NATIONAL CENTRE FOR CELL SCIENCE

ANNUAL REPORT 2000 - 2001

NATIONAL CENTRE FOR CELL SCIENCE

(An autonomous institution of the Department of Biotechnology,
Ministry of Science and Technology, Government of India).

Annual Report
2000 - 2001

NCCS Complex, Ganeshkhind, Pune - 411 007

CONTENTS

OUR MISSION

- To receive, identify, maintain, store, grow and supply: Animal and human cells / cell culture, cell lines of both. Existing (typed) and newly developed, hybrid cells including hybridomas; Tissues, organs, eggs (including fertilized) and embryos. Unicellular, obligate pathogens, parasites and vectors, plasmids, genes and genomic libraries.
- Develop, prepare, quality control and supply culture media, other reagents and materials and cell products independently and in collaboration with industry and other organisations.
- Research and development in the above and cell culture related materials and products.
- To establish and conduct post graduate courses, workshops, seminars, symposia.
- To serve as National Reference Centre for tissue culture, tissue banking cell products and data bank etc. and to provide consultancy services to medical, veterinary, pharmaceutical institutions, public health services and industries etc. in the country.
- To provide and promote effective linkages on a continuous basis between various scientific and research agencies / laboratories and other organisations including industries working in the country.
- To participate in such programs as required in the country for the betterment of society and advancement of science and technology.
- To collaborate with foreign research institutions and laboratories and other international organisations in the areas relevant to the objectives of the faculty.

FROM THE DESK OF THE DIRECTOR

It is my pleasure to present the Annual Report of the National Centre for Cell Science (NCCS) for the year 2000-2001.The mission of our organisation continues to be research and development, teaching and training and providing service as a national repository for cell lines and hybridomas in the country.

NCCS is a growing organisation and in our endeavour to acquire critical mass, our efforts for searching new talents have been successful. This year we have been able to attract three young scientists specialized in the areas of Molecular Genetics, Developmental Biology and Cell and Tissue Culture. We have also recruited twenty research fellows supported by five technical staff and three administrative personnel. I am confident that these scientific teams would contribute towards fulfilling the goals of NCCS.

The Centre focuses its research activities primarily in the areas of cell biology, cancer biology, diabetes, tissue engineering, insect molecular biology and infection & immunity.

Our endeavour in the area of cell biology focuses on cell migration which plays a central role in many physiological processes such as tissue formation during embryogenesis, angiogenesis, neovascularisation, tumourogenesis and wound healing. Epithelial-mesenchymal transition is important in cellular transformation leading to cell motility. Our studies using extra cellular matrix (ECM) gels have revealed the involvement of matrix metalloproteinases during the process of epithelial-mesenchymal transition. Data from such studies can enhance our understanding of wound healing. The matrix metalloproteinases or the ECM degrading enzymes play a vital role in tumour metastasis. In this regard, the osteopontin, a member of the ECM protein family was demonstrated to induce the activation of matrix metalloproteinase-2 and enhance cellular migration and ECM invasion.

Stem cell biology has attracted global attention due to its potential applications in transplantation. Bone marrow contains different cell types at different stages of maturation, some of which are of special significance during transplantation. NCCS has developed technologies for preservation and revival of bone marrow stem cells which led to a successful transplantation in a neuroblastoma patient. We continue to pour in rigorous efforts to improvise the existing technology for better recovery and full functionality of these cells. In addition, we are applying this technology to preserve cord blood cells which are an important source of stem cells. The hematopoietic stem cells are also precursors of osteoclasts, which are large, multi nucleated cells that resorb bone. Osteoclastogenesis is induced by RANKL (Receptor Activator of NF-κB Ligand), which is known to be modulated by various cytokines. Our studies have indicated that Interleukin-4 inhibits the RANKL induced osteoclastogenesis.

The central question in stem cell biology is what factor(s) determine the path towards self renewal or differentiation along a specific lineage or remain quiescent or die. Research in this area has highlighted that erythropoietin treatment of bone marrow derived mononuclear cells resulted in the

HRD

Training was imparted to twenty people in the area of animal tissue culture and basic techniques and twelve summer trainees in various ongoing research areas

Twenty six fresh research fellows have joined our ongoing research projects and four students have obtained the degree of doctor of philosophy.

Stem cell biology

Stem cells are immunologically naive cells which have the potential to differentiate into any type of cells. At NCCS, we successfully developed methods for their cryo preservation and revival for stem cell banking.

secretion of TGFβ1, a known modulator of hematopoiesis. Our results also emphasise the role of activation of nitric oxide in signalling pathway.

Insect Molecular Biology

The NCCS focuses on characterisation of the mosquito mid gut flora and important physiological enzymes at the genetic level to understand the physiology which in turn may help in developing new strategies for mosquito control.

Mosquitoes serve as vectors for many diseases and pose a serious threat to health and economy. Despite best efforts, it is difficult to control mosquitoes due to our lack of knowledge about the physiology of this insect. We believe that our approach towards the characterisation of mid gut flora of mosquitoes will aid in designing better control strategies in the future. In this regard, we have carried out extensive analysis and characterisation of mid gut flora of mosquitoes that consists of *Acinetobacter*, *Bacillus*, *Aureobacter*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Enterococcus*, *Lactococcus* and *Xanthomonas* genera.

We have also sequenced and characterised two different forms of hexokinases of *Drosophila melanogaster*. The idea behind this venture is to identify the promoter elements that control the differential expression of brain hexokinases.

Diabetes and Tissue Engineering

Study and exploitation of naturally occurring compounds like curcumin and marine extracts for better management of diabetes.

Incidence of diabetes is on the rise in India and NCCS is committed to carrying out research in this area for understanding and designing better strategies that involve traditional management practices and modern science. Several endeavours in the past and present are hampered severely due to lack of *in vitro* models of the disease. We have developed *in vitro* models for regeneration of islets from mouse ductal epithelial cells which have potential application for transplantation and screening of compounds for anti-diabetic activity. We found that the traditional compounds such as copper sulfate and curcumin, a yellow pigment of turmeric (*Curcuma longa*) protects RINm5F cells from the cytotoxic action of streptozotocin that induces type 1 diabetes in animal models.

In the area of cancer biology, we have earlier identified a 1.8 kb gene which causes tumours in mice. Further analysis of this gene revealed that a 600 bp segment of the 1.8 kb gene, which has the tumourogenic property, belongs to the family of riboregulators. We have also successfully cloned Dlxin-1 from mouse embryo fibroblasts that has been shown to possess growth suppressor activity.

Cancer Biology

Investigating the pathways that can be exploited for induction of programmed cell death for a cancer cell. In addition, we also study the process that aids metastasis and mechanisms involved at the cellular and molecular levels.

In our continuous efforts to understand the disruption of cellular phosphorylation signals by α-hemolysin and the proteins resulting from its fusion with TGFα, it was found that apart from delinking the EGFr, the fusion proteins also delink all downstream signals. The metastasis of breast cancer cells in bone is a major concern. The mechanisms and factors that contribute to this phenomenon are not known. The bone derived factor reported by us earlier is being biochemically characterised.

Induction of apoptosis and search for agents as apoptotic inducers has recently become the focus of research in cancer biology. We have studied the mechanisms of staurosporin induced apoptosis in pediatric malignant neuroblastoma cells in relation to activity of specific caspases. We found that staurosporin induced apoptosis in neuroblastoma cells is mediated by caspase-3 independent pathway. In yet another approach, studies on the therapeutic potential of wild type p53 expression in cancer cells is being pursued.

Infection & Immunity

How the immune system copes up with infectious agents such as leishmania, malarial parasites, HIV and other viral agents is the major focus at the NCCS. Currently attention is devoted to understanding the molecules involved in this process.

Cell surface molecules play an important role in regulating the activities of immunocompetant cells. One such group of molecules are called costimulatory molecules. Earlier we have identified two such molecules viz. M150 and B2, which induce Th1 and Th2 responses respectively. Further characterisation of M150 showed complete sequence homology with Lamp-1. Expression studies in different cell lines show that Lamp-1 undergoes differential glycosylation in macrophages. We have also shown that the B2 molecule activates the naive CD4$^+$ T cells and skews their differentiation towards the Th2 phenotype. Additionally, our studies on the role of CD28 in neutrophil function showed that CD28 signalling results in interferon-γ secretion and that PI-3 kinase is involved in this signalling.

The incidence of HIV infection has reached alarmingly high levels worldwide, including India. Understanding the pathogenesis of the virus is important for developing new strategies. We have been successful in making a reporter CD4$^+$ T cell line expressing GFP under the influence of HIV-1 LTR which will be invaluable in the screening of anti-HIV compounds.

SMAR1, a member of the matrix attachment region (MAR)-binding proteins, has received a lot of attention in recent years. We have shown that an alternatively spliced form of SMAR1 is anti-tumourogenic in mice. The critical role of MARs in transcription through HIV-LTR promoter was also demonstrated; such sequences may be important in the establishment of HIV pathogenesis.

Viruses are obligate intracellular pathogens, thus they must overcome all formidable defence mechanisms of the host. Sequencing of the genome of vaccinia, herpes virus simiri and Kaposi's sarcoma associated herpes virus have revealed the presence of ORFs similar to human complement control proteins. We have initiated studies to characterize the role of these proteins in immune evasion.

We have also studied the role of the insoluble pigment hemozoin in the induction of immunosuppression in lymphocytes and found that immunosuppression was mediated by defective monocyte-macrophage function.

Our research efforts have been reflected in 25 publications in national and international journals. We have also attracted peer-reviewed extramural funding from national and international agencies and initiated collaborative projects in various research areas.

We supply cell lines and cell derived products to researchers in the country. This year we have supplied 515 cell cultures to 102 research organisations. In our training programmes we have trained 20 individuals and about 12 young graduates from various universities. The library and documentation facility has procured 655 journals and 130 books in the frontier areas of biotechnology and it continues to be the member of Pune library network.

We are fully aware of the challenges ahead of us and I am confident that our scientific teams will be geared up to accept them and forge ahead.

G.C. Mishra
Director

REPOSITORY

The repository at the National Centre for Cell Science is the only repository that houses human and animal cells in India. This repository serves to receive, identify, maintain, store, cultivate and supply animal and human cell lines and hybridomas.

The repository has procured cultures from various sources within the country and abroad from 35 animal species. A major bulk of the cell lines stocked in the repository have been procured from the American Type Culture Collection (ATCC) and the European Collection of Animal Cell Cultures (ECACC). At present, the total number of culture strains is 1127, of which about 300 are available for distribution to users on registration. Approximately 510 researchers from 275 institutes have registered with NCCS for the same. During the year 2000 2001, the cell repository of NCCS has supplied 515 cell cultures comprising of 148 different cell types to 102 research organisations in the country. The repository has initiated programmes to develop, immortalise and characterize cell lines from different tissue/tumour types.

HUMAN RESOURCE DEVELOPMENT

One of the main objectives of the Centre is to enhance human resources by conducting symposia, workshops and tailor made training programes for individuals. To achieve this goal, the centre has conducted two workshops on tissue culture techniques as follows:

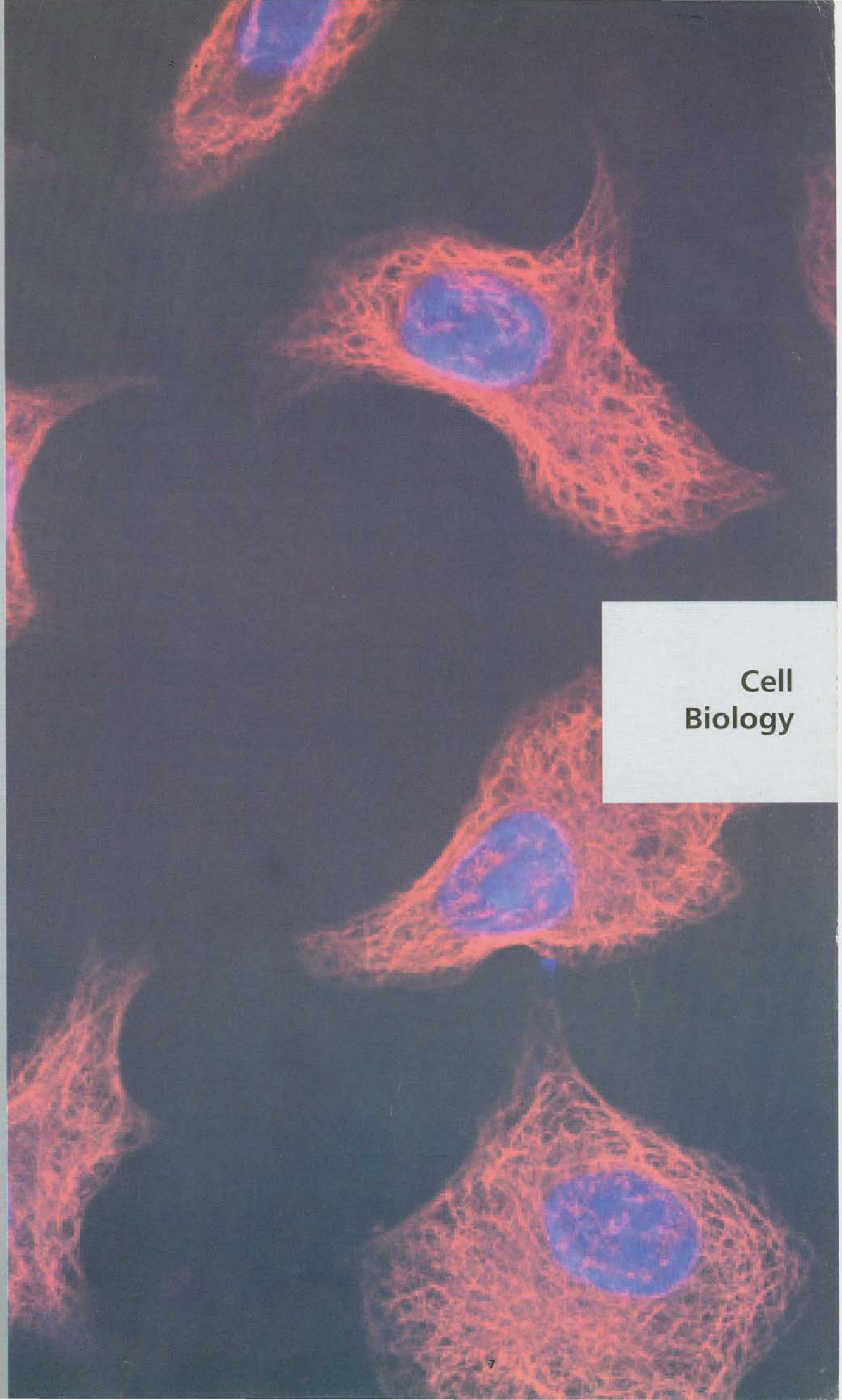
- Animal Tissue Culture: Principles and practice
- Basic techniques in animal tissue culture.

The total number of people trained in the above programmes during this year is twenty. In addition, the centre has also attracted several summer trainees from various universities which totals to twelve.

NCCS is pleased to welcome three new scientists, five technical staff and three administrative staff. Currently the total strength of the scientist at is twenty four.

NCCS scientists have actively participated in several teaching activities at various universities, colleges, departments (such as Departments of Zoology, Microbiology, and Biotechnology of University of Pune) and local colleges.

During this year twenty six junior research fellows have registered for Ph.D. and four students have obtained the degree of Doctor of Philosophy.



Cell Biology

Jayant M. Chiplonkar
jmchiplonkar@nccs.res.in

M. R. Vipra

STRUCTURE-FUNCTION ANALYSIS OF EUKARYOTIC CELLS: EPITHELIAL-MESENCHYMAL TRANSITION

Epithelial cells form a tight continuous layer of cells covering a body surface or lining a body cavity. However, cohesiveness of epithelial cells gets modulated at times and they are induced to dissociate, disperse and migrate. Cell migration plays a central role in many physiological processes such as tissue formation and remodeling in embryogenesis, angiogenesis and neovascularisation, wound healing and tumour invasion and metastasis. Many factors are thought to be involved in the acquisition of cell motility. Growth factors, primarily known to be regulators of cell proliferation, can also stimulate cell motility *in vitro*. Growth factors are also essential for tissue repair, morphogenesis and expansion, invasion and metastasis of tumour cells. Some types of tumour cells are reported to produce growth factor receptors thereby increasing their proliferative abilities.

Epithelial-mesenchymal transition (EMT) is a striking example of cellular transformation leading to cell motility and is characterised by the loss of epithelial and acquisition of mesenchymal features. Cells lose their cohesive nature and are converted into individual motile fibroblastic cells, which could result from interaction of more than one regulator. It has been reported that a scatter factor may promote EMT-like changes *in vitro*, for e.g. Haematopoietic Growth Factor influences both cell growth and motility. Each factor induces a particular transduction system and affects motility of cells; however, the net effect seen is a consequence of interaction of various transduction systems. Using *in vitro* wound healing models, it has been shown that repopulation of the denuded areas occurred, by the cells migrating from the edges of the wound, rather than by cell division. This leads to the question of how and when the signaling pathways triggered either a mitogenic or motogenic response.

Epithelial-mesenchymal transition (EMT) is a striking example of cellular transformation leading to cell motility and is characterised by the loss of epithelial and acquisition of mesenchymal features.

Aims

- To study the cellular response to external stimuli and occurrence of changes associated with epithelial-mesenchymal transition.
- To carry out a comparative analysis of migrating and non-migrating cells in *in vitro* wound healing models, cell migration and invasion.

Work achieved

An *in vitro* wound healing model has been developed to study the process of epithelial-mesenchymal transition (EMT), by SiHa and MDBK cell lines. We have earlier shown that cells synchronized at R in G1, could be induced to migrate. These migrating cells showed delayed time to reach G1/S phase as compared to the non-migrating cells in the monolayers. Further experiments were undertaken to analyze the response of the cells in S and G2 phases, which are committed to the progress of cell division. These cells were studied for the analysis of cell morphology, cell migration, and wound rehealing and further differentiation between migrating and non-migrating cells. Detection of cellular markers by immunocytochemistry was also studied for the comparative assessment of the cellular markers in migrating and non-migrating cells. Image analysis and confocal laser scanning microscopy have

revealed down regulation and up regulation in these two cellular variations, suggesting the occurrence of EMT-like changes in these cells. Degradation of the ECM proteins by migrating cells was detected by absence of ECM proteins like laminin and fibronectin.

Detection of cell motility and cell invasion was studied using uncoated and coated transwell membranes. Cell migration was assessed at 24, 48 and 72 hours. Induction of cell migration by foetal calf serum and growth factors FGF, EGF, and HGF occurred in uncoated membranes. On the other hand, lower degree of cell invasion occurred through membranes coated with thin layers of ECM gel. These observations have supported the occurrence of EMT in these cells.

Further characterisation to assess the possible role of matrix metalloproteinases in conditioned medium showed presence of MMP-2 as detected by zymography and fluorescence.

Future work

Efforts are underway to understand the role of cytoskeletal elements for conclusive support towards EMT-like changes in the same experimental system.

