis titled, '*Toxicological investigation of Tridax procumbens on Sprague Dawley rats*', Bundelkhand University, Jhansi (February 2013).

8. Participated as faculty for theory course of Ph.D. students registered with SIU, Pune (February 2013).

9. Reviewed STS project proposals for 'Short-Term Studentship Program' of ICMR (March 2013).

DR. USHA AGRAWAL

1. Annual Meeting of Delhi Chapter of IAPM at AIIMS, New Delhi on 23rd-24th February 2013.

2. Attended "Workshop on systems biology '13" from 16th-18th March 2013 at IIIT, Allahabad

DR. L K YERNENI

1. Attended one day Symposium on "Cord Blood Banking" held at Centre for Genetic Diseases and Molecular Biology, Pt. JNM Medical College, Raipur on 25th July 2012 and delivered invited talk on "Role of stem cells in epidermal regeneration.

DR. AVNINDER PAL SINGH

1. Delivered an invited talk on 'Neurocutaneous Melanosis' at the Neuropath 2013 conference held at GB Pant hospital, Delhi on 1st -2nd February 2013.

2. Invited to attend the IUSSTF Conclave and was felicitated with an Indo-US Research Fellowship Certificate and momento at the function hosted by DST at Hotel JW Mariott, Pune from March 15th -17th 2013.

3. Participated as an invited faculty for the slide discussion session in dermatopathology in International Dermatopathology Conference held at Father Muller Medical College, Mangalore from Aug 31st August – 2nd September 2012.

4. Participated as Judge for Poster session at the International Dermatopathology Conference held at Father Muller Medical College, Mangalore from 31st August – 2nd September 2012.

DR. RUCHI SINGH

1. Dr. Ruchi Singh participated in course on 'Principles and practice of clinical research' organized by Clinical Development Services Agency in partnership with the NIH Clinical Center, USA, at India International Centre, New Delhi from 29th October- 3rd November 2012.

2. Invited reviewer for manuscript submitted to Journal of Clinical Microbiology, Clinical Vaccine Immunology, Journal of Vector Borne Diseases.

3. Member, Editorial Board of Journal ISRN parasitology.

4. Reviewer for short-term studentship projects of ICMR (2012-13).

DR. SAURABH VERMA

1. Participated as scientific faculty and delivered lecture and training on FACS and CLSM in workshop on Research Methodologies for medical Scientists on 9th -12th October 2012 at NIP, New Delhi.

2. Particpated in Workshop on "Analysis of biological networks" organized by DBT from 6th -7th November 2012 at IIT, Guwahati.

DR. POONAM GAUTAM

1. Attended and presented a poster on **"Plasma-based markers for glioblastoma – an integrated resource for targeted exploration"** at International symposium on **"Proteomics beyond IDs and 4th Annual meeting of Proteomics Society (INDIA)"** held at National Chemical Laboratory (NCL), Pune from **22nd-24th November 2012**.

STUDENTS ACTIVITIES

1. Ms Vasundhra Bhandari won the Dr. S Sriramachari Young Scientist Award on 15 May, 2012.

2. Ms Vasundhra Bhandari participated and presented work in PGI Golden Jubilee Workshop on 'Molecular Diagnosis for Parasitic Diseases: Conventional & Real Time PCR techniques' on 10th September 2012 held in PGI, Chandigarh.

3. Ms Vasundhra Bhandari participated and presented work in Final Kaladrug Project Meeting held at Kathmandu, Nepal from 23rd -27th September 2012.

4. Mr Himanshu Kaushal participated and presented work in Immunocon Conference held From 9th–11th November 2012 on the topic "Enhanced IFN-γ and TNF-alpha levels in post kala-azar dermal leishmaniasis upon stimulation with *Leishmania* antigen".

5. Mr Kumar Avishek participated in Microcon Conference held from 22nd to 25th November 2012 in New Delhi and presented poster entitled "Use of slit aspirate specimens to diagnose Post-Kala-azar Dermal Leishmaniasis (PKDL): Minimally invasive sampling".