Lalita S. Limaye
lslimaye@nccs.res.in

Lalita Sassnoor
Nikhat Siddiqui

V. P. Kale
S.G.A. Rao
CRI, Mumbai
R. L. Marathe
Jehangir Hospital, Pune.

STUDIES ON CRYOPRESERVATION OF HAEMATOPOIETIC CELLS

Cryopreservation is now a common procedure to store cells, embryos and human tissues to use them at appropriate time for transplantation. Cryoprotectants such as dimethylsulfoxide (DMSO) and glycerol, together with serum are used to reduce deleterious crystal formation from free water. The use of various freezing devices allows a better control of the cooling rate and protects the cells from critical freezing stages. Bone marrow contains various cell types at different stages of maturation, some of which are especially important during transplantation. One major problem of preservation is the damage to the cells particularly during the phase transitions that take place during freezing and revival resulting in considerable cell loss. It is therefore important to optimize cell recovery and to study cell populations that are more affected during cryopreservation. The immediate goal of our project is to improve the recovery of haematopoietic cells in terms of viability and functionality as assessed by *in vitro* colony forming assays and engraftment potential of the frozen marrow. We have used certain bioantioxidants, membrane stabilizers, antifreeze proteins as additives in the conventional freezing medium to improve post thaw recovery. We are also studying the growth factor responsiveness, receptor status and progenitor subsets before and after freezing of haematopoietic cells. These studies will give us a deeper insight into the damage caused during freezing and suggest the ways and means to overcome the damage.

We have used certain bioantioxidants, membrane stabilizers, antifreeze proteins as additives in the conventional freezing medium to improve post thaw recovery. We are also studying the growth factor responsiveness, receptor status and progenitor subsets before and after freezing of haematopoietic cells.

Aims

- Standardisation of protocols for cryopreservation of haematopoietic cells and transfer of technology to hospitals that plan to undertake banking programmes.
- To study the mechanisms of cryodamage and standardise the composition of the freezing medium to achieve optimal recovery to suit indigenous conditions.
- To understand the low temperature biology of haematopoietic cells using primary human and murine cells and haematopoietic cell lines, so as to get a deeper insight into the cryopreservation phenomenon.

Work achieved

A total of 21 cord blood samples, 3 fetal samples and 22 murine bone marrow samples were used for various experiments as described below:

a) Large volume freezing in bags: Large sized cord blood and fetal liver samples were frozen in Baxter cryobags and the revival efficacy was assessed by total and differential colony counts. This is to extrapolate and strengthen our previous data of small volume freezing in vials. The results show that though there is a sample to sample variation, the overall post freezing recovery is good.

b) Recharging of irradiated stromal cell line with frozen cord blood /fetal liver cells and quantitating the longterm culture by CFU assay: The experiments are in progress and preliminary data needs further confirmation.

C) *In vivo* experiments on studying the engraftment potential of fresh and frozen marrow are being attempted in murine system. The time taken for neutrophils and platelets to reach normal counts in the peripheral blood lymphocyte smears is assessed and compared for different sets.

Future work

Further experiments are planned on *in vivo* engraftment and *in vitro* homing studies.

The effect of supplementation of certain membrane fatty acids with antioxidants to the cells after revival, will be studied extensively.

Bone marrow cryopreservation: Addition of membrane stabilizers and antioxidants in the conventional freezing mixture to conserve growth factor responsiveness and engraftment potential of the frozen marrow.

Our earlier work indicated that the growth factor responsiveness of frozen marrow cells is hampered during freezing and it is restored to some extent by addition of certain antioxidants. The aim of this project is to investigate this phenomenon in detail.

Aim

To study the behaviour of fresh *Vs* frozen cells with reference to following points:

- Analysis of progenitor subsets and growth factor receptor expression status of cryopreserved cells.
- Engraftment potential of stem cells *in vivo*.
- Reduction in DMSO concentration to a physiologically acceptable level by replacement / supplementation with membrane stabilizing agents.

Work achieved

The results show that additives like ascorbic acid, α-Tocopherol, catalase, trehalose and taurine help in restoring the proliferating response of the frozen cells to various CSFs.

Experiments were done using a human erythroleukemia cell line TF-1, known to be dependent on hGM CSF/ hIL-3 for its growth. The TF-1 is responsive to a variety of human haematopoietic growth factors like EPO, SCF, Interleukins etc. The cell line was used as a model for human haematopoietic cells. The cells were frozen with or without additives to the conventional freezing medium.

The viability and proliferative response of fresh vs. frozen cells were studied to detect the response of additives to DMSO in the freezing mixture by MTT assay and tritiated thymidine uptake. The results show that additives like ascorbic acid, α-Tocopherol, catalase, trehalose and taurine help in restoring the proliferating response of the frozen cells to various CSFs.

The changes in the expression of surface markers like CD15, CD34, CD45, adhesion molecules like CD43, CD44, CD49d, CD49e etc., before and after freezing are being analysed in cells by FACS (Fig.1) and preliminary results obtained on status of receptors to growth factors pre and post freezing are encouraging.

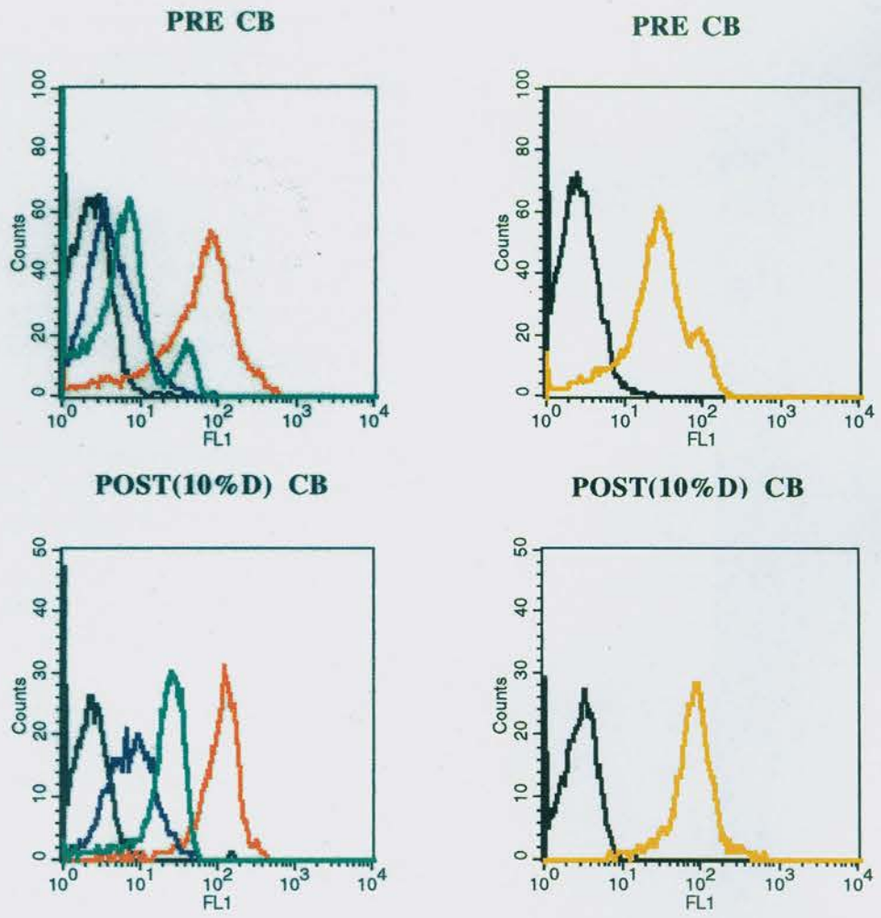


Fig 1. Expression of adhesion molecules (by cord blood cells) before and after freezing.

Future work

i) Attempts will be made to reduce or replace DMSO with other cryoprotectants.

ii) Experiments on growth factor receptors expression status of cryopreserved cells.

iii) *In vitro* LTBMC assay will be set up to study engraftment potential of fresh vs frozen haemotopoietic stem cells.

Vaijayanti P. Kale
vpkale@nccs.res.in

Suman Raphael
George Fernandes

L.C. Phadhy
TIFR, Mumbai

We are interested in exploring the factors responsible for bringing about the cellular cross talk in the marrow compartment and also to delineate the pathways involved in the regulation of HSC fate in either direction.

STROMAL CELL BIOLOGY

Development of hematopoietic stem cells (HSC) into fully differentiated blood cells is a multi-step process. This functional maturation takes place in the bone marrow microenvironment composed of many types of stromal cells and the extra-cellular matrix (ECM) molecules secreted by them. In a steady state condition, the HSC are believed to be present in a quiescent state. Recent reports, however, indicate that HSC do undergo a continuous turnover, albeit slowly. Since the body needs a constant supply of differentiated cells, it is necessary that a pool of HSC be maintained through out the life cycle. In order to achieve this, the processes of self-renewal and commitment to differentiation have to be balanced. Thus, a finely tuned and tightly controlled process is involved in the generation of blood cells from HSC.

How a stem cell decides whether to undergo self-renewal or to differentiate along a particular lineage or to remain quiescent or to die is poorly understood. Several experiments point towards the role of stromal cell derived signals in this decision making process, thus, underscoring the importance of cell-cell interactions. We are interested in exploring the factors responsible for bringing about the cellular cross talk in the marrow compartment and also to know the pathways involved in the regulation of HSC fate in either direction. Such knowledge would pave the way to generate cells of specific nature at least in culture and help identify the stromal cell mediated regulatory signals affecting the human haematopoietic stem cell fate.

Aims

- Elucidation of mechanistic aspects of TGFβ1 release from bone marrow derived mononuclear cells (BM-MNC).
- Identification of signals generated in the stromal cells in response to the treatment with TGFβ1 in the context of their involvement in hematopoietic support.

Work achieved

We have earlier found that the BM-MNC secrete a significant amount of TGFβ1 in the culture supernatant in response to erythropoietin treatment. The composition of the BM cells was found to have a profound effect on the levels of TGFβ1 secreted. Specific depletion of cells indicated that the process was critically dependent on T cell function. We found the presence of various phosphorylated signaling molecules like Jak 2,PT-PIC and Shc which are hallmarks of erythropoietin (Epo) mediated signaling pathway, in the lysates prepared from the $CD2^-$ enriched cell preparation after erythropoietin treatment. However, whether this is a direct action of Epo on $CD2^-$ cells or is mediated through some indirect mechanism is not certain. Since removal of granulocytes was found to increase the sensitivity of MNC to erythropoietin we examined this issue further. We immuno-precipitated the conditioned media prepared from the cell fraction enriched for $CD15^-$ granulocytes with anti-EpoR antibody. The western blot analysis of these precipitates showed that the cells secreted a large amount of soluble EpoR in the medium. It is likely that this soluble receptor could be responsible to control the action of erythropoietin on the BM cells.

Since TGFβ1 is a known modulator of hematopoiesis and also a potent morphogen, it's secretion from the MNC in response to growth factors is likely to have an important physiological significance. Secondly, the observation that the non-erythroid cells have the ability to respond to erythropoietin might have a wider implication in the development of hematopoiesis and also in the clinical application of this hormone.

Our results indicate that activation of nitric oxide signaling pathway could be one of the important down stream events in the TGFβ1 mediated signal transduction in the context of hematopoietic support.

Our earlier experiments have shown that treatment of BM derived stromal cells with TGFβ1 makes them more competent to support the growth of stem cells. We, therefore, carried out experiments to footprint the signaling pathways involved in the process. We have used pharmacological modulators of various pathways and colony formation or $CD34^+$ cell proliferation as the read out systems. The treatment of stromal cells with inhibitors of nitric oxide (NO) signaling such as cPTIO, inhibited the proliferation of $CD34^+$ stem cells co-cultured with them while the treatment with stimulators like SNN or SNAP stimulated the $CD34^+$ cells. Our results indicate that activation of nitric oxide signaling pathway could be one of the important down stream events in the TGFβ1 mediated signal transduction in the context of hematopoietic support.

Future work

Examination of the physiological significance of erythropoietin mediated TGFβ1 release.

Study of the role of activation of nitric oxide pathway in the stromal cells on the stem cell fate.

Mohan R. Wani
mohanwani@nccs.res.in

Latha Mangasetti
Shruti Khapli
Satish Pote

The lack of clear mechanisms for many bone-activate cytokines is largely attributable to the complexity of cell system previously used for the analysis of osteoclastic responsiveness. The recognition of RANKL will lead to new insights into the control of bone resorption and osteoclast formation by interleukins.

CELLULAR AND MOLECULAR REGULATION OF OSTEOCLAST DIFFERENTIATION AND ACTIVATION BY INTERLEUKINS

Osteoclasts are large multinucleated cells, which resorb bone. Osteoclasts differentiate from haemopoietic precursors of the monocyte/macrophage lineage through a cell-to-cell interaction with osteoblastic/stromal cells in the presence of calciotropic hormones. Receptor activator of NF-κB ligand (RANKL), a novel membrane-bound molecule is expressed by osteoblastic/bone marrow stromal cells. Recombinant soluble RANKL induces osteoclast formation and bone resorption in stroma free populations of haemopoietic precursor cells in the presence of macrophage-colony stimulating factor (M-CSF). RANKL replaces the need for both osteoblastic cells and calciotropic hormones in this system.

The lack of clear mechanisms for many bone-active cytokines is largely attributable to the complexity of cell system previously used for the analysis of osteoclastic responsiveness. The recognition of RANKL will lead to new insights into the control of bone resorption and osteoclast formation by interleukins. Interleukin 4 (IL-4) is a pleiotropic immune cytokine secreted by activated Th2 cells and mast cells which influences immunologic and haemopoietic processes. In addition, this cytokine has the potential to antagonise inflammatory and destructive mediators of the rheumatoid arthritis process. It is also known that IL-4 treatment can prevent cartilage destruction; however, the role of IL-4 in osteoclast differentiation and activation is not clear. We, therefore, propose to delineate the role of IL-4 in RANKL-induced osteoclastogenesis.

Aims

- To identify the target cells for the action of IL-4 on osteoclast differentiation and activation.
- To study the mechanism of action of IL-4 in the regulation of lineage commitment in bipotential osteoclast-macrophage precursors.

Work achieved

In the present study, we first examined the effect of recombinant mouse IL-4 on RANKL-induced osteoclast formation in spleen cells. Recombinant soluble RANKL induced the formation of multinuclear tartarate-resistant acid phosphatase (TRAP) positive osteoclasts in the presence of M-CSF in these cultures. These cells form resorption pits when incubated on bovine cortical bone slices. IL-4 inhibited osteoclast formation in a dose dependent manner in mouse spleen haemopoietic cells cultured for 7 days. IL-4 significantly inhibited osteoclast formation at minimum concentration of 1ng/ml (Figure1).

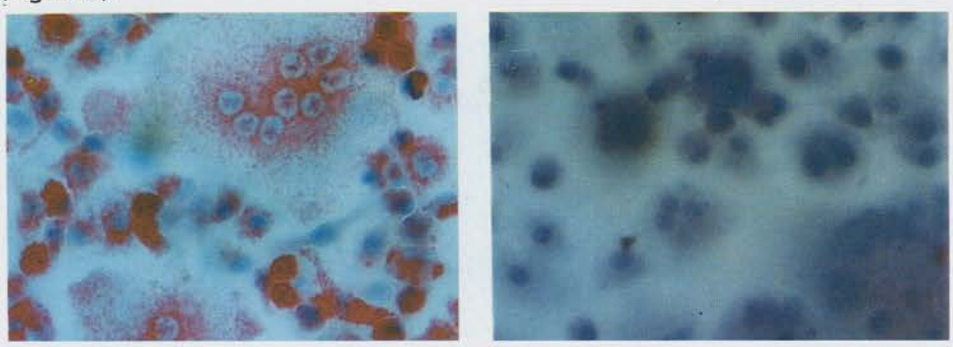


Fig 1. IL-4 inhibits RANKL - induced osteoclast formation in M-CSF- dependent haemopoietic precursors. Left: Haemopoietic precursors treated with M-CSF (30ng/ml) + RANKL (30ng/ml). **Right:** M-CSF + RANKL + IL-4 (1ng/ml) stained for TRAP. TRAP positive cells appeared red.(Magnification x 360)

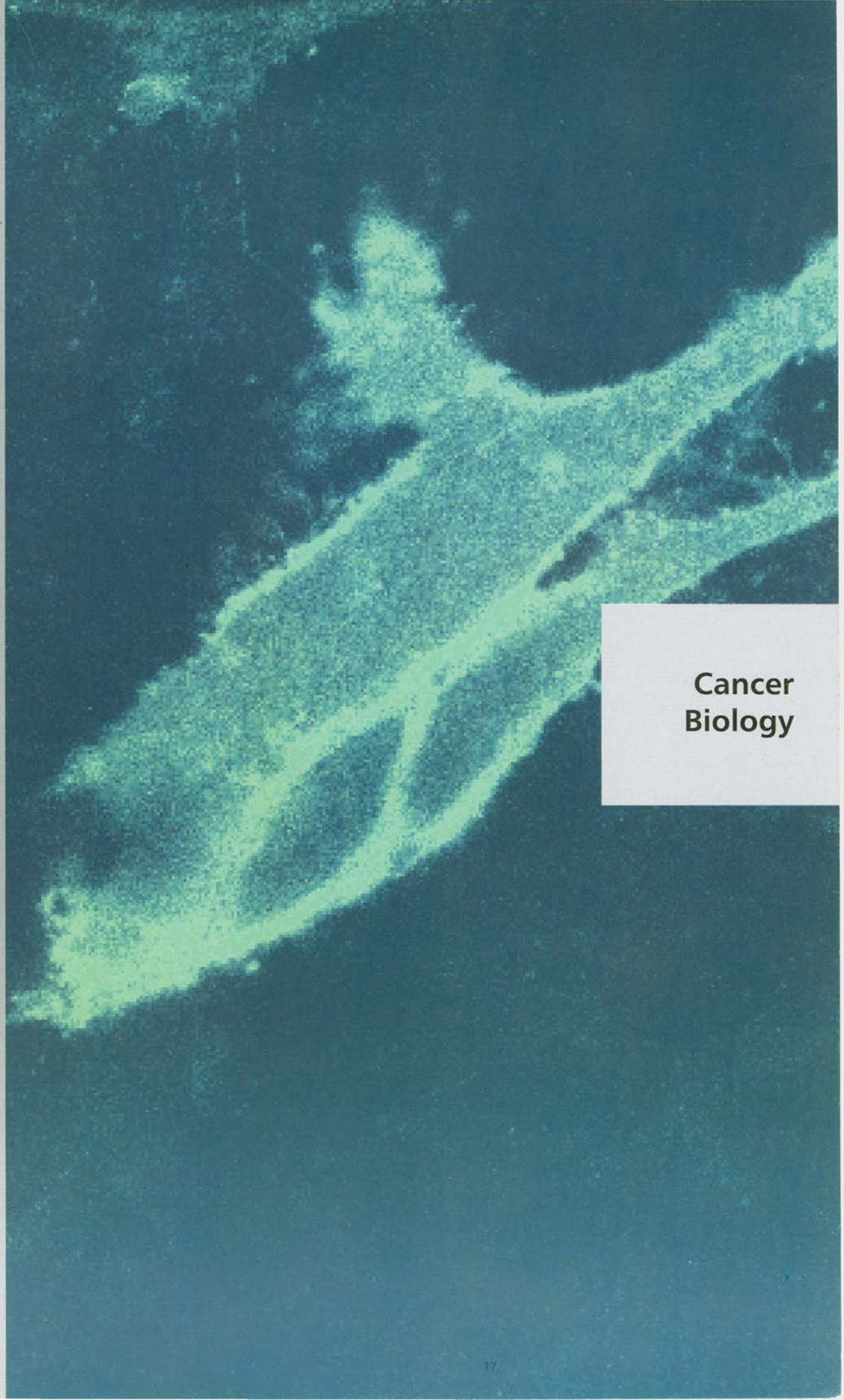
Spleen cells contain many other cell types including stromal cells and T cells. To determine the target cells for the action of IL-4 we further examined the effect of IL-4 on stroma free M-CSF-dependent mouse bone marrow cells. Absence of contaminating stromal cells was confirmed in cultures in which M-CSF was omitted. In M-CSF-dependent non-adherent bone marrow cells, RANKL induced osteoclast formation in the presence of M-CSF. IL-4 inhibited both osteoclast formation and bone resorption in these cells.The inhibitory effect of IL-4 on osteoclast formation in M-CSF-dependent mouse bone marrow cells was prevented by mouse anti-IL-4 antibody.

In the present study we show that IL-4 exerts potent dose-dependent inhibitory effect on osteoclast formation and bone resorption. This study also shows that IL-4 has a direct action on osteoclast precursors in the absence of stromal cells.

We have previously shown that PGE_2 synergise with RANKL to induce osteoclast differentiation. We found that IL-4 also inhibits the osteoclast formation induced by PGE_2. Thus in the present study we show that IL-4 exerts potent dose-dependent inhibitory effect on osteoclast formation and bone resorption. This study also shows that IL-4 has a direct action on osteoclast precursors in the absence of stromal cells. We are further investigating the mechanism of action of IL-4 in the regulation of lineage commitment in bipotential osteoclast-macrophage precursors.

Future work

Activated T cells also secrete RANKL and support osteoclast formation; however, the mechanism of this action is not known. Cytokine-mediated bone loss is a major feature of rheumatoid arthritis, osteoporosis and diseases of the immune system. Our future plan is to investigate the role of IL-4 in activated T cell induced osteoclastogenesis.



Cancer Biology

Anjali Shiras
anjalishiras@nccs.res.in

Varsha Shepal
Suvarna Deshmukh
Angana Sengupta

IDENTIFICATION AND CHARACTERISATION OF ONCOGENES IMPLICATED IN MELANOMA

Melanoma, is a highly metastatic and invasive cancer which occurs due to uncontrolled proliferation of melanocytes. The genetic and molecular mechanisms that lead to progression of melanoma are poorly understood. Mutations of p16 (CDKN2), p53, ras, neurofibromatosis type I gene (NF-1), bcl2 and the retinoblastoma gene have been described, but none are common, implicating for heterogeneous mechanisms of carcinogenesis. Both familial inheritance of potential tumour suppressor genes, e.g. p16, and differences in DNA-repair capacity contribute to the individual risk for melanoma. The genetic events, particularly aberration of cell cycle control and transcriptional control mechanisms are also implicated in the pathogenesis of melanoma. The project is aimed at the study of transforming genes implicated in melanoma using an expression cDNA library constructed from Clone M3, a mouse melanoma cell-line. We have identified a novel stretch of a 1.8 Kb region from M3 cells with tumourigenic potential and sequenced the molecule .

The project is aimed at the study of transforming genes implicated in melanoma using an expression cDNA library constructed from Clone M3, a mouse melanoma cell-line.

Aims

- Characterisation of the 1.8 Kb sequence to identify the regionresponsible for transformation.
- Expression Cloning of the identified region, sequencing, transfection and tumourigenicity studies to understand the mechanisms of transformation induced by the gene.

Work achieved

We have isolated, cloned and sequenced a 1.8 Kb gene with tumourigenic potential from mouse melanoma cells referred as Clone M3. Sequence analyses of the gene revealed that the molecule lacked long open reading frame. We isolated different clones by PCR from the 1.8 Kb stretch using internal primers. Interestingly amongst the various clones studied, we identified a 600 bp region with tumourigenic potential. The 600 bp sequence was cloned in both sense and antisense orientations. Using transfection, stable cell-lines were generated with insert in both orientations and assayed for functionality and growth potential (Fig.1). Only cells with insert in sense orientation could rapidly proliferate as was assayed by growth curve in culture (Fig.2) and were tumourigenic in nude mice. The 600 bp stretch cloned in reverse orientation was not tumourigenic. Expression studies by RT-PCR with primers from the 600 bp sequence revealed presence of the transcript in Clone M3, and transfectant cell-line but not in NIH3T3 cells . Interestingly, the specificity of expression to only Clone M3 and not NIH3T3 cells explains for the transforming potential of this clone. The sequence determination confirmed that the clones were novel, did not possess a long ORF and belonged to a novel family of proteins termed as *riboregulators.* Recently, a number of riboregulators with transforming potential are being identified like BORG-1 in Osteosarcomas. However, here we have isolated a class of molecules with unique structural and biological features.

The sequence determination confirmed that the clones were novel, did not possess a long ORF and belonged to a novel family of proteins termed as riboregulators.

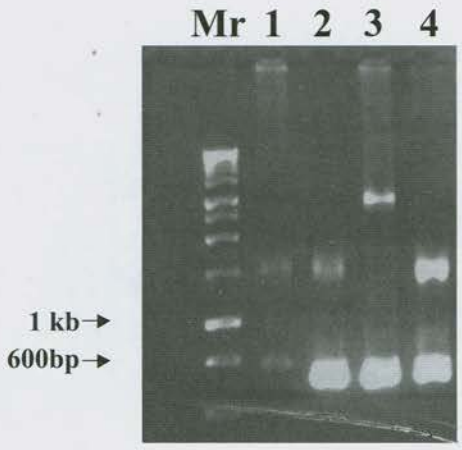


Fig 1. Genomic PCR of transfectants. Mr: DNA ladder. Lane 1: Vector transfected NIH 3T3. Lane 2: 600 bp sense transfected NIH 3T3 (T-7 and AS 12). Lane 3: 600 bp antisense transfected NIH3T3 (T7 and AS-7); Lane 4: Tumour tissue 600 sense cell line.

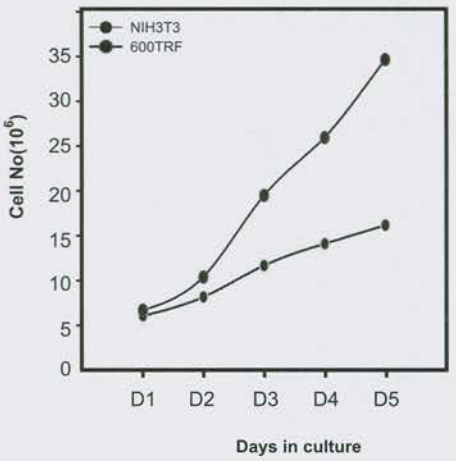


Fig. 2. Cell proliferation analyses

Future work

Study the tissue specific distribution of the 600 bp region in different mouse tissues and established cell-lines .

Understand the mechanism of transformation induced by the Riboregulator class of molecules with special emphasis on the 600bp clone.

Cloning and tissue specific expression studies with a member of mouse homeobox gene dlxin-1

Our study demonstrated for the specific expression of Dlxin-1 in primary and transformed cells of neuroectodermal and mesodermal origin with absence of expression to haematopoietic and epithelial cells.

The Dlxin gene family comprises of at least six mammalian homologues of the Drosophila homeodomain protein, distal-less (DLL) which is expressed strongly in the forebrain and limbs of developing fruit-fly. We have generated a 1 Kb Dlxin-1 cDNA clone from mouse embryo fibroblasts, sequenced the clone, and analyzed the tissue specific distribution. Our study demonstrated for the specific expression of Dlxin-1 in primary and transformed cells of neuroectodermal and mesodermal origin with absence of expression to haematopoietic and epithelial cells. The major emphasis of work this year was on study of the cell growth properties in NIH3T3 clones with ectopic expression of Dlxin-1 in sense and anti-sense orientations with respect to markers of cell proliferation like Ki67 using FACS and confocal microscopy. NIH3T3 cells transfected with sense Dlxin-1 constructs demonstrated a 60% decrease in proliferation potential as evidenced by Ki67 expression suggesting for a tumour suppressor function of Dlxin-1(fig.3).

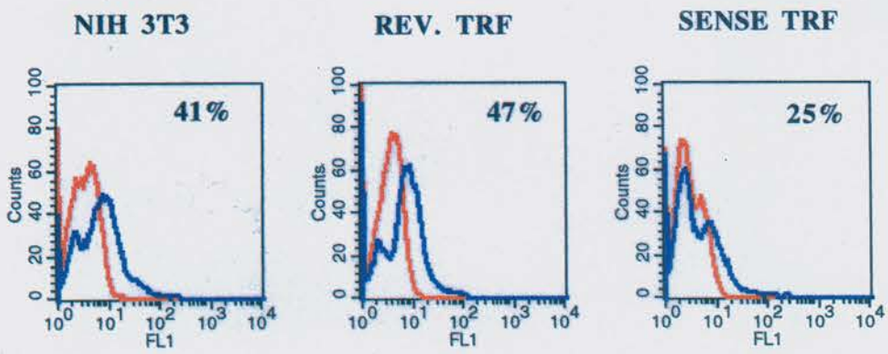


Fig 3. Ki 67 expression analyses in Dlxin-1 transfected clones.

The Dlxin-1 sequence amplified and cloned by us shows 75% homology to Necdin, a neuron specific growth suppressor. This region more or less spans the entire homology region of Dlxin-1 with Necdin. The growth inhibition with sense expression into NIH3T3 cells may be related to the growth suppressor properties of Dlxin-1.

Future work

Full length cloning of Dlxin-1 gene and expression analyses.

Study the tumour suppressor functions and adhesion properties of Dlxin-1 cDNA clone in relation to extracellular matrix components.

Musti V. Krishnasastry
mvks@nccs.res.in

Seshagiri Rao
Navneet Sangha
Surinder Kaur
Ravi Vijayavargia
Devapriya N Samanta
Anag Sahasrabuddhe
Anil Lotke

By studying the mechanism of action of a-hemolysin at the molecular level, we hope to redesign the molecule(s) in such a way that its lethal properties will be substituted with desirous ones.

THE αHLTGFα IS CAPABLE OF ENGAGING A PROTEIN TYROSINE PHOSPHATASE FOR AN EFFICIENT DEPHOSPHORYLATION OF EGFR

Intra and inter cellular signals play a vital role in proliferation and differentiation. Our group primarily focuses on how pathogens and other agents exploit the weakness in signal transduction pathways designed by nature. These pathways were investigated by employing a model pore forming protein, α-hemolysin (α-HL) to understand its influence on the signal transduction pathway initiated at the Epidermal Growth Factor receptor (EGFr). Our recent studies in this direction have revealed a total loss of phosphorylation signal of EGFr in the presence of α-HL. Further attempts were made to examine whether EGFr is capable of retaining the signal even when both α-HL and Transforming Growth Factor α (TGFα), a ligand for EGFr are joined together by genetic engineering. By studying the mechanism of action of α-hemolysin at the molecular level, we hope to redesign the molecule(s) in such a way that its lethal properties will be substituted with desirous ones.

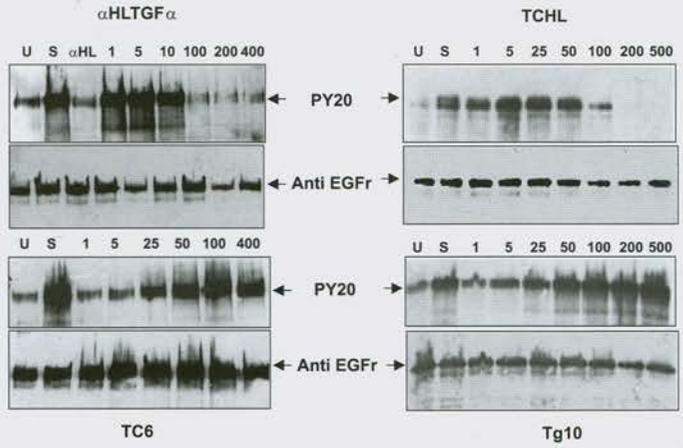
Aim

- To design various fusion proteins of α-HL, a pore forming protein from *Staphylococcus aureus* and TGFα and EGFr binding peptide for studying the functional ability of fusion proteins at the molecular level.

Work achieved

The α-HL soon after binding to target cells disengages the cellular communication pathway completely. The disengagement is done by activating a cell surface associated protein tyrosine phosphatase that removes the signal associated with the EGFr selectively. This feature is extremely important because α-HL is able to achieve the reversal of EGFr signal just by staying outside the cell unlike several kinase inhibitors that need to cross the cell membrane. Several of our initial studies have indicated the presence of a molecule that helps the α-hemolysin in this process.

In order to examine the process further, we have constructed several fusion proteins of α-HL and TGFα to study the ability of EGFr to retain the phosphorylation signal. All the fusion proteins have been able to bind to target cells and form oligomers like they do on rabbit red blood cells and lyse them. The phosphorylation assays carried out in the presence of these fusion proteins have revealed a concentration and time dependency as shown in Figure 1.



Effect of αHLTGFα, TCHL On tyrosine Phosphorylation of EGFr : A431 cells were incubated with indicated amount (nM)of fusion protein for 30 minutes at 37° C. U and S represents cells untreated and stimulated with TGFα (70 nM) respectively. α-HL represents cell treated with the toxin for 30 minutes. Top and bottom panels represent the blots probed with anti-phosphotyrosine and anti-EGFr respectively. The EGFr band is marked with an arrow. Tg10 and TC6 represent control protein where the signal is retained at the EGFr.

This data indicates that EGFr is unable to retain the phosphorylation signal even when its own ligand is present during the entire stimulation process in the presence of α-HL. It is clear from our other experiments that α-HL is able to take the help of a cell surface associated protein tyrosine phosphatase during this process.

This data indicates that EGFr is unable to retain the phosphorylation signal even when its own ligand is present during the entire stimulation process in the presence of α-HL. It is clear from our other experiments that α-HL is able to take the help of a cell surface associated protein tyrosine phosphatase during this process. It is equally interesting to note that the downstream signals from EGFr are also down regulated completely.

Future work

Examination of finer details of the loss of phosphorylation signals and the consequences that arise due to the observed phenomenon.

Padma Shastry
padma@nccs.res.in

Anmol Chandele
Ravi Shukla
Jayshree Jagtap

STUDY OF MECHANISMS AND PATHWAYS OF APOPTOSIS INDUCED BY STAUROSPORIN IN NEUROBLASTOMA CELLS.

Neuroblastoma is an extra cranial solid malignant pediatric tumour originating from neural crest cells and accounts for 12-15% of cancer related deaths globally. Neuroblastoma is refractory to conventional therapeutic regimens and therefore, alternative model of therapy/approaches are being explored for better outcome of treatment. Induction of apoptosis and search for agents/compounds as apoptotic inducers has recently become the focus of research in cancer biology. In NB also therapeutic approaches that are aimed at selectively activating apoptosis are under development and are thought to lead to a more effective and less toxic approach to the treatment. Protein kinase C (PKC) family plays an important role in key functions as growth, differentiation and survival and perturbations in signaling pathways thus, involving PKC inhibitors as apoptotic inducers in tumours is a topic of investigation. Staurosporin (STR) is a microbial alkaloid and is a broad-spectrum inhibitor of many PKCs. Though the ability of STR to induce apoptosis is reported, the exact mechanisms of apoptosis in different cell types are not clearly understood.

Induction of apoptosis and search for agents/compounds as apoptotic inducers has recently become the focus of research in cancer biology. In NB also therapeutic approaches that are aimed at selectively activating apoptosis are under development and are thought to lead to a more effective and less toxic approach to the treatment.

Aim

- To study the mechanisms of STR induced apoptosis in neuroblastoma cells in relation to the role of antiapoptotic and proapototic molecules of the BCL2 family and activity of specific caspases.

Work achieved

The study is done using two human neuroblastoma cell lines SK-N-MC and SK-N-SH. In our earlier work, we demonstrated that STR induced apoptosis in these two cell lines. The project was continued to decipher the pathway and STR induces mechanisms of apoptosis. Arrest of cells in G2/M is reported to precede apoptosis in many cell systems. We therefore analyzed the distribution of cells in different phases of cell cycle at time points preceding apoptosis. SK-N-SH showed a higher proportion of cells in G2/M and higher number of apoptotic cells at 12h and 24h respectively.

The caspase 3 activity was comparable in both the cell lines; SK-N-SH showed elevated levels earlier than SK-N-MC (Fig: 2). Interestingly, an inhibitor of caspase 3 - Z-As(ome)-Gln-Met-Asp(ome)-CH2F, did not inhibit apoptosis or cleavage of PARP though caspase 3 activity was inhibited.

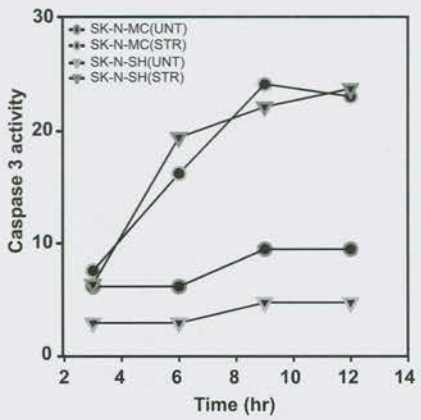


Fig 1. Effect of STR on caspase activity in neuroblastoma cells.

Poly ADP-ribose Polymerase (PARP, Mr 116 KD) plays an important role in repair of damaged DNA. Inactivation of PARP by its cleavage into an 89 KD product is the hall mark of apoptotic cascade. In NB cells treated with STR, cleavage of PARP was observed at 12h in SK-N-MC compared with 3 hr in SK-N-SH (Fig:1).

The cells positive for cleaved fragment of PARP was higher in G2/M compared to Go/G1, or S phase of cell cycle. Further studies were done to detect the enzyme activity of caspase 3, which plays a central role in apoptosis.

The caspase 3 activity was comparable in both the cell lines; SK-N-SH showed elevated levels earlier than SK-N-MC (Fig: 2).

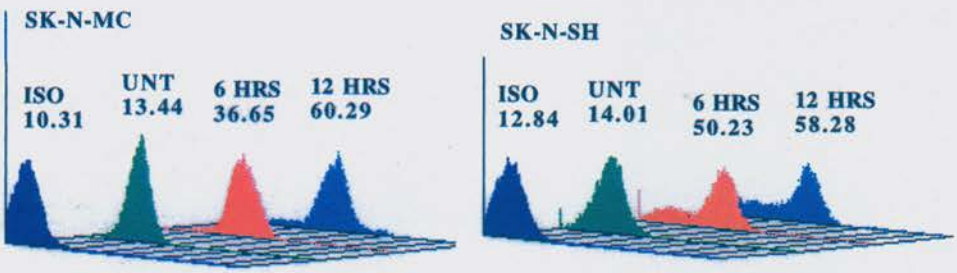


Fig 2. Cleavage of PARP with STR treatment

Interestingly, an inhibitor of caspase 3 - Z-As(ome)-Gln-Met-Asp(ome)-CH2F, did not inhibit apoptosis or cleavage of PARP though caspase 3 activity was inhibited. The data suggest that STR induced apoptosis in neuroblastoma cells is mediated by a caspase 3 independent pathway.

Further work

The role of other members of caspase family and release of cytochrome c will be investigated in apoptosis.

Gopal C. Kundu
kundu@nccs.res.in

Neeraj Tiwari
Subha Philip
Nitin Agarwal
Ganapati H.M.
Anuradha Bulbule

IDENTIFICATION AND CHARACTERISATION OF A NOVEL BONE DERIVED CHEMOATTRACTANT FACTOR THAT REGULATES BREAST CANCER CELL MIGRATION AND BONE METASTASIS

Breast cancer is a major cause of cancer-related deaths among women and its incidence is increasing rapidly. Although a number of genes like BRCA1, BRCA2, Syk are known to play significant roles in the regulation of breast cancer, the molecular mechanism(s) of breast cancer and its metastasis to different organs including bone is still unclear. Previous studies have shown that the inoculation of human breast cancer cells to nude mice developed osteolytic bone metastasis. Therefore, we proposed that bone or bone derived tumour cells might express chemoattractant like factor that might regulate breast cancer cell migration and bone metastasis. We have purified a chemoattractant protein (factor) from bone that showed the induction of breast cancer cell migration. Our recent data indicated that this factor interacts with its receptor in breast adenocarcinoma cells. Further characterisation of this factor and its receptor are in progress.