6. Mr Deepak Kumar Deep participated in Microcon Conference held from 22nd to 25th Nov, 2012 in New Delhi presented poster entitled "Identification of miltefosine resistance- associated genes in *Leishmania donovani*".

7. Ms. Nitu Kumari presented poster *entitled*, "*Role of cytokines in recurrence-free survival of bladder cancer patients*" in award category at 32nd Annual Convention of Indian Association for Cancer Research on 13th -16th February 2013.

8. Ms. Nitu Kumari attended the workshop "*In silico* skills for 'Omics': 3 Hands-on Courses" 5th -14th August 2012 at IBAB/ Shodhaka, Bangalore.

DNB/ Ph.D. PROGRAMME (2012-13)

DNB Program

The Institute has been accredited for training in DNB Pathology course for the last 16 years.

The Institute runs a DNB course for which the admissions are through a Common Entrance test conducted by NBE.

The following Two primary candidates and two post-DCP candidates joined the Institute in this session.

1. Dr Reena Jain
2. Dr Manju Bhamu
3. Dr Mariya Ansari
4. Dr Reetu Jadhav

The students are working on the following topics as part of their dissertation:-
Role of NFκB pathway in bladder urothelial carcinoma - **Dr Reena Jain**

Study on expression of cancer stem cell markers and epithelial-mesenchymal transition markers in breast cancer - **Dr Manju Bhamu**

Immunohistochemical expression of chemokine receptor CXCR3 and its ligands in basal cell carcinoma- **Dr Mariya Ansari**

Study on immunohistochemical expression of ERG, wnt, and βcatenin in prostate cancer - **Dr Reetu Jadhav**

Ph.D. Program

The Institute has been recognized by the following universities:

- BITS, Pilani
- Indraprastha University, New Delhi.
- Jamia Hamdard, New Delhi.
- Symbiosis International University, Pune.

The following students successfully completed Ph.D. during this period :

1. Mr. Gajender Katara
2. Ms. Arpita Kulshreshtra
3. Mr. Pradeep Singh Chauhan
4. Ms. Abha Soni

The following students has submitted their Ph.D. Thesis :-

1. Ms. Regina Devi
2. Mr. Dhirender Singh Yadav
3. Mr. Sandeep Verma
4. Ms. Vasundhara Bhandari

	2012-13
Research Associate	6
Senior Research Fellow	20
Junior Research Fellow	3
CSIR – JRF & SRF	5
UGC - JRF & SRF	3
Total	**37**

SCIENTIFIC ADVISORY COMMITTEE

SCIENTIFIC ADVISORY COMMITTEE

1	**Dr. Indira Nath** Emeritus Professor of Raja Ramanna Fellow Chairperson, Scientific Advisory Committee, National Institute of Pathology, New Delhi	Chairperson
2	**Dr. Rajiv Sarin, Director,** Tata Memorial Centre, Advance Center for Treatment, Research & Education in Cancer, Kharghar, Navi Mumbai – 410 210.	Member
3	**Dr. Kusum Joshi, Professor & Head** Department of Pathology Post Graduate Institute of Medical Sciences, Chandigarh	Member
4	**Dr. Chitra Sarkar, Professor,** Department of Pathology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-29	Member
5	**Dr. Subhroto Sinha, Professor, Director,** National Brain Research Centre (NBRC), Near NSG Campus, Nain Mode, Manesar, Gurgaon, Haryana-122050	Member
6	**Dr. N.K. Mehra, Professor & Head,** Department of Transplant Immunology & Immunogenetics All India Institute of Medical Sciences, Ansari Nagar, New Delhi – 110 029.	Member
7	**Dr. R.R. Bhonde, Professor & Dean,** Manipal Institute of Regenerative Medicine (MIRM), 10, Service Road, Near Union Bank, Domlus Layout, Bangalore - 560071	Member
8	**Dr. Ravi Sirdeshmukh, Scientist** Distinguished Scientist & Ass. Director, Institute of Bioinformatics, Unit-1, Discoverer, 7th Floor, International Tech. Park Ltd. Whitefield Road, Bangalore-560 066	Member
9	**Dr. Sudha Bhatacharya, Professor & Dean,** Dept. Environmental Sciences, Jawahar Lal Nehru University, New Delhi.	Member