We proposed that bone or bone derived tumour cells might express chemoattractant like factor that might regulate breast cancer cell migration and bone metastasis. We have purified a chemoattractant protein (factor) from bone that showed the induction of breast cancer cell migration.

Aims

- To purify and sequence the factor from human bone extract and bone derived osteosarcoma cells that induces breast cancer cell migration and ECM invasion.
- To generate the specific antibody and clone the cDNA and characterize the putative receptor of the factor.
- To study the molecular mechanism of cell motility, invasiveness and bone metastasis in breast cancer cells.

Work achieved

We have purified a chemoattractant protein (factor) from bone extract by FPLC. The molecular weight of this factor is ~55 kDa as judged by SDS-polyacrylamide gel electrophoresis. This factor induces the dose dependent migration of breast adenocarcinoma cells (MDA-MB-231). The sequence of the 30 amino acids from the N-terminal region of the factor showed 67% homology with human serum albumin. An antibody against this protein has been generated and western blot analysis data identified a single band, indicating the specificity of the antibody. Receptor binding studies revealed that radiolabeled form of this protein binds with a putative receptor in breast adenocarcinoma cells. Recently, we have purified a similar protein from the conditioned medium of osteosarcoma cells that also induces the breast cancer cell motility.

Receptor binding studies revealed that radiolabeled form of this protein binds with a putative receptor in breast adenocarcinoma cells. Recently, we have purified a similar protein from the conditioned medium of osteosarcoma cells that also induces the breast cancer cell motility.

Future work

Further characterisation of this protein, cloning the cDNA of the factor and identification and characterisation of the receptor are in progress.

Osteopontin induced expression of matrix metalloproteinase-2 (MMP-2) correlates with enhanced invasiveness and tumour growth in melanoma cells

Osteopontin (OPN) is a member of extracellular matrix (ECM)-protein family

that plays a major role in cell adhesion, migration, ECM-invasion and metastasis in various cell types. Matrix metalloproteinases (MMPs) are ECM degrading enzymes that play a significant role in cell migration and metastasis. Our current investigation is focused on how OPN regulates the expression and activation of MMP-2 and controls the cellular migration, ECM-invasion and tumour growth using melanoma cells. These findings indicated that OPN induces the activation of MMP-2 and enhances the cellular migration, ECM-invasion and tumour growth in these cells. The mechanism of OPN induced MMP-2 activation, invasion and tumour growth will be studied.

Aims

- To investigate the effect of purified human OPN on the regulation of the MMP-2 production and activation in melanoma cells.
- To examine the role of OPN on cell motility and invasion and establish the functional correlation between OPN induced MMP expression and OPN-enhanced migration and ECM-invasion in these cells.
- To delineate whether OPN controls the tumour growth and to explore how MMP-2 is mechanistically involved in promoting tumour growth in nude mice.

Work achieved

In our earlier studies, we have shown that purified OPN from human milk induces the expression and activation of MMP-2 in a dose dependent manner in melanoma cells. Our data also demonstrated that treatment of these cells with purified OPN enhances the cellular migration and ECM-invasion by interacting with $\alpha v\beta 3$ integrin. Recently, we have shown that the OPN-enhanced migration and invasion are drastically reduced when OPN-induced MMP-2 expression is suppressed by transfecting the cells with MMP-2 specific antisense S-oligonucleotide. Moreover, nude mice injected with OPN treated cells developed large tumours and the levels of pro and active MMP-2 in tumours were significantly higher than those in control mice. (Fig 1. & Fig 2.)

Juxta -tumoural injection of MMP-2 antibody or MMP-2 specific antisense S-oligonucleotide dramatically reduced the OPN induced tumour growth as well as MMP-2 activation in nude mice.

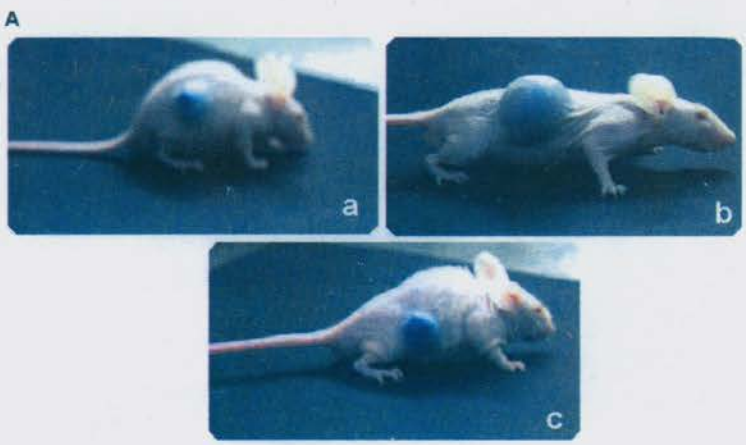


Fig 1. Typical photograph of OPN induced tumour growth in nude mice (a) control (b) treatment with OPN (10μM) and treatment with OPN (10μM) and MMP -2 antibody (50 μg/ml)

Juxta -tumoural injection of MMP-2 antibody or MMP-2 specific antisense S-oligonucleotide dramatically reduced the OPN induced tumour growth as

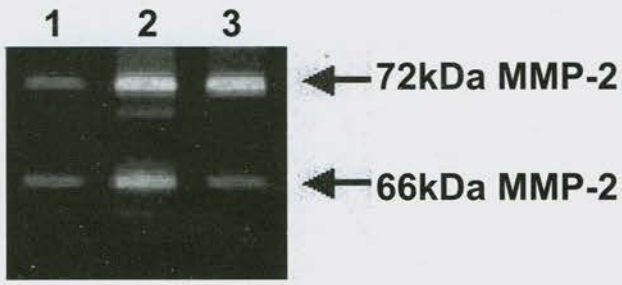


Fig 2. MMP- 2 expression in tumour of nude mice by zymography. (1) control (2) treatment with OPN (10μM) and (c) treatment with OPN (10μM) and MMP-2 antibody (50μg/ml)

well as MMP-2 activation in nude mice. This data indicates that MMP-2 plays a significant role in controlling invasiveness and tumour growth.

Future work

The molecular mechanism and pathways by which OPN regulates MMP-2 activation and tumour growth will be delineated.

Manoj Kumar Bhat
manojkbhat@nccs.res.in

Rishi Raj Chhipa
M.V. Vijay Kumar
Sachin Survae
Sandeep Singh
Reena
Pallavi Dhoot

REGULATED EXPRESSION OF EXOGENOUS WILD-TYPE TUMOUR SUPPRESSOR GENE IN HUMAN CANCER CELLS: ITS ROLE IN ANTI-CANCER THERAPEUTICS

Cancers appear at a substantial frequency in the human populace-implicating malfunction of specific components of genomic "care taker" systems leading to increased mutability of the human genome. The most prominent member of these systems is the p53 tumour suppressor gene. p53 plays a pivotal role in the control of normal cell growth and survival. It is a multifaceted transcription factor regulating multiple cellular processes including cell cycle progression, apoptosis, DNA repair and differentiation. Alteration of the p53 gene is most commonly reported genetic abnormality in a number of cancers. In tumours, the prevalence of p53 alterations varies, ranging from 10 to 60% in most cancers and topping up to 80% in some subsets. In order to contribute to the recent ongoing research in the area of cancer therapeutics, we are exploring the effect of wild-type p53 expression in human tumour cell lines. Inducible gene expression systems are being used to modulate such exogenous gene expression. The system allows regulated expression of the desired gene *in vivo* and biological consequences can be effectively monitored. Employing this system we desire to study the drug sensitivity in a set of isogenic cell lines in which the p53 gene is either over expressed or disrupted. Because the only difference among these cell lines is the absence or presence of activator, the interpretation of results will be particularly straightforward and uncomplicated.

Aim

- To study role of p53 over expression or deletion on anticancer therapeutic drugs.

Work achieved

We have standardised a regulated gene expression system and constructed a vector for developing suitable cell culture based model for studying the role of wild-type p53 in cancer therapeutics. To develop cell culture model(s) in which either p53 could be over expressed exogenously or function of endogenous p53 could be abrogated, we developed stable clonal cell line of human breast cancer and hepatocarcinoma cells. Developing such stable clones is two-step process. The first step involves transfection of cells with plasmid containing cDNA coding for inducer binding transcriptional activator and selecting antibiotic resistant clones. The second step involves transfection of stable clones with plasmid coding for wild-type p53 and selecting clones for a second antibiotic selection. Binding of inducer to the regulator protein enables it to transcribe genes downstream of regulator protein binding response element. We have successfully obtained stable clones constitutively expressing regulatory protein. Initially these clones were screened for their ability to drive expression of β-galactosidase reporter gene in the presence of the inducer. The β-galactosidase positive clones were further characterised. By transiently co-transfecting p53 expression plasmid and CAT reporter plasmid into these stable clones we have shown that p53 mediated CAT reporter activity is significantly higher in stable clone cells treated with the inducer compared to untreated cells. However, the degree of inducible

By transiently co-transfecting expression plasmid and CAT reporter plasmid into these stable clones we have shown that p53 mediated CAT reporter activity is significantly higher in stable clone cells treated with the inducer compared to untreated cells.

reporter acitivity is different among clones generated from same parental cells (Figure 1). We have also confirmed the CAT reporter activity is indeed regulated by the amount of inducer added to the cells (Figure 2).

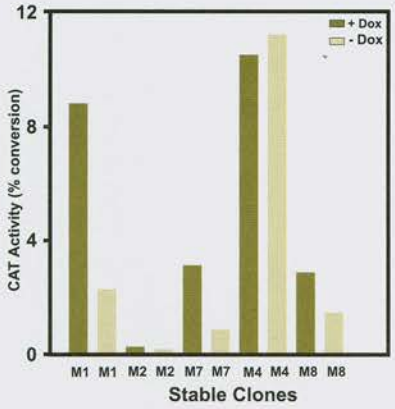


Fig 1. Modulation of p53 transcriptoinal activity in stable clones by inducer.

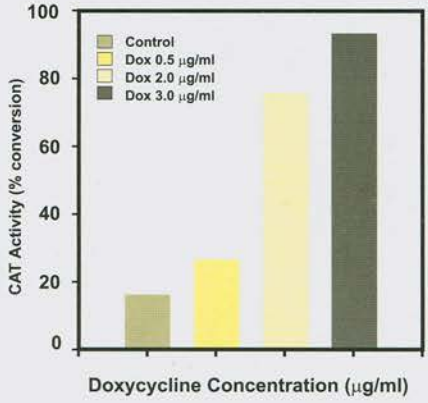


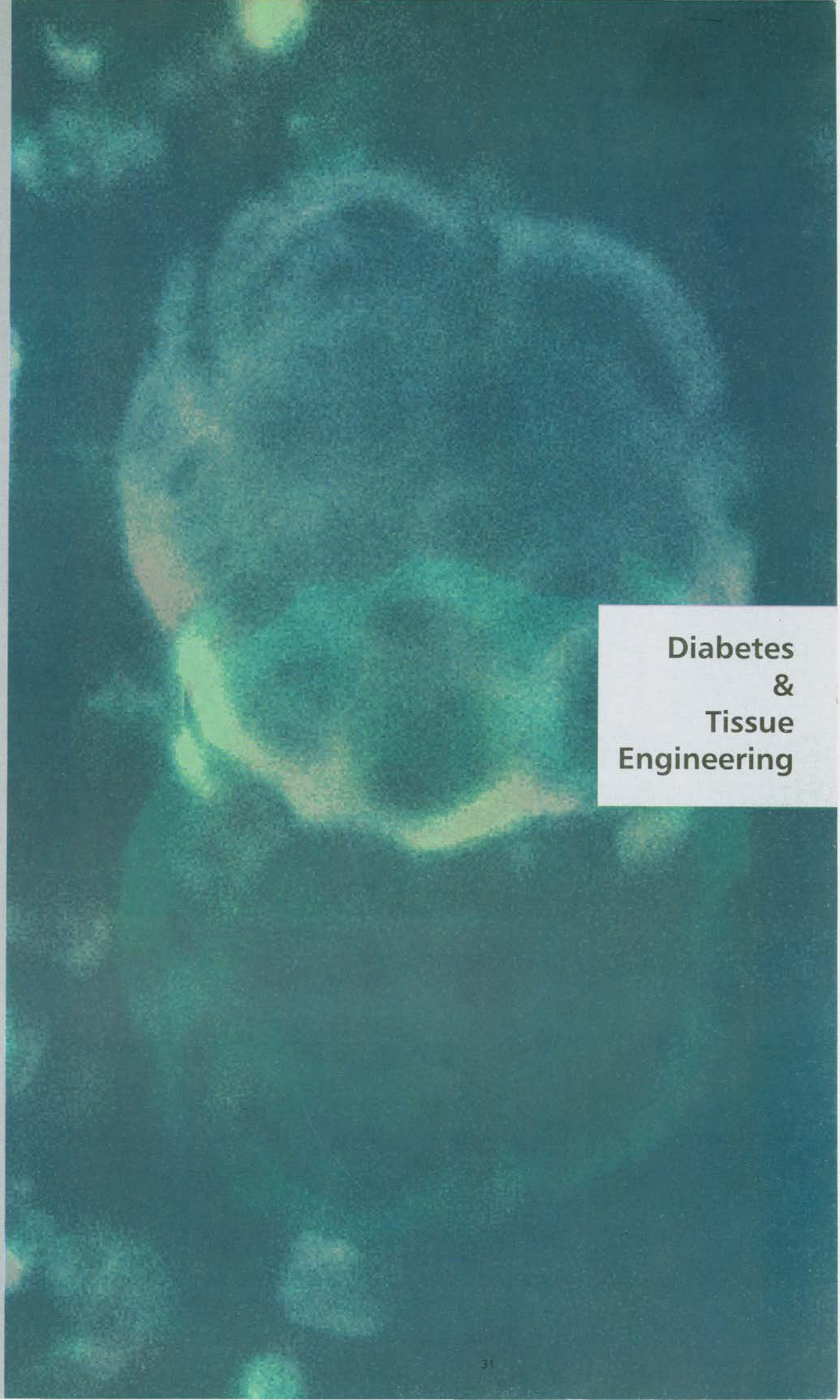
Fig 2. Transcriptional activity of exogenous p53 in stable clone.

Future work

Characterisation of double stable transfectants thus obtained.

Screening of individual clones for inducible expression, transcriptional activation by CAT reporter assays. Characterization with respect to growth properties and drug responses will be carried out.

Induction/down regulation of downstream proteins upon treatment with anticancer drugs by western analysis, and / or FACS analysis will be studied in these clones.



Diabetes & Tissue Engineering

Ramesh R. Bhonde
rrbhonde@nccs.res.in

Savita Kurup
Yogita Shewade
Malati Umrani

DEVELOPMENT OF EXPERIMENTAL AND THERAPEUTIC MODELS IN DIABETES AND PANCREATITIS

All the major events such as the development, morphogenesis, differentiation, and growth of epithelial organs for pancreas are regulated by factors derived from the fetal mesenchyme. These factors may be specific but are often ubiquitous in nature. Since regenerating liver contains growth factor(s), which directly or indirectly can induce islet cell proliferation and differentiation, exploration of such trophic regulatory mechanisms may have profound implications for the management of diabetes and the future of islet transplantation. Hence present studies were undertaken to evaluate the potential of regenerating liver extract in triggering islet neogenesis and thereby pancreatic regeneration in experimental diabetic mice.

Among various animal species, several syndromes resembling pancreatitis either occur spontaneously or can be induced experimentally by different procedures. None of these syndromes is able to truly reproduce the complexities of human pancreatitis; nevertheless each one of these can be very helpful in understanding at least some aspects of the pathogenesis and evaluation of the disease and a window for testing new antipancreatitc drugs. Many models have been described to elucidate the pathomechanism of acute pancreatitis under experimental conditions. The acute pancreatitis remains a disease with significant morbidity and lethality.

Among various animal species, several syndromes resembling pancreatitis either occur spontaneously or can be induced experimentally by different procedures. None of these syndromes is able to truly reproduce the complexities of human pancreatitis; nevertheless each one of these can be very helpful in understanding at least some aspects of the pathogenesis and evaluation of the disease and a window for testing new antipancreatitc drugs.

Despite significant merits of existing models for elucidating the mechanisms involved in early cellular events and pathogenesis of acute pancreatitis, these models have not lent themselves to the development of effective new therapies. Further progress seems to be hampered by the limitations of existing animal preparations.

Herein we have developed a method of producing acute pancreatitis in the BALB/c mice that we believe overcomes the shortcomings of previous pancreatitis models. Here we report a novel way of inducing and modulating pancreatitis in mice by injecting conditioned medium (CM) from MIA Pa Ca-2 (ATCC CRL-1420), a human pancreatic carcinoma cell line. The CM injection reproducibly causes an acutepancreatitis. The model appears to be superior for the study of innovative therapy aimed at preventing or limiting the necrotizing phase of pancreatitis.

Aims

- To evaluate the islet neogenesis potential of cytosolic extract from regenerating rat liver for their ability to cure experimental diabetes in mice, as regenerating pancreatic extract has been shown to possess nesidioblastotic activity.
- To develop a method of producing acute pancreatitis in the BALB/c mice that we believe would overcome the shortcomings of previous pancreatitis models.

Work achieved

Swiss albino mice (n=115) were rendered diabetic with streptozotocin (200mg/kg body weight) and randomized into three groups so as to receive either cytosolic extract (intraperitonially 100ul) of regenerating liver or

cytosolic extracts from normal liver for fifteen consecutive days and normal control. Mice treated with cytosolic extract from regenerating liver became euglycemic and attained normal body weight on day 22 and remain normoglycemic during 120-day follow-up. Diabetic controls remained hyperglycemic throughout the period of study with around 90% mortality by the end of one month following sustained uncontrolled hyperglycemia. Islet neogenesis was confirmed in mice treated with cytosolic extract from regenerating liver by increasing circulating insulin concentration (8.69±0.56 to 24.54± 1.77 μU/ml) (Fig1), islet area (1101±143μ² on day 7 to 13633.9 ± 685.43μ² on day 120), increase in body weight (12.57± 0.611 (day 1) to 22.29± 0.60 g (day22)) and subsequent decrement in plasma glucose (635.9±25.0 on day 1 to 187±18.93mg/dl on day 22) (Fig 2). Histological analysis of pancreas in the regenerating liver extract treated group revealed numerous tiny neo-islets as compared to the larger mature islets in the non-diabetic controls.

Histological analysis of pancreas in the regenerating liver extract treated group revealed numerous tiny neo-islets as compared to the larger mature islets in the non-diabetic controls.

Cytosolic extract from regenerating liver carry the potential to initiate islet neogenesis and restore normoglycemia in streptozotocin diabetic mice. Our findings could have important clinical implications in the management of diabetic mellitus.

It was found that repeated injections of CM from MIA Pa Ca-2 cell line for seven consecutive days led to acute pancreatitis in BALB/c mice as revealed by histological examination (showing edema, inflammatory infiltration etc.) and hyperamylesimia (Fig 3) irrespective of the day of collection of culture medium, indicating its potential to induce pancreatitis.

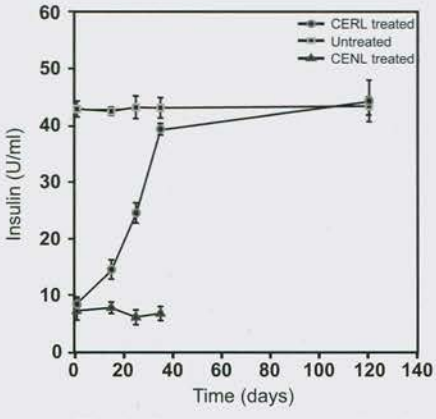


Fig.1. Serum Insulin profile in mice treated with CERL & CENL treated animals.