10	**Dr. Dhananjaya Saranath,** 601-B, Kalpak Gulistan, 9A, Perry Cross Road, Bandra West, Mumbai – 400 050	Member
11	**Dr. Kiran Katoch, Director** National Jalma Institute of Leprosy & Other Mycobacterial Diseases, Tajganj, Agra, UP	Member
12	**Dr. Chandrima Saha, Deputy Director,** National Institute of Immunology Aruna Asaf Ali Marg, New Delhi	Member
13	**Dr. Syamal Roy** Indian Institute of Chemical Biology, Council of Scientific & Industrial Research, 4, Raja SC Mullick Road, Kolkata – 700 032., West Bengal.	Member
14	**Dr. Ashok Sehgal** Director, In-charge, Institute of Cytology & Preventive Oncology, Research-cum-clinical complex, 1-7, Sector-39, Near Degree College, NOIDA, UP - 201301	Member
15	**Dr. Vijay Kumar, Scientist F & Head,** Division of BMS, Indian Council of Medical Research, New Delhi	Member
16	**Dr. Ashwini Kumar, Head** Indian Institute of Toxicology Research, Post Box No. 80, Mahatma Gandhi Marg, Lucknow – 226 001	Member
17	**The Principal** Vardhman Mahavir Medical College, Safdarjang Hospital, New Delhi – 110 029	Member
18	**Dr. B D Athani** Addl. DG & Medical Superintendent, Safdarjang Hospital, New Delhi	Member
19	**Dr. Sunita Saxena, Director** National Institute of Pathology, New Delhi	Member Secretary

Institutional ANIMAL ETHICAL COMMITTEE (IAEC)

INSTITUTIONAL ANIMAL ETHICAL COMMITTEE

NIP Members

Dr. Sangita Rastogi, Scientist 'E' & InCharge, Animal House, NIP - *Member Secretary*

Dr. Poonam Salotra, Scientist 'F'

Dr. Usha Agrawal, Scientist 'E'

Dr. L K Yerneni, Scientist 'E'

Scientist member

Dr. Harmeet Singh Rehan, Prof., & Head, - *Chairman*
Dept. Of Pharmacology, LHMC, New Delhi

Social Scientist member

Dr. B B Batra, Chief Medical Officer, NFSG, CGHS, New Delhi

Veterinay Consultant

Dr. P K Yadav, Sr. Veterinary Officer, Experimental Animal Facility, AIIMS, New Delhi

CPCSEA Nominees

Dr. D N Rao, Professor, Dept. Of Biochemistry, - *Main CPCSEA Nominee*
AIIMS, New Delhi

Dr. Smriti Rekha Dutta - *Link CPCSEA Nominee*

INSTITUTIONAL STEM CELL RESEARCH AND THERAPY COMMITTEE

INSTITUTIONAL STEM CELL RESEARCH AND THERAPY COMMITTEE

CHAIRPERSON

Prof. N K Mehra, MD, Ph.D.
Immunologist,
AIIMS, New Delhi

MEMBERS

Dr. Suneeta Mittal, MD
Assisted Reproductive Technologist
AIIMS, New Delhi

Prof. Ritu Priya Mehrotra, MBBS, MD
Social Medicine Expert
JNU, New Delhi

Dr. C D Tripathi, MD
Pharmacologist
Safdarjang Hospital
New Delhi

Dr. Ashok Mukhopadhyay, M.Tech., Ph.D.
Stem cell Researcher
NII, New Delhi

Dr. Goutham Bhattacharya, M.Sc., Ph.D., LLB
IPR Attorney
K & S Partners, Gurgaon

Dr. Rolli Mathur, M.Sc., Ph.D.
Biomedical Research & Ethics,
ICMR, New Delhi.

Dr. Sunita Saxena, MD, DCP
Pathologist and Scientist,
NIP, New Delhi

MEMBER SECRETARY

Dr. L K Yerneni, M.Sc., Ph.D.
Biomedical Scientist,
NIP, New Delhi

INSTITUTIONAL COMMITTEES

INSTITUTIONAL COMMITTEES

Purchase Committee

Dr. Poonam Salotra
Dr. A. K. Jain
Dr. L K. Yerneni
Mrs. R. Saratha
Mr. V.K. Khanduja (till 30/9/2012)
Mr. Raja Ram (w.e.f. 07/12/2012)

Transport Committee

Dr. Poonam Salotra
Dr. B. S. A. Raju
Ms. R. Saratha
Mr. Raja Ram

Library Committee

Dr. Sujala Kapur
Dr. Saurabh Varma
Dr. Usha Agrawal
Dr. L K Yerneni
Dr. Avninder P Singh
Mrs. R Saratha
Ms. Anita Sharma

Maintenance/Security and Sanitation Committee

Dr. Poonam Salotra
Dr. A.P. Singh
Dr. Ashwani Kr. Mishra (till 08/06/2012)
Dr. Saurabh Varma
Mrs. R. Saratha

Condemnation Committee

Dr. Saurabh Varma
Dr. Avninder Pal Singh
Dr. B. S. A. Raju
Mrs. R. Saratha
Mr. V.K. Khanduja
Mr. V.K. Khanduja (till 30/9/2012)
Mr. Raja Ram (w.e.f. 07/12/2012)

Tender Committee

Dr. Sangita Rastogi
Dr. Saurabh Varma
Dr. B. S. A. Raju
Mrs. R Saratha
Mr. VK Khanduja (till 30/9/2012)

Renovation/Building Committee

Mr. B. Bose
Mr M.M. Prasad
Mr. A.K. Srivastava
Dr. Poonam Salotra
Dr. Sangita Rastogi
Dr. S. Kapur
Dr. A.K. Jain
Dr. L K Yerneni
Mrs. R. Saratha
Mr. V.K. Khanduja (till 30/9/2012)

Technical Committee

Dr. Ashok Mukherjee
Dr. D. N. Rao
Dr. A.K. Jain
Dr. L.K. Yerneni
Mrs. R. Saratha
Mr. V.K. Khanduja (till 30/9/2012)

Canteen Committee

Dr. Sangita Rastogi
Dr. Anju Bansal
Dr. Ashwani Kr. Mishra (till 08/06/2012)
Mrs. R Saratha
Mr. Ravi Kapoor
Ms. Madhu Badhwar

REFERRAL SERVICES

REFERRAL SERVICES

HISTOPATHOLOGY/CYTOLOGY LABORATORY

The Histopathology/Cytology laboratory provides diagnostic services for Safdarjung hospital patients and routinely performs histopathology, cytology of fluid specimens and FNACs on palpable lumps in patients. It is a tertiary review centre for patients outside Delhi and NCR. Diagnostic techniques such as frozen sections both as intraoperative support and for research purposes is performed. Immunohistochemistry is performed routinely and an extensive panel of antibodies is used for confirming histogenesis in undifferentiated tumours and lymphomas. Immunoflourescence is performed routinely for renal biopsies as an adjunct to histopathology. The diagnostic spectrum constitutes operative specimens and biopsies of all organs including lymph nodes alongwith skin, renal and bone marrow biopsies. The Department received approximately 5000 specimens in the year under report. Research support is also provided for Postgraduate and PhD students of NIP as well as other Institutes.