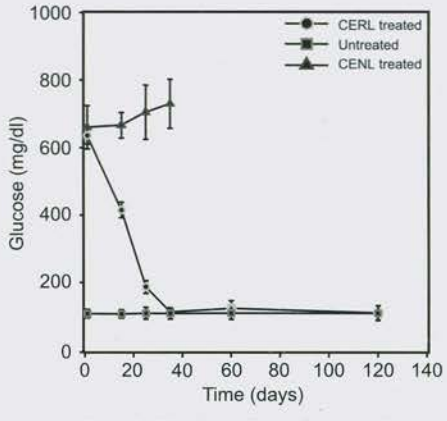


Fig. 2. Plasma Glucose profile in mice treated with CERL & CENL treated animals.

Pancreatitis model

In an effort to elucidate potential of secretory products of a pancreatic carcinoma cell line to induce acute pancreatitis, we injected BALB/c mice of either sex i.p. 0.1ml/day for a week with CM obtained from MIA Pa Ca-2, a human pancreatic carcinoma cell line. CM were collected from MIA Pa Ca-2 cell line at different post culture period viz. 24 h, 48 h and 72 h referred to as CM-24, CM-48, CM-72 respectively. The control and experimental mice were monitored for the changes in body weight, pancreatic weight, pancreatic volume, serum levels of amylase and histological examination was performed at specified time intervals on 1, 7, 14 and 28 days. The pancreatic weight/body weight ratio (pw/bw) was calculated in each case.

It was found that repeated injections of CM from MIA Pa Ca-2 cell line for seven consecutive days led to acute pancreatitis in BALB/c mice as revealed by histological examination (showing edema, inflammatory infiltration etc.)

and hyperamylesimia (Fig 3) irrespective of the day of collection of culture medium, indicating its potential to induce pancreatitis. Further it was shown that it is possible to modulate course of pancreatitis and induce regeneration of pancreas to restore normal architecture and serum amylase levels by selecting conditioned medium of specific day. The serum levels of cytokines such as IFN-γ, TNF-α, IL-6, IL-12 p40 and IL-6 (Fig 4) were found to be increased in the treated group indicating acute inflammation. The RNA expression of TNF-α and IL-6 confirmed the above results.

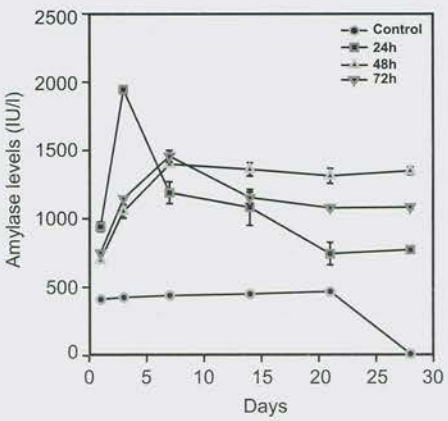


Fig. 3 Kinetic of Serum Amylase levels during pancreatitis.

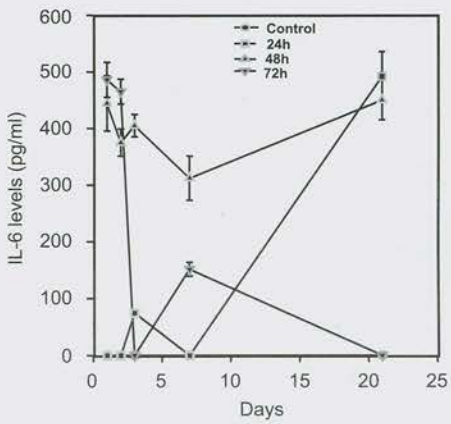


Fig. 4 Serum IL-6 profile during pancreatitis.

The peritoneal fluid from the treated and untreated animals was quantitated for $CD4^+$ $CD8^+$, MAC-1 and B220 cell population on different days by flow cytometric analysis. Which revealed increased levels of CD4 cells and MAC-1 during pancreatitis.

These studies led us to development of a new, easy and simple model for pancreatitis.

Since curcumin is also known to induce expression of HSPs we were interested in testing the hypothesis whether curcumin pretreatment to rat insulinoma cell line RINm5F may protect these cells from cytotoxic action of streptozotzocin (STZ) through the induction of stress proteins.

Role of curcumin in protecting RINm5F cells from cytotoxic action of Streptozotocin: It has been hypothesized that adequate stimulation of β-cells coupled with reduced oxidative stress and supplementation of antioxidants may prevent loss or even restore the β-cell activity from environmental diabetogenic insults. Curcumin, a yellow pigment of turmeric (Curcuma longa) used commonly as a spice has been shown to possess a wide range of therapeutic utilities in the traditional Indian medicine having antimutagenic, anticarcinogenic and antiinflammatory activity. Dietary curcumin has been shown to be beneficial in modulation of progression of renal lesions in diabetes.

The heat shock proteins (HSPs) have also been shown to participate in islet recovery from nitric oxide (NO) mediated islet damage. Role of HSPs for treatment of autoimmune diabetes and insulitis has also been suggested. Since curcumin is also known to induce expression of HSPs we were interested in testing the hypothesis whether curcumin pretreatment to rat insulinoma cell line RINm5F may protect these cells from cytotoxic action of streptozotzocin (STZ) through the induction of stress proteins.

In order to elucidate possible mechanism of cytoprotection offered by curcumin we studied induction of HSPs in curcumin treated cells. Studies revealed that the curcumin treatment led to up regulation of HSP-70 in a time dependant manner as confirmed by western blot analysis.

Aims

- To determine the role of curcumin in the induction of heat shock proteins.
- To ascertain the role of HSPs in the protection of RINm5F cells.

Work achieved

In order to investigate whether curcumin exerts any protective action against STZ induced cytotoxicity, we exposed RINm5F (Rat insulinoma) cells to 20 μM curcumin for different time intervals prior to STZ treatment. It was observed that viability of RIN cells was not affected by curcumin, alone which was comparable to that of control.

Streptozotocin (1 mM) treatment decreased the viability to 50% as compared to controls. However, RIN cells exposed to curcumin pretreatment followed by STZ challenge, exhibited higher viability in time dependant manner, the optimum being obtained at 7 hrs. treatment, indicating cytoprotection offered by curcumin. FACS analysis showed that curcumin pretreatment offered protection against STZ induced apoptosis in RIN cells. In order to elucidate possible mechanism of cytoprotection offered by curcumin we studied induction of HSPs in curcumin treated cells. Studies revealed that the curcumin treatment led to up regulation of HSP-70 in a time dependant manner as confirmed by western blot analysis. The upregulation of HSP-70 corresponded with viability of cells conferring the cytoprotective action of curcumin to the induction of HSP-70. The study emphasises the need of curcumin as a dietary supplement to protect β-cell mass and prevent their apoptosis from diabetogenic insults.

Future plans

It is planned to extend these studies in the islets in vitro as well as in vivo to assess the effect of curcumin on induction of HSPs and thereby protecting islets from diabetogenic insults.

Pradeep B. Parab
parab@nccs.res.in

Mamata Katdare
Puroshottam Kumar

STUDIES ON TYPE 1 DIABETES MELLITUS (IDDM)

Insulin dependant diabetes mellitus is an autoimmune disease characterised by a progressive T cell mediated β-cell destruction. In both human and animal models of type 1 diabetes, modulation of this destructive response can be affected by tolerance or by immunization of β-cell specific autoantigens such as insulin and Glutamic acid decarborylase (GAD65) either during the prediabetic stage or at the manifestation of the disease. The other way to prevent overt diabetic condition in type 1 diabetes is to increase the biomass of insulin producing β-cells either through islet transplantation or by stimulating new pancreatic islet tissue regeneration.

In both human and animal models of type 1 diabetes, modulation of this destructive response can be affected by tolerance or by immunization of β-cell specific autoantigens such as insulin and Glutamic acid decarborylase (GAD65) either during the prediabetic stage or at the manifestation of the disease.

Aims

To study experimental models of IDDM with reference to :

- *In vitro* regeneration of islets from mouse ductal epithelial cells which might have applications for transplantation.
- Induction of diabetes in rats by immunization with 1 Fluoro 2-4-dinitrobenzene (FDNB) tagged RIN cells.
- Identification and isolation of proliferative and differentiating factor(s) of insulin producing cells with potential application in diabetes management.

Work achieved

Islets were generated from mouse pancreatic ductal epithelial cells and stained with insulin specific stain i.e. DTZ. Expression of mRNA transcripts of insulin, PDX-1 and Reg-1 gene were identified on regenerated islets. Glucose challenge studies on islet's ability to secrete insulin are underway.

Rat insulinoma cells were tagged with 1-Fluoro2-4-dinitrobenzene (FDNB) and used for immunization. Diabetes was produced in rats after long exposure with the FDNB tagged RIN cells (Fig.1). The findings in the present study suggests that it is possible to chemically modify the surface of the RIN cells, which initiates inflammatory process in pancreas leading to insulitis in experimental animals. We have identified a 112 kDA antigen on the surface FDNB-tagged RIN cells: this auto antigen might be a candidate antigen for vaccination for the delay or prevention of type 1 diabetes.

The findings in the present study suggests that it is possible to chemically modify the surface of the RIN cells, which initiates inflammatory process in pancreas leading to insulitis in experimental animals.

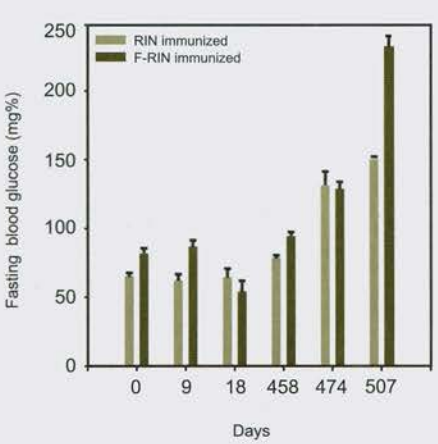


Fig 1. Fasting glucose levels in F-RIN & RIN immunized rats.

We have also developed three mouse monoclonal antibodies against human insulin that could be used in developing a sandwich ELISA for detection of circulating insulin levels.

Rat pancreatic acinar cell line AR42J is known to differentiate into insulin producing cell line in the presence of Activin A and Betacellulin. We are studying the proliferative and differentiating activities of perivitrulline fluid of the fertilised eggs of Horse Shoe

crab on AR42J cell line. Preliminary studies showed the presence of proliferating activity in perivitrulline fluid as assessed by [^{3}H]-thymidine incorporation. Protein profile of perivitrulline fluid was studied by SDS-PAGE.

Future work

Regenerated islets will be isolated from culture and their functionality will be assessed by challenging with glucose to measure amount of insulin release. Various other growth factors will also be used in pancreatic ductal epithelial cell culture to accelerate islet cell regeneration. Further studies to investigate the effect of immunzation with FDNB tagged MIN cells on STZ-induced type 1 diabetic model will be undertaken.

Purification and characterisation of various fractions from perivitrulline fluid will be done. These fractions will be tested for their proliferative and differentiating activities *in vitro*.

Sandhya Sitasawad
ssitasawad@nccs.res.in

Tanuja Bankar

Ramesh Bhonde
Debashis Mitra

Infiltrated macrophages and T cells were found to secrete cytokines such as IFN-, TNF-a and IL-1, which induce destruction of b-cells in vitro by production of ROI's.

OXIDATIVE STRESS IN DIABETES MELLITUS AND DEVELOPMENT OF THERAPEUTIC STRATEGIES

Insulin dependent diabetes mellitus (Type 1) is a T cell mediated autoimmune disease localized to the endocrine pancreas that occurs spontaneously in genetically predisposed individuals. A common pathological feature is the infiltration of inflammatory cells into the islets, followed by the selective destruction of β-cells. Infiltrating cells are composed of T and B lymphocytes, macrophages and NK cells among which T cells and macrophages are believed to play the major role in β-cell destruction. Earlier reports suggest that locally produced reactive oxygen intermediates (ROI's) are involved in this effector mechanism of β-cell destruction. Infiltrated macrophages and T cells were found to secrete cytokines such as IFN-γ, TNF-α and IL-1, which induce destruction of β-cells *in vitro* by production of ROI's. ROI's are further known to disrupt antioxidant enzymes by releasing trace elements. The copper and zinc status in the blood of diabetic patients and their descendants is found to be decreased, providing further evidence for a role for these anti oxidant trace elements in this disease.

Aim

- To evaluate the potential usefulness of copper sulfate in the prevention of STZ-induced type I diabetes.

Work achieved

We used C57BL6 female mice, in which copper sulfate treatment was started at 6 weeks of age (fifteen doses on alternate days) followed by an IP injection of 40 mg/kg body weight STZ for five consecutive days. The efficacy of the treatement was evaluated at 10 weeks of age. The treatment with copper sulfate significantly decreased blood glucose levels (Fig.1), levels of lipid peroxidation (Fig. 2) and mRNA expression of the enzyme iNOS and the cytokines IFN-γ and IL-4.

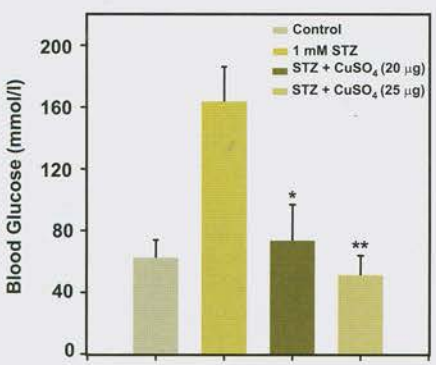


Fig 1. Fasting plasma Glucose levels in C57BL6 female mice (10 weeks of age) treated with and without physiological concentrations of copper sulphate. Data are means ± SD (n=5). * Significantly different from Group 1 (P<0.05). ** Significantly different from Group2 (P<.005).

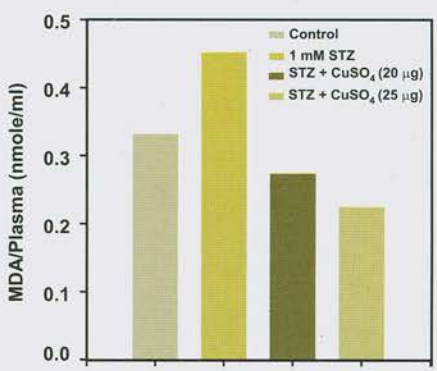


Fig. 2. Effect of CuSO4 on STZ induced lipid peroxidation in C57BL6 female mice. Six to eight week old C57BL6/ female mice were given physiological dose of $CuSO_4$ i.e. 20µg/0.2ml and 25 µg/0.2 ml dissolved in distilled water respectively on every alternate day for 30 days followed by intraperitoneal injections of STZ (Sigma) at a dose of 40mg/kg body weight for 5 consecutive days in Groups 2, 3 and 4. Lipid peroxidation was estimated and expressed in terms of mole MDA/ml plasma. Data are means ± SD (n = 7). * Significantly different from Group 1 (P < 0.05). ** Significantly different from Group2 (P < 0.05).

Generalized increase in the peripheral circulating IgG_1 and IgG_{2a} levels was observed in all groups as compared to the control group. However no particular trend of shifting of the ratio of IgG_1/IgG_{2a} was evident (Fig. 3). Histological analysis of the pancreas revealed that, three out of five animals in copper sulfate treated groups showed absence of mononuclear cell infiltration and no change in the shape and size of islets as compared to pancreas of STZ-induced diabetic group of animals (Figs. 4a and 4b).

Histological analysis of the pancreas revealed that, three out of five animals in copper sulfate treated groups showed absence of mononuclear cell infiltration and no change in the shape and size of islets as compared to pancreas of STZ-induced diabetic group of animals

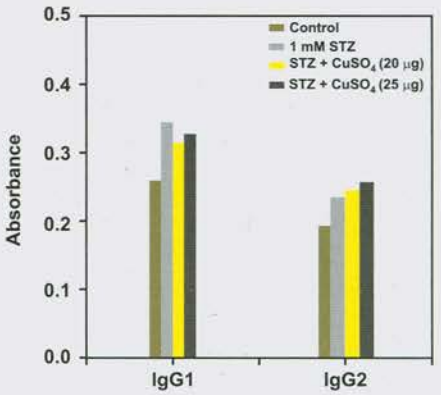


Fig 3. IgG1 and IgG2a levels in C57Bl6 female mice treated with and without physiological concentrations of copper sulphate.

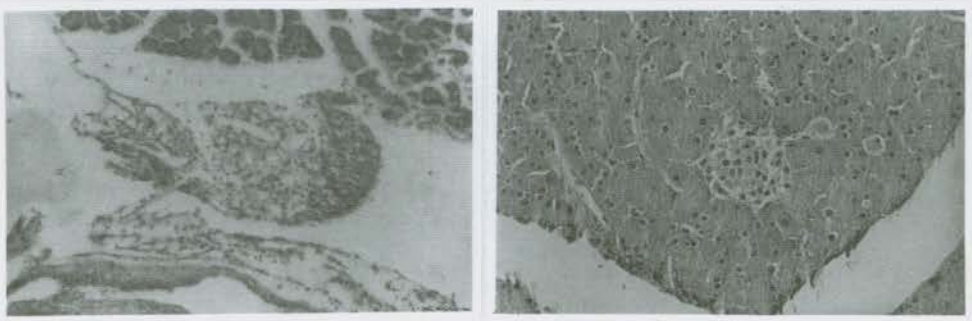


Fig.4. Pancreas from C57BL6 mice (a) Group 1, STZ-induced diabetic animal. HE and light green stained. Large islet showing infiltration of mononuclear cells (X100). (b) Group 3, 20µg/0.2ml $CuSO_4$ treated animal. Shape and size of the islet is normal (X400).

Tissue engineering approach may ultimately allow the development of viable autologous vascular grafts for clinical use. Novel approaches for producing small-caliber arterial grafts have been developed, however, the approaches have suffered problems either with mechanical properties or the utilization of neonatal cells.

Tissue engineering of functional pulmonary arteries/veins for transplantation: pattern of gene expression in monolayer and 3-D culture

"Repair" of many congenital cardiac defects requires the use of conduits to establish right ventricle to pulmonary artery continuity. Common treatments for coronary artery blockage include by-pass grafting and balloon angioplasty. Angioplasty avoids the need for major surgery and is usually preferred. The drawback of the procedure is that in 30-40% of patients, the treated vessels reocclude (restonosis) within six months due to smooth muscle cell overgrowth.

At present surgical mainstays of therapy of affected vessels which is less than 6 mm in diameter include bypass grafting with autologous veins or arteries, however, adequate tissue for bypass conduits is lacking in many patients. Artificial materials, when used to bypass arteries less than 6 mm in diameter, have thrombosis rates greater than 40% after 6 months. Therefore, tissue engineering approach may ultimately allow the development of viable autologous vascular grafts for clinical use. Novel approaches for producing

small-caliber arterial grafts have been developed, however, the approaches have suffered problems either with mechanical properties or the utilization of neonatal cells. Production of small-caliber autologous arteries *in vitro* from vascular cells grown on a biodegradable polymer matrix such as polyethylene glycol, by means of pulsatile perfusion system for vessel culture is another novel strategy. However, implantation of such biomaterial for a longer periods may lead to change in the polymer structure due to its interaction with proteins that may lead to toxicity, risk of bacterial infection, limb loss and death. Development of a technique to produce small- caliber autologous arteries *in vitro* and *in vivo* by using vascular endothelial growth factor (VEGF)/basic fibroblast growth factor (bFGF), gene targeting and development of *in vitro* blood vessels are the newer approaches hereby taken.