Staff: Dr Sunita Saxena, Dr Sujala Kapur, Dr Usha Agrawal, Dr Anju Bansal, Dr Avninder P Singh, Dr Fouzia Siraj, Ms Krishna, Ms Karuna, Mr Satpal Singh, Mr Anil Verma, Mr Madan Lal, Mr Raj Singh, Mr Shyam Sundar, Mr Sanjay, Ms Jyoti, Ms Sharda.

MOLECULAR BIOLOGY LAB

In the year under report, total of 150 clinical samples were received from VL patients (blood, bone marrow), PKDL patients (blood, tissue biopsy and slit aspirates) and CL patients (tissue biopsy). Samples were processed for detection and species identification of *L. donovani*/ *L. tropica* infections by PCR, PCR-RFLP, LAMP, Q-PCR and rk39 strip tests.

Staff: Dr. Poonam Salotra, Dr. Ruchi Singh, Mr. Sandeep Verma, Ms. Vasundhra Bhandari, Ms.Vanila Sharma, Mr. Himanshu Kaushal, Mr. Kumar Avishek, Mr. Deepak Kumar Deep, Ms. Aditya, Mr. Uday, Ms. Kamlesh Sharma, Mr. Anish Saxena, Mr. RC Chhetri.

ELECTRON MICROSCOPE DIVISION

The EM Facility at National Institute of Pathology is a centralized core laboratory which provides High Resolution Analytical Transmission Electron Microscopy application for biological tissues to all users. The facility is used for diagnostic as well as basic research activities by several scientists of the Institute.

The Facility also provides EM research support to other researchers at regional and national level.

The Electron Microscopy Division is equipped with state of art 120kV Hitachi H7500 High Resolution Transmission Electron Microscope with side-mount Gatan and bottom mount AMT digital camera systems along with Thermo Noran Energy Dispersive Microanalysis System. The processing laboratory is equipped with latest ultra-microtomes and cryo-ultra microtomes for cutting semi and ultrathin sections, glass knife makers, diamond knives and can process tissues samples for both scanning as well as transmission electron microscopy.

Staff: Dr. AK Jain, Mr. Banajit Bastia, Mrs. Asha Rani Srivastava, Mrs. Anita Bhatia, Mr. Suresh

ENVIRONMENTAL TOXICOLOGY DIVISION

The Environmental Toxicology Division at National Institute of Pathology is a centralized core laboratory with capabilities of High Resolution Mass Spectrometry in Real Time. The division is equipped with JEOL Direct Analysis in Real Time (DART) Time Of Flight Mass Spectrometer, Shimadzu GC 2014 Gas Chromatograph with Flame Ionization and Electron Capture Detectors along with Auto-Sampler and Shimadzu High Performance Liquid Chromatography with UV and PDA detectors and Auto Sampler facilities. The Division also has an ARL Beckman Direct Current Plasma Spectrometer for trace metal analysis, Microwave Sample Digestion and Extraction Systems.

The Division is undertaking extensive research in Human Environmental Biomonitoring

Program and is involved in monitoring of toxic metals phthalates and organic pollutants in human samples. The Institute has developed a modest Environmental Specimen Bank with over 200 placental specimen.

Staff: Dr. AK Jain, Dr. Nida Akhtar, Ms. Rashmi Tomar, Mr. Ravi Deval, Mr. Shashi N Kumar, Mr. Manoj

ANIMAL HOUSE FACILITY

(CPCSEA Registration number: 102-1999/ CPCSEA)

The Animal House Facility caters to the scientific community of the Institute for small animal experimentation in accordance with the CPCSEA (*Committee for the Purpose of Control & Supervision of Experiments on Animals*) guidelines. National Institute of Pathology has an IAEC (*Institutional Animal Ethics Committee*) duly constituted as per the CPCSEA guidelines. During 2012-13, 4 research projects submitted by the Institute's scientists were given animal ethical clearance by IAEC in its meeting dated 21st November 2012.

Staff: Dr. Sangita Rastogi, Mr. Kuldeep Kumar Sharma, Mr. Madan Lal, Mr. Bala Dutt.