Aims

- To use acellularized placental vessels as a natural scaffolding material on which endothelial cells (EC) and smooth muscle cells (SMC) isolated from a small fraction of the patients blood vessel will be cultivated and a blood vessel will be grown by pulsating the tube.
- To study the changes in expression of genes involved in the blood vessel formation in isolated monolayered endothelial cells in culture and in 3-D system
- To study neovascularisation using cancer cells and VEGF genes near the plaque formation to the *in vitro* methods and also using animal models.

Work achieved

Method of acellularization of the tube using mice aorta was done by the repeated freezing and thawing method. Human mammary artery samples were obtained and processed for the isolation of endothelial cell cultures. Endothelial cells were isolated using dispase II treatment but the cells did not grow further due to lack of the growth media.

However, endothelial cells were isolated from the rat aorta by using the combination of dispase II and collagenase IV. These cultures were grown in the EC culture media which showed good cultures. Mouse aorta explant cultures were also standardised successfully.

Future work

Endothelial cells and smooth muscle cells will be isolated from a small fraction of the patients blood vessel, cultivated and a blood vessel will be grown by pulsating the tube. In the parallel studies, development of new blood vessels (angiogenesis) from the existing ones will also be done. This will be achieved, by transfecting the isolated endothelial cells with the VEGF (VEGF 121/VEGF 165) and bFGF genes and injecting/ targeting these cells near the site of the plaque.

Histologic examination of the blood vessel with Verchoff's elastin stain, Masson trichome stain and immunostaining for the EC markers platelet endothelial cell adhesion molecule (PECAM), von Willebrand factor as well as for the markers for SMC's such as smooth muscle α-actin, calopin and

myosin heavy chains will be done by confocal laser microscopy. characterisation of the blood vessel will be done for wall thickness, collagen content, suture retention by scanning electron Microscopy and other techniques. Mechanical strength will be tested by study of contractile forces and quantitation of stress-strain relationship. Rabbit will be used as a model in this study.

It is observed that, there is a change in the pattern of gene expression when the isolated EC's and SMC's forms a 3-D structure, such as changes in the secretion of non coagulating/plaque forming products etc. The pattern of gene expression in the isolated EC's and SMC's and the change during a 3-D culture will be investigated.

Manoj Mojamdar
manojmojamdar@nccs.res.in

Vaishai Chaubal
Sujit Nair

REGULATION OF MELANOGENESIS IN B16 MELANOMA CELLS: ROLE OF γ-GLUTAMYL TRANSPEPTIDASE AND MELANOCYTE-SPECIFIC GENE-1

Mammalian pigmentation is regulated at many different developmental and cellular levels and is influenced by many genes, either directly or indirectly. Two kinds of melanin, eumelanin and pheomelanin are synthesized by melanocytes within specialized organelles called the melanosomes. The availability of thiol compounds, namely cysteine and glutathione regulates the eumelanin-pheomelanin switch and hence represents an important control point in pigment synthesis. While the function of γ-glutamyl transpeptidase is understood in several tissues, it is still ambiguous in melanomas and melanocytes and several controversies shroud its role.

The melanocyte-specific gene-1 (MSG1) was initially identified as a candidate pigmentation-related gene product by mRNA differential display between highly pigmented B16F1 and poorly pigmented B16F10 mouse melanoma cell lines. Expression of MSG1 in adult animals is relatively confined to normal melanocytes, nevocytic nevus cells, retinal pigmented epithelial cells and low-malignancy melanocytes. Melanocytic differentiation has been shown to be a prerequisite for high levels of MSG1 expression in human melanocytes and melanoma cells. These observations have suggested a role for MSG1 in melanogenesis. Biochemically, MSG1 is a 27-kDa nuclear protein belonging to the MSG1/CITED family of transcriptional co-activators.

Melanocytic differentiation has been shown to be a prerequisite for high levels of MSG1 expression in human melanocytes and melanoma cells. These observations have suggested a role for MSG1 in melanogenesis.

Aims

- To elucidate the role of γ-glutamyl transpeptidase in melanogenesis. To understand the prooxidant role of γ-glutamyl transpeptidase and its effect on melanogenesis.
- The regulation of γ-glutamyl transpeptidase in B16 melanoma in different pigmentary conditions.
- To elucidate the regulation of the pigment-cell specific genes by MSG1
- To understand the role of MSG1 in tumourigenesis.

Work achieved

A. Role of γ-glutamyl transpeptidase in melanogenesis:

Work done in our lab has shown that contrary to the earlier studies, membrane bound γ-glutamyl transpeptidase activity does not significantly contribute to cellular cysteine levels required for pheomelanin synthesis within B16 melanoma cells. Our results have clearly shown that inactivation of γ-Glutamyl transpeptidase did not affect levels of pheomelanin as studied by HPLC analysis. We have shown that melanosomes purified from B16 melanoma have a very negligible γ-GT activity, thus our studies tend to support the view that 5-S-cysteinyl dopa rather than glutathionyl dopa is the relevant precursor of melanogenesis.

In B16 melanoma cells, γ-GT activity leads to the production of hydrogen peroxide in the extracellular space, thus subjecting the cells to a mild oxidative stress. Inhibition or activation of γ-GT activity led to a decrease or increase in the nuclear levels of redox sensitive transcription factor NF-κB (p65).

Our results clearly indicate that activated NF-κB led to a decrease in the tyrosinase levels apparently through the activation of other melanocyte specific transcription factors. In addition to the regulation through the redox reactions we have shown that γ-GT is itself subject to regulation (activity and RNA levels) in the melanotic and amelanotic phases of B16 melanoma cells: γ-GT activity as well as RNA levels decrease in the amelanotic conditions. Thus we have shown that γ-GT is regulated in B16 melanoma cells and the levels depend on the pigmentation status of B16 melanoma.

Our studies have shown that in B16 melanoma cells γ-GT is regulated by type 1 promoter. We have been able to identify the type of γ-GT mRNA transcript present in B16 melanoma cells by RT-PCR and sequence analysis.

Levels of γ-GT (activity and mRNA) were significantly less in normal murine melanocytes as compared to melanoma cells .A low γ-GT activity in normal cells versus high activity in malignant cells has been described for other tissues as well. Earlier reports have shown that γ-GT is transcribed from six different promoters. γ-GT is a single copy gene that encodes for six mRNAs, which have 5 unique untranslated regions. Our studies have shown that in B16 melanoma cells γ-GT is regulated by type 1 promoter. We have been able to identify the type of γ-GT mRNA transcript present in B16 melanoma cells by RT-PCR and sequence analysis.

B. Role of MSG1 in melanogenesis

B16 melanoma cells oscillate between melanotic and amelanotic stages as determined by the cell pellet colour, melanin assays and enzyme assays. Western Blot analysis showed that the expression of MSG1 was strong in melanotic B16 melanoma cells and weak in amelanotic cells. Thus the amount of cellular MSG1 correlates with pigmentation. To further investigate the possible link of MSG1 expression and cellular pigmentation, B16 F10 clones stably expressing full length MSG1 (HA-MSG1) and MSG1 deletion mutants (HA-MSGΔ1SID, HA-MSGΔ1CR2) were generated. Interestingly, it was seen that cells over-expressing MSG1 protein and its mutant lacking the transcriptional activating domain (HA-ΔCR2) were significantly more pigmented than the control cells, as determined by cell pellet colour and melanin assays. Thus our results have shown that the smad interacting domain of MSG1 (SID domain) plays an important role in regulation of pigmentation specific genes. Cells over-expressing MSG1 showed an increase in the specific activity of tyrosinase and dopachrome tautomerase (Dct).

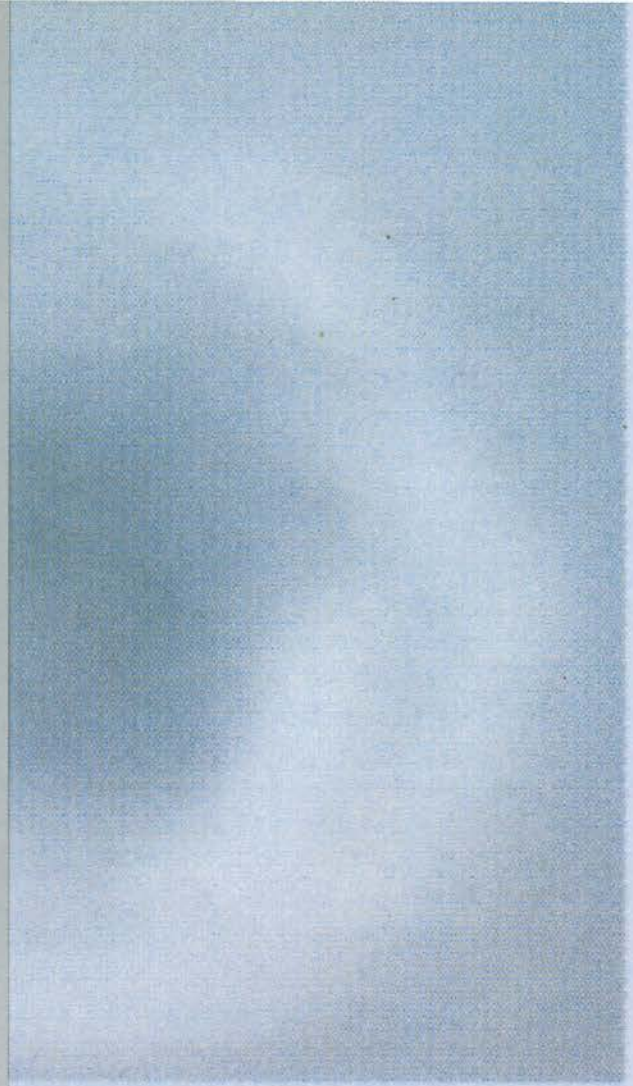
Experiments performed in our lab indicate that B16 melanoma cells over-expressing MSG1 when injected subcutaneously in C57 mice gave rise to bigger tumours as compared to the control tumours.

In order to further investigate the mechanisms of MSG1-induced enhancement of pigmentation, B16 melanoma cells were transiently transfected with expression plasmids for MSG1 and its effect on Dct were determined. Over-expression of HA-tagged MSG1 was confirmed by western blot analysis and total RNA was isolated at 0 and 6 hr after transfection. Northern blot showed a 60% increase in Dct transcripts, thus providing evidence that MSG1 enhances Dct expression.

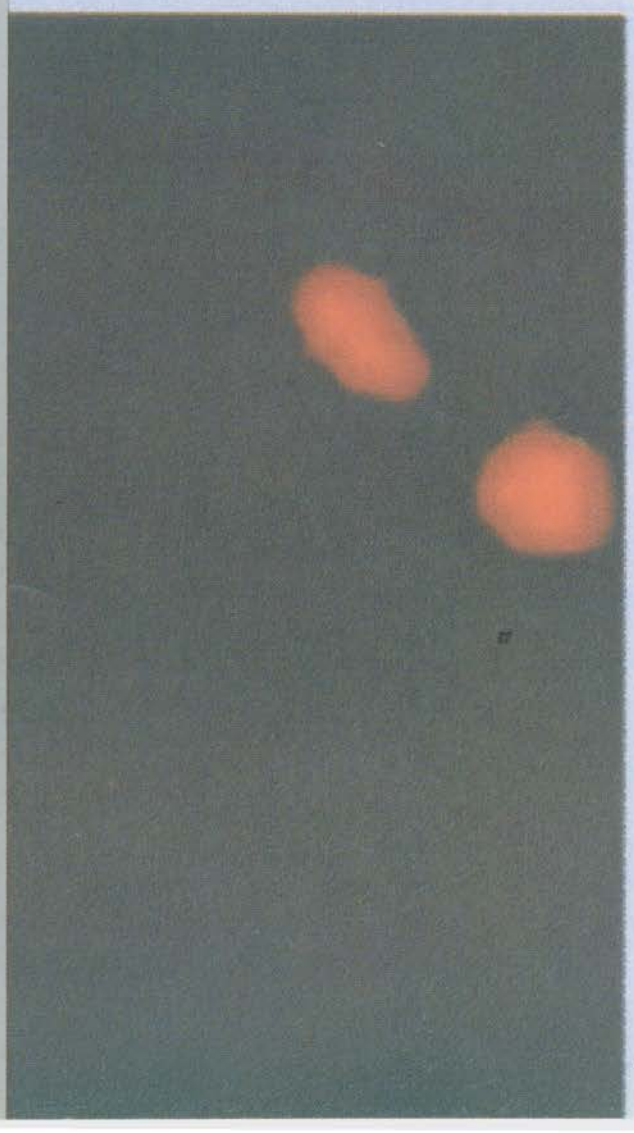
Experiments performed in our lab indicate that B16 melanoma cells over-expressing MSG1 when injected subcutaneously in C57 mice gave rise to bigger tumours as compared to the control tumours.

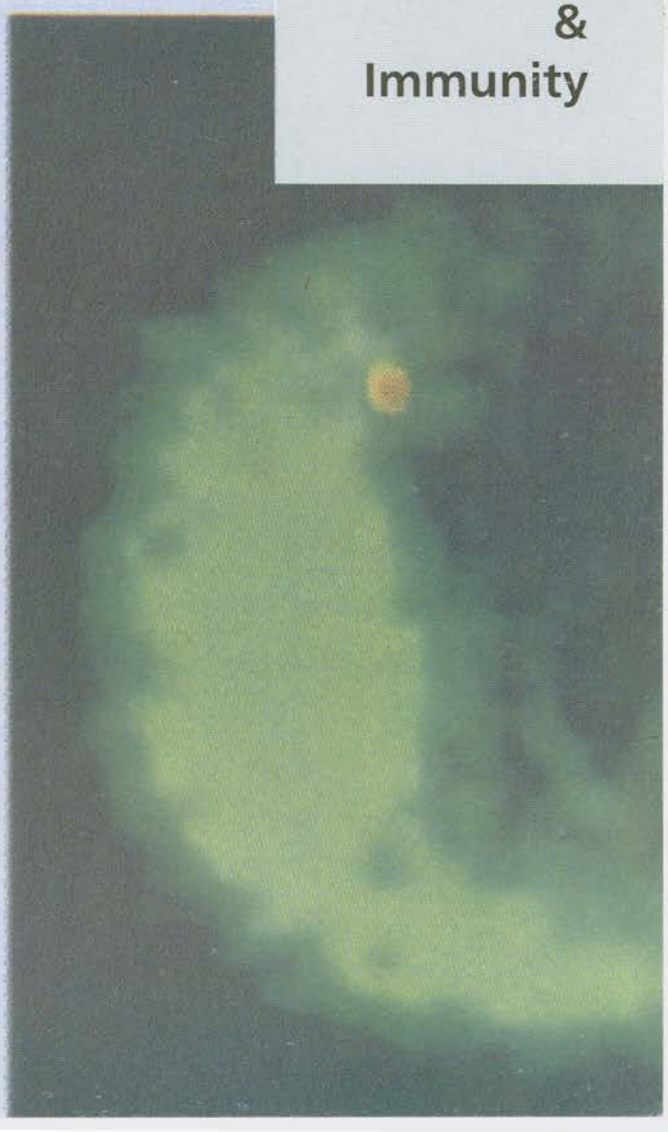
Future work

- To study the regulation of Dct by MSG1.
- To elucidate the mechanism of MSG1-induced tumourigenicity.



Infection & Immunity





Gyan C. Mishra
gcmishra@nccs.res.in

B. Joshi
D.V.R.Prasad
Pinaki P. Banerjee
Vrajesh Parekh

Debashis Mitra
Samit Chattopadhyay
Pradeep B. Parab

The differentiation of naïve CD4$^+$ Th cells into Th1 and Th2 has important biological implication in terms of susceptibility or resistance to a particular disease. Over past few years a number of reports indicated that individual molecules can selectively regulate differentiation into Th1 or Th2 subsets.

CHARACTERISATION OF TH1 AND TH2 SPECIFIC CO-STIMULATORY MOLECULES

The hallmark of the adaptive immune system is the generation of tremendous diversity to provide the appropriate receptors needed to recognize all the possible pathogens or antigens that an individual may encounter. As the frequency of any specific clone is the reciprocal of the diversity, the requirement for clonal expansion of T cells, which play a pivotal role in immune regulation, is of paramount importance. The T helper cell activation for clonal expansion is proposed to be a two-signal model. The first signal is accomplished by recognition of antigen-Ia complex, expressed by antigen presenting cells (APCs). The second signal or costimulation is MHC non-restricted and is delivered by accessory molecules present on the surface of the professional APCs. The two-signal model predicts that in the TCR engagement by specific antigen, the presence of costimulatory ligands leads to T cell activation, proliferation and differentiation. However, T cell encounter with cognate antigen in the absence of costimulatory signal results in anergy. A number of co-stimulatory molecules are discovered both on APC and their counter receptors on T cells. The prominent one include B7.1- and B7.2CD28, 4-1BBL4-1BB and B7RPICOS. On the basis of their ability to secrete specific lymphokines, CD4$^+$ T cells have been divided into Th1 and Th2 subsets. Th1 lymphocytes are characterised by the production of IL-2 and IFN-γ contributing to cellular immunity, whereas Th2 lymphocytes mainly involved in humoral immunity produce IL-4, IL-5, IL-10 and IL-13 cytokines. Th1- and Th2-associated cytokines tend to be reciprocally regulatory. IFN-γ inhibits Th2 associated functions and IL-4 and IL-10 have negative effect on Th1 associated functions. There are ample of examples wherein co-stimulatory molecules that preferentially activate T cells either to Th1 or Th2 phenotypes. B7.1/CD80 predominantly activates Th1 cells; in contrast to its closely related family member B7.2/CD86, which also acts through CD28 induces Th2 phenotype. The differentiation of naïve CD4$^+$ Th cells into Th1 and Th2 has important biological implication in terms of susceptibility or resistance to a particular disease. Over past few years a number of reports indicated that individual molecules can selectively regulate differentiation into Th1 or Th2 subsets.

Aim

- Characterisation and functional evaluation of two costimulatory molecules namely M150 and B2, which we previously identified as Th1 and Th2 specific costimulatory molecules respectively.

Work Achieved

We discovered a 150 kDa surface protein present on macrophage (M150) as costimulatory molecule, which drives Th cell differentiation towards Th1 subset. Further we also showed that macrophages predominantly employ M150, in comparison to B7.1 for co-stimulating T helper cells to proliferate and produce IFN-γ. The M150 was shown to restore normal Th1 function upon bystander co-stimulation in suppressed Th1 disease conditions like Leishmaniasis and Tuberculosis. This 150 kDa membrane protein isolated from SDS-PAGE gel was eluted and subjected to FPLC, which showed a single peak confirming that it is a homogenous single entity. The profile of 2-D gel

electrophoresis of M150 also showed a single spot. In our efforts to clone the M150 gene we sought to have partial sequence of the M150 protein. The N-terminal and two stretches of internal sequences revealed 100% homology with the LAMP-1. Interestingly LAMP-1 is highly heterogeneous in mature forms with oligosaccharides differing markedly both qualitatively and quantitatively in various cells. Only in macrophages LAMP-1 attains 130-150 kDa in comparison to 105-115 kDa in NIH 3T3 cells. Heteroduplex analysis to trace any internal sequence differences in a stretch of 500 nucleotides reveled that LAMP-1 gene is similar in both the cells indicating that heterogeneity is possibly because of glycosylation.

Electron microscopy and radiolabeling studies revealed the binding of liposomised B2/gp96 to the surface of anti-CD3 activated CD4$^+$T cells. Such binding was specific and was shown to be competed by unlabelled liposomised form of the same protein. Taken together our data suggest that gp96 binds, activate and skews the naïve CD4$^+$T cell differentiation towards Th2 phenotype.

Polyclonal antibodies developed in rabbit and rat against purified M150, did not probe the lower range of LAMP-1 in Western blot. Anti-LAMP-1 antibody picks up a approximately 120-145kDa proteins but not M150. IgM monoclonal, which we raised against M150, may be recognizing a very unique glycosyl moiety on M150 (Fig 1A). Double staining FACS analysis with G1 and anti-Mac1, anti-B220, anti-CD4 and anti-CD8 showed that M150 is predominantly expressed on macrophages.

We expressed LAMP-1 gene as a fusion protein both in macrophage cell-line and CHO cells. Western blot with transfected protein, results showed that anti-M150 monoclonal antibody G1 specifically recognizes the M150/LAMP-1 only when expressed in macrophage cell line, but not in CHO, suggesting that LAMP-1 might be undergoing differential glycosylation yielding high molecular weight version of LAMP-1, which we previously described as M150 (Fig 1).

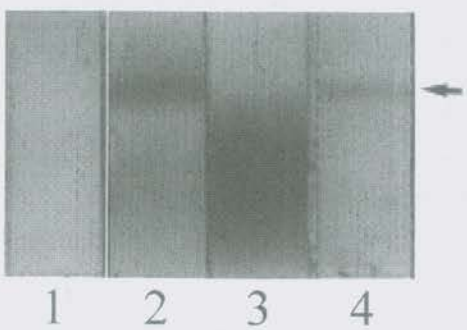
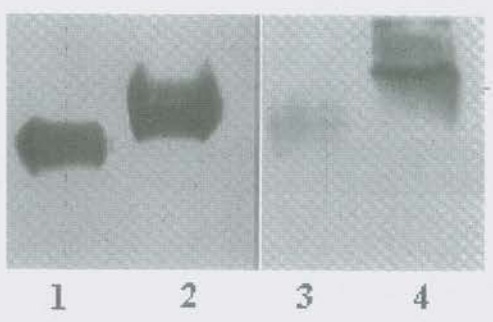


Fig 1: Western blotting of macrophage membrane proteins, transfected fusion proteins and deglycosylation of fusion proteins. Surface membrane proteins isolated and transferred to nitrocellulose membrane. NC membrane was cut into strips and incubated with separate primary antibodies. Lane 1 is control sera, lane 2 is polyclonal M150, lane 3 is anti-LAMP-1 (ID4B) and lane 4 is G1 (antiM150 mAb), position of M150 is indicated by arrow.



Western blot with soluble transfected proteins. Lane 1 and 2 represent CHO and Macrophage purified LAMP-1-Fc proteins respectively, probed with anti-LAMP-1 antibody (ID4B). Lane 3 and 4 represent CHO and Macrophage purified LAMP-1-Fc proteins respectively, probed with anti-M150 mAb (G1).

Naïve CD4$^+$T cell proliferation was induced only with macrophage LAMP-1 Fc, but not with CHO-LAMP-1-Fc (Fig 2A). The monoclonal G1 mAb specifically blocked the proliferation induced by macrophage-LAMP-1-Fc but not ID4B, indicating involvement of high molecular weight LAMP-1 (M150) which is

expressed perhaps only in macrophages (Fig 2B). The proliferation induced by LAMP-1/M150 and cytokines in the supernatants i.e. IL-2 and IFN-γ but not IL-4 and IL-13 clearly demonstrate LAMP-1/M150 induces Th1 polarity of Th cell differentiation in this culture system.

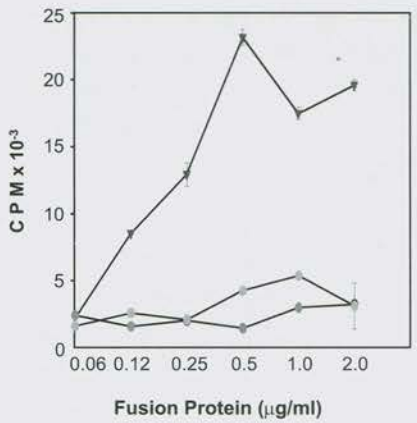


Fig 2A.: In vitro T cell proliferation assay. Non Fused Fc control (closed circle), CHO-LAMP-1-Fc (open circle), Macrophage-LAMP-1-Fc (filled triangle) used at varying concentrations for CD4 T cell proliferation assay. 1μCi [H] thumidine was added for last 12 hours of culture and harvested for analyzing the incorporation.

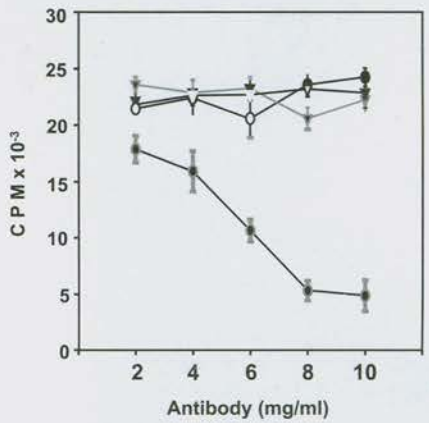


Fig 2B.: Blocking of T cell proliferation by anti-M150mAb. The T cell proleferation induced by 0.5mg/ml of Macrophage-LAMP-1-Fc and 1μg/ml of anti-CD3 was challenged with increasing concentrations of control Rat IgM (open triangle), isotype control IgG2a (filled triangle), anti-LAMP-1 i.e. ID4B (filled circle) and anti-M150 i.e. G1 (open circle). The thymidine incorporation was used to plot the blocking function of monoclonal antibody against M150.

In response to stress stimuli from the environment the synthesis of different stress proteins occurs in the cells. Differential expression of these proteins can be induced through a range of variable stresses including heat shock, nutrient deprivation, and also by pathogenic infection. Effect of such pathogenic infection on cells of the immune system and expression of stress proteins by them is a virgin arena of immunology to be explored in order to provide not only an effective preventive strategy against the diseases but also for better understating the basics of host defense mechanisms. To further elucidate this fact, administration of a gram negative bacterial endotoxin (lippopolysaccharide) on resting B cells resulted in isolating a 99-105 kDa costimulatory molecule (B2) from LPS activated B cell surface which is proven to be "stress protein" gp96 not only by partial protein sequence homology but also through immunoprecipitation and immunoblotting experiments. In the present study we demonstrate that gp96 activates the naïve CD4 T cells (Fig. 3) by delivering the co-stimulatory signal to them and can skew its differentiation towards Th2 phenotype (Fig. 4) . Moreover stimulation dependent, non MHC bound surface expression of gp96 on APCs like splenic B cells also strengthen the possible role of gp96 in costimulation (Fig. 5). Electron microscopy and radiolabeling studies revealed the binding of liposomised B2/gp96 to the surface of anti-CD3 activated $CD4^+$ T cells. Such binding was specific and was shown to be competed by unlabelled liposomised form of the same protein. Taken together our data suggest that gp96 binds, activate and skews the naïve $CD4^+$ T cell differentiation towards Th2 phenotype.

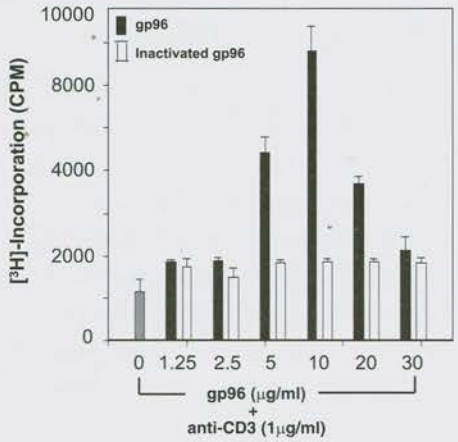


Fig 3. GP 96 mediated co-stimulation: GP96 activates CD4 T cells in a dose dependent manner H incorpoation by the proliferating CD4 T cells at the last 12 h of 60h culture. Cells were cultured in the presence of activated gp96 at various concentrations using soluble anti-CD3 (1μg/ml) as the first signal.

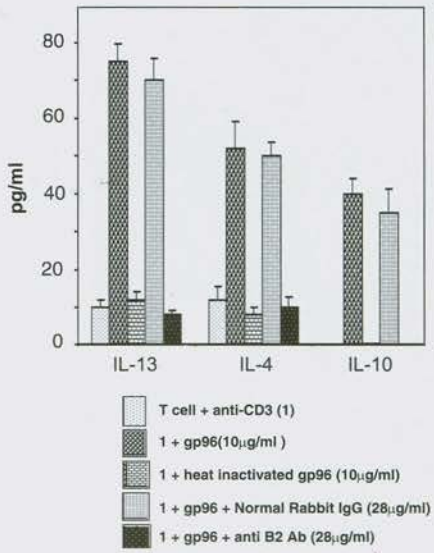


Fig 4. GP 96 induced Th2 type cytokine secretion by CD4+T cell. Levels of Th1 and Th2 type of cytokines (IL-4, IFN-γ,IL-10 and IL-13) from selected combinations of 60h culture supernatants was measured. IFN-γ level was undetectable. Results are representative of three independent experiments.

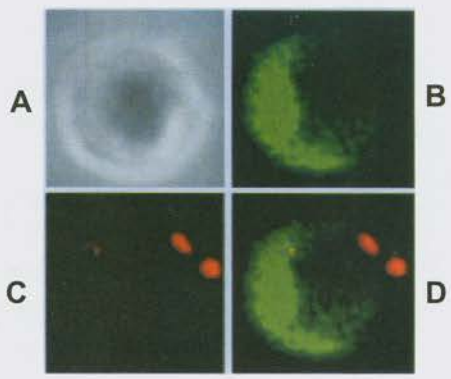


Fig 5. MHC independent surface expression of gp96. Confocal image of LPS activated, non permeabilized B cell showing- Unstained phase contrast view (image A); staining with anti-gp96 followed by anti-goat FITC (image B), or by anti IA-PE alone (image C), surface labeling with anti IA-PE and anti-gp96 + anti-goat FITC antibody (image D).

Future plans

Further characterisation of B150 a Th2 subset specific costimulatory molecule

Regulation of the immune system during experimental stress and autoimmune diseases.

Bhaskar Saha
sahab@nccs.res.in

K. Venuprasad
Meenakshi Jadhav

ROLE OF T CELLS AND NON-T CELLS IN DETERMINING THE SUSCEPTIBILITY TO *LEISHMANIA* INFECTION

The cells of the innate immune system (e.g., neutrophils, macrophages, mast cells, etc.) and adaptive immune system (T and B cells) go hand in hand to initiate and regulate an immune response. The cell surface molecules play a big role in regulating the activity of all these immuno-competent cells. One group of such molecules is called costimulatory molecules of which CD40, CD80 and CD86 and their respective ligands CD154 and CD28/CD152 are implicated in regulating an immune response. Therefore, the role of CD28-CD80/CD86 and CD40-CD154 mediated costimulation in the regulation of anti-leishmanial immune response has been investigated.

Aims

- Role of CD28 in neutrophil function
- Role of neutrophils in initiating T cell response

Work achieved

Earlier we have shown that neutrophils express CD28 and that CD28 signaling modulates neutrophil migration in response to IL-8, a neutrophil activating chemokine. We have shown that CD28 signaling regulates the ligand-independent expression of the chemokine receptor to modulate this IL-8 responsiveness.

The role of CD28-CD80/CD86 and CD40-CD154 mediated costimulation in the regulation of anti-leishmanial immune response has been investigated.

We followed our investigation on what neutrophil does at the site of infection using *Leishmania major* as the model infection. We have found that *Leishmania major* infection of human peripheral blood monocytes turned macrophages secrete IL-8 in a parasite infection dose dependent manner. Corroborating this finding, we observed that significantly more neutrophils migrated towards the parasite infected macrophages. The migrated neutrophils interact with macrophages to induce IFN-γ secretion and reduction of intra macrophage parasite burden. *In vitro* studies have shown that the neutrophil-macrophage co-culture in presence of CTLA4-Ig (a fusion protein which blocks CD28 interaction with its ligands, CD80 and CD86 on macrophages) inhibited IFN-γ secretion and prevented the anti-leishmanial function of neutrophils. Therefore, this observation suggests that the CD28 signaling in neutrophils results in IFN-γ secretion.

We have explored the signaling pathway involved in CD28-induced IFN-γ secretion by neutrophils. Since PI-3 kinase is proposed to be a CD28-signaling intermediate, albeit inconclusive, we investigated whether PI-3 kinase was involved in CD28 signaling in neutrophils. PI-3 kinase is found to mediate the CD28 signaling as pre-incubation of neutrophils with wortmannin, an inhibitor of the enzyme, results in reduction of the cytokine secretion in a dose-dependent manner. Immunoprecipitation of CD28 followed by Western blots with anti-PI3 kinase shows that CD28 associates with PI-3 kinase in neutrophils. Since the secretion of IFN-γ peaks six hours after CD28cross-linking, it may be from the pre-formed pool. Intracellular staining of IFN-γ immediately after CD28 cross-linking fails to detect the cytokine while actinomycin D inhibits the cytokine secretion in a dose-dependent manner suggesting that CD28 signaling through PI-3 kinase increases IFN-γ

gene transcription which eventuates in the cytokine secretion. RT-PCR with the RNA isolated from control, CD28-signaled and CD28-signaled but wortmannin treated shows that the message for IFN-γ RNA is increased in CD28 signaled neutrophils but significantly reduced in the wortmannin-treated neutrophils confirming the role of PI-3 kinase in the induction of IFN-γ secretion in neutrophils.

We have designed in vitro model to test if neutrophils play any role in initiating the T cells response. We have observed that the culture supernatants of CD28 signaled neutrophils induce chemotaxis of T cells.

It has been shown that neutrophils are the first cells to migrate into the site of infection or inflammation. In contrast, in case of *Leishmania* infection, especially *L. major*, where the disease initiates in the cutaneous tissue, T cells but not neutrophils are shown to play an exclusive role in the regulation of the anti-leishmanial immune response. Therefore, we have designed *in vitro* model to test if neutrophils play any role in initiating the T cells response. We have observed that the culture supernatants of CD28 signaled neutrophils induce chemotaxis of T cells. Currently experiments are being performed to test the role of these neutrophil secreted chemokines/cytokines in setting T-helper subset bias.

Future Work

The mechanism of IL-8 secretion by *Leishmania major* infected macrophages will be investigated as IL-8 secreted rapidly after *L. major* infection is the major neutrophil chemotactic factor. The CD28 signaled neutrophil-secreted chemokines/cytokines, which induce T cell chemotaxis, would be identified. T cell regulation by neutrophils will also be investigated.

Prakash Deshpande
prakashdeshpande@nccs.res.in

M. S. Deval

Padma Shastry

ROLE OF HEMOZOIN IN IMMUNE RESPONSE IN MALARIA

Intraerythrocytic malaria parasite uses hemoglobin as a major nutrient source of amino acids. However, parasites are unable to degrade hemoglobin-heme and instead polymerizes it into an insoluble dark brown pigment called hemozoin. This pigment is released into the blood circulation along with merozoites as the schizont stage parasitized erythrocytes burst.

Plasmodium falciparum associated immunosuppression and a poor proliferative response of lymphocyte to Concanavalin A and other mitogens has been observed. Impairment of phagocytic function of macrophages by hemozoin is reported. On the basis of this evidence, we have hypothesized that the malarial pigment, hemozoin might be a crucial factor in the impairment of the host immune system.

Aim

- To understand the role played by hemozoin and the mechanism involved in immunosuppression.

Work achieved

Plasmodium falciparum associated immuno-suppression and a poor proliferative response of lymphocyte to Concanavalin A and other mitogens has been observed. Impairment of phagocytic function of macrophages by hemozoin is reported.

Two isolates of human malaria parasite *P. falciparum* were cultured *in vitro* in candle jar desiccator. Malarial pigment hemozoin was isolated from trophozoite enriched parasitized erythrocytes. Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors and were stimulated with T-cell mitogen (PHA-L) in the presence of different concentrations of malaria pigment. The proliferation of cells was assessed by [^{3}H] thymidine incorporation. Hemozoin inhibits T-cell mitogen induced proliferation of PBMCs in a dose dependent manner. Except hemozoin, none of the other components i.e., malarial parasite soluble extract, parasite culture supernatant, hymosal, hemoglobin or hemin chloride inhibits the PBMCs proliferation. There is no toxic effect of the pigment on the PBMCs. The inhibition is mediated through adherent cells) phagocytosed with hemozoin pigment.

Except hemozoin, none of the othe components i.e., malarial parasite soluble extract, parasite culture supernatant, hymosal, hemoglobin or hemin chloride inhibits the PBMCs proliferation.

Cytokine profiles: Hemozoin treated and untreated PBMCs stimulated with PHA-L culture supernatants were assessed for different cytokines i.e, IL-2, IL-β, TNF-α, IFN-γ and IL-12. Hemozoin treated PBMC culture supernatants show 3-4 fold increase in the TNF-α and IL-1β as compared to controls. On the other hand, a 3-4 fold decrease of IFN-γ and IL-2 levels were observed in pigment treated cell culture supernatants as compared to controls. Moreover, addition of exogenous recombinant T-cell growth factor (rhIL-2) or neutralization of TNF-α did not abrogate hemozoin induced inhibition of proliferation of PBMCs stimulated with PHA. Hemozoin also inhibited the cell proliferation induced by Concanavalin A. On the basis of these results, we propose that a similar mechanism of hemozoin mediated immunosuppression might be responsible for the impairment of immune response in malaria patients.

Future work

Hemozoin interaction with human monocyte/macrophages will be studied using THP1 and U937 cell lines. Hemozoin induced impairment of different functions of these cells will also be analysed.

Debashis Mitra
dmitra@nccs.res.in

Ajith Mathew
Jayashree Ladha
Sujata Bhade
Chunaram Choudhary
Neelakshi Jog
Shraddha Bubana
Snigdha Ghosh

Bhaskar Saha
Gyan C. Mishra

MOLECULAR AND CELLULAR BASIS OF HIV PATHOGENESIS

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS), which is defined by a dramatic reduction in the number of CD4+ T cells (to less than 200 cells/μl) and the onset of opportunistic infections. The incidence of HIV infection has reached alarmingly high levels worldwide including India. The therapeutic strategies being pursued at present can reduce the viral load remarkably but are not the ultimate answer to AIDS patients. Thus, it is very important to understand the pathogenesis of the virus and develop new strategies to overcome the deadly disease. Keeping this in mind, we have initiated studies on three different aspects of HIV-1 pathogenesis. The overall goal is to gain more understanding of the virus and its interaction with the host cell, which may lead us to new antiviral strategies.

We have initiated studies on three different aspects of HIV-1 pathogenesis. The overall goal is to gain more understanding of the virus and its interaction with the host cell, which may lead us to new antiviral strategies.

Aims

- Role of viral regulatory proteins Tat and Nef in HIV pathogenesis and differential gene expression studies in HIV-1 infected cells.
- Immune response to HIV infection towards generation of DNA vaccine.
- Identification of anti-HIV activity in plant extracts and marine animals.

Work achieved

Role of viral regulatory proteins Tat and Nef in HIV pathogenesis and differential gene expression studies in HIV-1 infected cells

The HIV-1 encodes for Nef, a 27 KDa protein, which has come a long way from being termed as a negative factor to being one of the most important proteins of HIV-1. The Nef protein of HIV-1 is now regarded as a regulatory protein responsible for establishment of infection and for pathogenesis. However, its role in HIV-1 replication is not yet clear. The current study has been undertaken to understand the role of Nef in viral transcription and replication, in presence of the transactivating protein, Tat, and also to determine whether these two proteins interact and thereby influence HIV replication and pathogenesis. Using transient transfection assay, co-immunoprecipitation and pull down analysis, we have been able to demonstrate that HIV-1 Nef protein physically interacts with Tat, the premier transactivating protein of HIV-1. Our observations further indicate that the interaction results in positive regulation of Tat induced HIV-1 LTR mediated gene expression. Furthermore, as the role of Nef in HIV induced neuropathogenesis is yet to be clearly understood, we have initiated studies to identify Nef interacting host cell factors.