MICROBIOLOGY LABORATORY

The focus of research in Microbiology laboratory is on female genital chlamydiasis and *Chlamydia trachomatis* induced reactive arthritis. During the reporting period, clinical collaborations were continued with Department of Obstetrics & Gynecology (SJ hospital, New Delhi), Army hospital (R&R, New Delhi) and Central Institute of Orthopedics (VMMC & SJ hospital, New Delhi). Endometrial curettage tissue was collected from 20 spontaneous aborters undergoing dilatation and evacuation while synovial fluid and blood were collected from 12 patients with seronegative spondyloarthropathies (4 with reactive arthritis and 8 with undifferentiated spondyloarthropathy), 6 patients with rheumatoid arthritis and from 9 patients with osteoarthritis.

Staff: Dr. Sangita Rastogi, Mr. Praveen Kumar, Ms Namita Singh, Ms Priya Prasad, Mr. Kamal Dev

FLOWCYTOMETRY LABROTARY

In total, **1159** samples of blood, cervical cells, leukaemic patients, urinary bladder samples were acquired and analysed for surface antigen expression, intracellular cytokine response and apoptosis.

Scientific Staff: Dr. Saurabh Verma, Mr. P.D. Sharma

CONFOCAL LASER SCANNING MICROSCOPY LABROTARY

Total of **89** images were acquired on confocal laser scanning microscope.

Scientific Staff: Dr. Sunita Saxena, Dr. Saurabh Verma, Mr. P. D. Sharma

LIBRARY

The Institute library continues to cater to the needs of the Institute's staff, students and the research staff by subscribing to 20 (Foreign print) and 15 (Indian) journals. 42 online journals are subscribed through M/s Wiley's and Science Direct. The faculty of Safdarjung hospital and others Institute's Library are also using the facility. The library serves the scientific staff, DNB, MSc, Ph.D. students, Project Staff, WHO fellows and trainees by getting books and other Library services. The users access the online journals and reprints through inter-library loan from Safdarjung Hospital Library, NIC, National Medical Library, INSDOC and others as requested by the scientific and administrative staff of the Institute. The library also receives inter-library loan request on e-mail to send photocopies of the same by post free of cost. The photocopy facility for the scientists and students is being continued.

The Institute library has more than 10,000 books, bound journals,CDs, thesis, Annual Reports, WHO and AFIP Fascicles, Govt Publications, Newsletter pertaining to Pathology, Cancer, Computer Science, Immunology, Infectious Diseases, Toxicology, Statistics, Electron Microscopy, Confocal Microscopy, sub-branches of Pathology and other specialized medical subjects and Hindi books have also been added to the Library collection, PUB-MED, NML-ERMED, JCCC@ ICMR (consortium between NML ICMR and AIIMS) and other online

services are also provided for the scientific and technical staff of the Institute. JCCC and J-Gate Custom content for Consortia through informatics India Ltd. is used for resource sharing of other ICMR Libraries. The facility allows the user to be more flexible in meeting their needs. The library also provides other services like indexing and abstracting of books and journals. The Library displays the recent scientific publications, Annual Reports, Newsletters of other Institutes and newspaper clippings of scientific, technical and govt. policies.

Local Area Network (LAN) facility with Six nodes terminal internet connection has been installed in the library for internet browsing and e-mail access. Online journals which are subscribed from Science-Direct, Wiley, ICMR Consortia, NML-ERMED can be accessed on the desktop by institute's scientists (6 terminals).

The NIP Newsletter (Quarterly) is being continued since 2003. This is published and distributed to ICMR Institutes and other medical Institutes. The list of publications 1952-2013 and updated regularly and PDF of most of the publications are available.

Staff: Dr. Sujala Kapur, Mrs. Anita Sharma, Mrs. Sangeeta Batra, Mr. Dharmender

E-Mail: ioplibrary@rediffmail.com

COMPUTER DIVISION

Computer Division of the Institute serves as the backbone for communication by all other divisions and department of the Institute through Local Area Networking and proving internet services. The Computer Division is equipped with the three servers for LAN, antivirus and application programs alongwith latest computer systems, a laser printer, color inkjet printers, scanner and up to date software. The department helps the students in conducting weekly journal club meeting as well as other data entry and formatting for their thesis. Through maintenance of Histopathology software, it helps in recording, storage and archival of histopathology data.

The department helps in the compilation and generation and printing of Annual Report, Highlights and other documents. The Computer Division also takes care of day to day

assistance and maintenance of almost 70 computers in different divisions of the Institute. Computer Division is also responsible for maintaining the Institute's website, uploading latest information and updating the different web pages.

It is actively involved in financial accounting, i.e generation of pay, paybills, schedules, pay slips, compilation of income tax, filing of quarterly and annual tax returns, etc.

Staff: Dr. AK Jain, Mr. Shiv Prakash, Ms. Seema Sharma

STAFF LIST

NATIONAL INSTITUTE OF PATHOLOGY

NEW DELHI

Sl. No.	Name of the Employee	Designation
1.	Dr. Sunita Saxena	Director
2.	Dr. Sujala Kapur	Scientist 'F'
3.	Dr. Poonam Salotra	Scientist 'F'
4.	Dr. A. K. Jain	Scientist 'F'
5.	Dr. Nasreen Z. Ehtesham	Scientist 'F'
6.	Dr. Sangita Rastogi	Scientist 'E'
7.	Dr. Usha Agrawal	Scientist 'E'
8.	Dr. L.K. Yerneni	Scientist 'E'
9.	Dr. Anju Bansal	Scientist 'D'
10.	Dr. Avninder Pal Singh	Scientist 'D'
11.	Dr. Ruchi Singh	Scientist 'D'
12.	Dr. Saurabh Verma	Scientist 'C'
13.	Dr. Fouzia Siraj	Scientist 'C'
14.	Dr. S. Appala Raju Bagadi	Scientist 'B'
15.	Dr. Poonam Gautam	Scientist 'B'
16.	Mrs. R. Saratha	Administrative Officer
17	Sh. Raja Ram	Accounts Officer

Group 'B'

Sl. No.	Name of the Employee
1	Mr. Yogendra Kumar
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4	Mr. Jagdish Prashad
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7	Mr. V.S. Rawat
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10	Mr. Mangey Ram
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13	Dr. L.C. Singh
14	Mrs. Madhu Badhwar
15	Mr. Shiv Prakash
16	Mrs. Seema Sharma
17	Mrs. Anita Bhatia
18	Mr. Chandi Prasad

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1	Mr. Pushp Raj
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3	Mrs. Krishna
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5	Mrs. Valsamma Mathew
6	Mr. Jagdish Pant
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8	Mr. Suresh Bhimrao Kamble
9	Mr. Satyapal Singh
10	Mr. Madan Lal
11	Mr. Shiv Bahadur
12	Mr. Manwar Singh
13	Mr. Puran Singh
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18	Mr. Rajesh Kumar
19	Mr. Brijender Singh
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23	Mrs. E. Sharda
24	Mrs. Sangeeta Batra
25	Sh. Bijendra Kumar
26	Ms. Jyoti
27	Sh. Kamal Dev
28	Sh. Daya Sagar
29	Sh. Shyam Sunder
30	Sh. Ajit Singh Lehra

Group C (Attendant (Services)

Sl. No.	Name of the Employee
1	Mr. Bala Dutt
2	Mr. Jagdish Ram
3	Mr. Dharmendar Singh
4	Mr. Sajid Hussain
5	Mr. Ram Chander Das
6	Mr. Puran Chand
7	Mr. Rajendra Kumar
8	Mr. Manoj Kumar
9	Mr. Anish Kumar Saxena
10	Mr. Raj Singh, Attendant
11	Mr. Chandrika Prasad
12	Mr. Sanjay Dutt Upreti
13	Mrs. Charanjit Kaur

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