Despite significant advancement in our understanding of the pathogenesis of AIDS, the mechanism by which HIV-1 infection induces CD4 T cell depletion is not clearly understood. The objective of the present work is to identify differentially expressed molecules in HIV infected cells and to elucidate the interaction of those molecules in the signaling cascade leading to cell death.

Despite significant advancement in our understanding of the pathogenesis of AIDS, the mechanism by which HIV-1 infection induces CD4 T cell depletion is not clearly understood. The objective of the present work is to identify differentially expressed molecules in HIV infected cells and to elucidate the interaction of those molecules in the signaling cascade leading to cell death. In order to distinguish HIV infected cells from uninfected cells, we have been successful in making a reporter CD4 T cell line expressing GFP under the influence of HIV-1 LTR. The vector has been stably transfected in Jurkat T cell line, and is able to express green fluorescent protein at low levels, which

increases dramatically after HIV-1 infection. This cell line will also be useful in preliminary screening of anti-HIV compounds without any expensive assays. These cells will be used in differential gene expression studies using RNA from infected and uninfected cells.

Immune response to HIV infection towards generation of DNA vaccine

Development of immunological intervention against HIV-1 has been severely hampered due to lack of understanding on the concept as to what constitutes protective immunity to the virus.

The plausible reason could be that high rate of replication, mutation and recombination of HIV enable the virus to evolve rapidly in the host in such a way that it outsmarts the immune response evoked by natural immunity or a vaccine. At present, our knowledge of cytotoxic T lymphocyte response in HIV infection and the role of neutralizing antibody in limiting virus replication is far from clear. The present study, therefore, aims at defining the most critical components involved in the anti HIV immune response, which results in elimination of the virus, using DNA immunization protocol. In order to identify candidate molecules for generating protective immune response, we plan to use a murine model and eukaryotic expression vectors encoding different HIV proteins like Tat, Nef, gp120, etc. We have started DNA immunization studies in mice using the pCDNA-Tat vector and have been able to observe antibody response. In addition to Tat, we are also trying to clone the gp120 gene of subtype C in expression vector. Our aim is to clone Tat and gp120 in a bicistronic vector and then use it in DNA immunization studies.

The present study, therefore, aims at defining the most critical components involved in the anti HIV immune response, which results in elimination of the virus, using DNA immunization protocol.

Identification of anti-HIV activity in plant extracts and marine animals

The current therapeutic strategy involving the use of reverse transcriptase and protease inhibitors in combination (HAART) is too expensive a regimen to be used in Indian context, and is not the ultimate answer for AIDS patients. Extensive work is being done throughout the globe to identify new anti-HIV therapeutic strategies. One of the strategies has been to identify anti-HIV compounds in natural resources. Antiviral activity has been shown to be present in a number of plants and marine animals. We have started looking into anti-HIV activity in marine bivalves of Indian coastline and also in trees of the genus Calophyllum.

Future work

The future work involves identification of the domains in Nef or Tat proteins, which are important in the interaction and also elucidation of the signal transduction pathway. We are also trying to identify novel and efficient delivery systems for DNA immunization as a part of the DNA vaccine work.

14. Sandhya S. L., Yogita Shewade, and Ramesh Bhonde (2000) Role of Bittergourd Fruit Juice in STZ-induced diabetic state in vivo and in vitro, *J. Ethnopharmacology,* 73, 71-9

15. Sarrias, M.R., Franchini, S., Canziani, G., Moore, W.T., Sahu, A., Lambris, J.D. (2001) Kinetic analysis of the interactions of complement receptor 2 (CR2, CD21) with its ligands C3d, iC3b and the Epstein Barr virus glycoprotein gp350/220. *J. Immunol.,* 167,1490-1499.

16. Savita, K. and Bhonde, R. R. (2000) Combined effect of nicotinamide and streptozotocin on diabetic status in partially pancreatectomized adult BALB/c mice, *Hormone and Metabolic Research,* 32, 330-334

17. Shastry, P., Basu, A., Rajadhyaksha, M. S.(2001) Neuroblastoma cell lines - A versatile in vitro model in neurobiology (Review article), *International Journal. of Neurosciences* 108, 1-18

18. Shewade Y. M. and Bhonde R.R. (2001) Differential action of fatty acids against streptozotocin induced in vitro cytotoxicity in islets, *In vitro Cell and Develop. Biol., (in press)*

19. Shiras, A., Shepal, V., Bhonde, M. and Shastry, P. (2001) Expression Analyses of S100 in Primary and immortalised Mouse Fibroblast cultures, *In vitro Cell and Dev. Biol-Animal,* 37, 172-174

20. Shiras A. S., Sengupta A., Shepal V. S. (2001) Cloning and Expression analyses of a novel homeobox gene clone Dlxin-1 with tumour suppressor properties. *Mol. & Cell Biol. Res. Commun.* (in press)

21. Shouche, Y. S. and Patole, M. S. (2000) Mitochondrial rRNA sequence analysis of some mosquito species, *J. Biosci.,* 25, 361 -366

22. Shukla, M. S., Pidiyar, V., Bhave, N. A., Patole, M. S. and Shouche, Y. S. (2000) PCR amplification and sequencing of mitochondrial 12S rRNA gene fragment from Muntiacus muntjak (Indian Muntjac), *Current Science,* 80, 101 102

23. Sitasawad, S.L, Shewde, Y.M and Bhonde R.R. (2000) Role of Bittergourd Fruit Juice in STZ-Induced Diabetic State in vivo and in vitro. *J. Ethenopharmacol.,* 73, 71-79

24. Sitasawad, S.L, Deshpande, M., Katdare, M., Tirth, S. and Parab P. (2001) Beneficial effect of supplementation with copper sulfate on STZ-diabetic mice (IDDM), *Diabetes Research and Clinical Practice,* 52, 77-84

25. Venuprasad, K., Parab, P., Prasad, D.V.R., Sharma, S., Banerjee, P., Deshpande, M., Mitra, D. K, Pal, S., Bhadra, R,. Mitra, D., and Saha, B. (2001) Immunobiology of CD28 expression on human neutrophils I : CD28 regulates neutrophil migration by modulating CXCR-1 expression, *Eur. J. Immunol.,* 31, 1536-1543

26. Nair, S. S., Chaubal, V. A., Shioda, T., Coser, K. R., Mojamdar, M. (2001) Over-expression of MSG1 transcriptional co-activator increases melanin in B16 melanoma cells: a possible role for MSG1 in melanogenesis. *Pigment Cell Research,* 14, 206-209

27. Chaubal, V. and Mojamdar, M. (2000) Inter-relationship between tyrosinase and TRP-1 with -Glutamyl transpeptidase in B16 melanoma cells. *Pigment Cell Research,* Suppl 7, 48

14. Sandhya S. L., Yogita Shewade, and Ramesh Bhonde (2000) Role of Bittergourd Fruit Juice in STZ-induced diabetic state in vivo and in vitro, *J. Ethnopharmacology,* 73, 71-9

15. Sarrias, M.R., Franchini, S., Canziani, G., Moore, W.T., Sahu, A., Lambris, J.D. (2001) Kinetic analysis of the interactions of complement receptor 2 (CR2, CD21) with its ligands C3d, iC3b and the Epstein Barr virus glycoprotein gp350/220. *J. Immunol.*, 167,1490-1499.

16. Savita, K. and Bhonde, R. R. (2000) Combined effect of nicotinamide and streptozotocin on diabetic status in partially pancreatectomized adult BALB/c mice, *Hormone and Metabolic Research,* 32, 330-334

17. Shastry, P., Basu, A., Rajadhyaksha, M. S.(2001) Neuroblastoma cell lines - A versatile in vitro model in neurobiology (Review article), *International Journal. of Neurosciences* 108, 1-18

18. Shewade Y. M. and Bhonde R.R. (2001) Differential action of fatty acids against streptozotocin induced in vitro cytotoxicity in islets, *In vitro Cell and Develop. Biol., (in press)*

19. Shiras, A., Shepal, V., Bhonde, M. and Shastry, P. (2001) Expression Analyses of S100 in Primary and immortalised Mouse Fibroblast cultures, *In vitro Cell and Dev. Biol-Animal,* 37, 172-174

20. Shiras A. S., Sengupta A., Shepal V. S. (2001) Cloning and Expression analyses of a novel homeobox gene clone Dlxin-1 with tumour suppressor properties. *Mol. & Cell Biol. Res. Commun.* (in press)

21. Shouche, Y. S. and Patole, M. S. (2000) Mitochondrial rRNA sequence analysis of some mosquito species, *J. Biosci.*, 25, 361 -366

22. Shukla, M. S., Pidiyar, V., Bhave, N. A., Patole, M. S. and Shouche, Y. S. (2000) PCR amplification and sequencing of mitochondrial 12S rRNA gene fragment from Muntiacus muntjak (Indian Muntjac), *Current Science,* 80, 101 102

23. Sitasawad, S.L, Shewde, Y.M and Bhonde R.R. (2000) Role of Bittergourd Fruit Juice in STZ-Induced Diabetic State in vivo and in vitro. *J. Ethenopharmacol.,* 73, 71-79

24. Sitasawad, S.L, Deshpande, M., Katdare, M., Tirth, S. and Parab P. (2001) Beneficial effect of supplementation with copper sulfate on STZ-diabetic mice (IDDM), *Diabetes Research and Clinical Practice,* 52, 77-84

25. Venuprasad, K., Parab, P., Prasad, D.V.R., Sharma, S., Banerjee, P., Deshpande, M., Mitra, D. K, Pal, S., Bhadra, R,. Mitra, D., and Saha, B. (2001) Immunobiology of CD28 expression on human neutrophils I : CD28 regulates neutrophil migration by modulating CXCR-1 expression, *Eur. J. Immunol.*, 31, 1536-1543

26. Nair, S. S., Chaubal, V. A., Shioda, T., Coser, K. R., Mojamdar, M. (2001) Over-expression of MSG1 transcriptional co-activator increases melanin in B16 melanoma cells: a possible role for MSG1 in melanogenesis. *Pigment Cell Research,* 14, 206-209

27. Chaubal, V. and Mojamdar, M. (2000) Inter-relationship between tyrosinase and TRP-1 with -Glutamyl transpeptidase in B16 melanoma cells. *Pigment Cell Research,* Suppl 7, 48

PUBLICATIONS (2000-2001)

1. Deshpande, M., Mitra, D., and Parab, P. (2000). Polyacrylamide gel as a matrix for the delivery of a layer or coat of other molecules, *Biotechnique*, 30, 258-262

2. Hardikar, A. A., Risbud, M.V., Remacle, C.R., Reusens, B, Hoet, J. J., Bhonde, R.R. (2001) Islet Cryopreservation: Improved recovery following taurine pretreatment, *Cell Transplantation (in press)*

3. Jacob, S. S., Shastry, P., and Sudhakaran, P. R. (2001) Influence of nonenzymatically glycated collagen on monocyte-macrophage differentiation, *Atherosclerosis (in press)*

4. Jaykumar, P. C., Souche, Y.S. and Patole, M. S. (2001) Cloning of two hexokinase isozyme sequences from Drosophila melanogaster, *Insect Biochemistry and Molecular Biology (in press)*

5. Kurup, S., Risbud, M.V. and Bhonde R.R. (2001) Selective cytotoxicity of Mia Paca-2 conditioned medium to acinar cells : A novel approach to reduce acinar cell contaminants in isolated islet preparations, *Transplant International (in press)*

6. Limaye, L. S. and Kale, V. P. (2001) Cryopreservation of human hematopoietic cells with membrane stabilizers and bio-antioxidants as additives in the conventional freezing medium. *J. Hematotherapy and Stem Cell Research* 10, 709 - 718

7. Prasanna, I, Lakra, W. S., Ogale, S.N. and Bhonde, R. R. (2000) Cell culture from fin explant of endangered mahasheer, Tor putitora (Hamilton), *Current Science*, 79, 93-95

8. Risbud, M.V., Saheb, N. D, Jyoti, J. and Bhonde, R.R. (2001) Preparation, characterisation and in vitro biocompatibility evaluation of poly (butylene terephthalate)/Wollastonite composites, *Biomaterials*, 22, 1591-1597

9. Risbud, M.V., Bhonde, M.R. and Bhonde R. R. (2001) Chitosan-Polyvinyl pyrrolidone hydrogel does not activate macrophages: Potentials for transplantation applications, *Cell Transplant.*, 10, 195-202

10. Risbud, M.V. and Bhonde, R. R. (2001) Polyamide-6 composite membrances : Properties and in vitro biocompatibility evaluation, *J. Biomaterial Sc. Polymer Edition*, 12, 125-136

11. Risbud, M.V. and Bhonde, R.R. (2001) Suitability of cellulose molecular dialysis membrane for bioartificial pancreas: In vitro biocompatibility studies., *J. Biomed. Materials Res.*, 54, 436-444

12. Risbud, M.V., Ringe, J., Bhonde R. R. and Sittinger. M. (2001) In vitro expression of cartilage specific markers by chondrocytes on chitosan hydrogel : Implications in cartilage engineering, *Cell Transplantatiion (in press)*

13. Sahu, A. and Lambris, J. D. (2001) Structure and biology of complement protein C3, a connecting link between innate and acquired immunity, *Immunoogical Reviews*, 180, 35-48

Samit Chattopadhay
samit@nccs.res.in

Ruchika Kaul
Asavari Kulkarni
Farid Ahmed
Shravanti Rampalli
Sujoy Mukhopadhyay
Devraj Mogare

ROLE OF MARS AND MAR-BINDING PROTEINS IN CELL CYCLE REGULATION AND VIRAL TRANSCRIPTION

Many of the matrix attachment region (MAR)-binding proteins have caught attention in the recent years. The MARs are a class of cis-regulatory elements that are about 200-300 bp long AT-rich sequences, occurring in about 30kb of eukaryotic chromosomal DNA. These sequences are often located next to the transcriptional promoters and enhancers. The MAR-binding proteins bind to these sites, attach to the nuclear matrix through a DNA loop, and regulate transcriptions of various genes by maintaining the accessibility. Thus, a proper interaction between MARs and their respective binding proteins play an important role in the gene expression in a tissue and cell stage specific manner. SMAR1 is one such protein that we have identified from mouse T cells. An alternatively spliced form of SMAR1 is the functional form that shows a strong anti-tumourigenic function in mice. Now we have evidence showing that SMART1 spliced form activates p53 mediated transcription upon direct interaction with it. Very recently, we have been able to show that such MARs also play a critical role in transcription through the HIV-LTR promoter and we hypothesize that such AT- rich MAR sequences play a pivotal role in the establishment of HIV pathogenesis.

Very recently, we have been able to show that such MARs also play a critical role in transcription through the HIV-LTR promoter and we hypothesize that such AT- rich MAR sequences play a pivotal role in the establishment of HIV pathogenesis.

Aims

- To delineate the molecular basis of anti-tumourigenic properties of a novel MAR binding protein SMAR1.
- Studies on interactions of SMAR1 with other MAR binding proteins e.g., Cux, SATB1, Bright and mutant p53.
- Role of AT-rich MAR sequences in the regulation of HIV transcription mediated through LTR promoter.

Work achieved

SMAR1, an anti-tumourigenic cell cycle regulatory protein: Matrix associated protein SMAR1, a 68 kDa nuclear protein, was isolated from mouse thymocytes at the Double Positive (DP) stage. There are at least three variants of SMAR1, of which one of the alternately spliced variants (SMAR1^{S}) is shorter than the full-length form (SMAR1^{L}) by 39 amino acids (aa). This 39 aa region is located at the N-terminus of the protein. By employing a single tube RT-PCR assay, we show that SMAR1^{S} is downregulated in various tumour cell lines. Interestingly, SMAR1 overexpressing mouse melanoma cells when injected into mice, delays the tumour progression, suggesting that it is strongly anti-tumourigenic (Figs. 1 and 2).

We show that overexpression of SMAR1 specifically upregulates BTG3 indicating that the antitumourigenic property of SMAR1 stems from both upregulation as well as direct protein-protein interaction with BTG3.

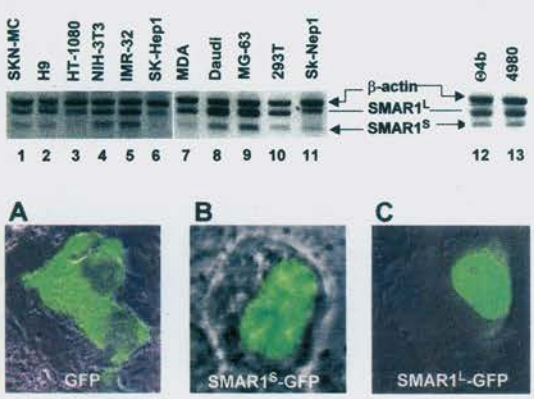


Fig 1 The upper panel depicts differential expression of the spliced form of SMAR1 (SMAR1-S) in various tumor cell lines. Lower panel shows nuclear expression of SMAR1 within mouse melanoma cells. **(A)** Only GFP expression in both nucleus and cytoplasm. **(B)** Expression of spliced form of SMAR1 fused with GFP. **(C)** GFP fusion with SMAR1-L

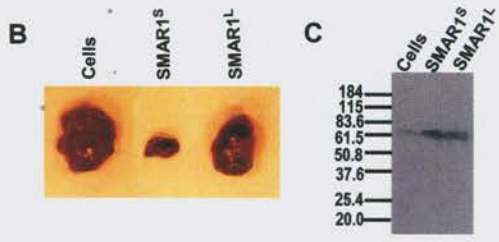


Fig2. Left panel shows tumor regression by over-expression of the spliced form of SMAR1 (SMAR1-S). No significant regression occur upon expression of either vector or the long form of SMAR1(SMAR1-L); Right panel; Western blot showing over-expression of SMAR1 in the melonoma cell lines upon stable transfection.

Previously, it has been shown that BANP, the mouse homolog of SMAR1, directly interacts with BTG3 (B cell translocation gene 3). BTG3 belongs to the BTG family of proteins that are involved in the negative control of cell cycle. We show that overexpression of SMAR1 specifically upregulates BTG3 indicating that the antitumourigenic property of SMAR1 stems from both upregulation as well as direct protein-protein interaction with BTG3.

Role of a novel MAR sequence as a cis element in the regulation of HIV transcription at a distance: The transcription of HIV provirus is characterised by an early Tat-independent and a late Tat-dependent phase. Since the LTR promoter of HIV is otherwise a poor promoter, in the absence of the transactivator protein Tat, majority of the transcripts are transcribed as short or truncated forms. Thus, during the early phase of HIV life cycle, the site of integration of the provirus into various places in the genome is critical. We believe that MARs are some such hotspots where the HIV-LTR get activated and full-length processive transcription occur in the absence of Tat. We have evidence clearly demonstrating that the correct positioning of a novel MAR sequence can drive the transcription through LTR at a long distance. This novel concept will also help us in designing new generation of viral vectors for gene therapy.

Future plans:

We have shown that SMAR1 upregulates p53 mediated transcription. Currently, we are interested in understanding the mechanism of inhibition of p53 by SMAR1 and its direct interaction with wild type as well as mutant p53.

The newly isolated MAR sequence will be used to construct the lentiviral vectors that will efficiently express transgenes upon integration into the chromosome.

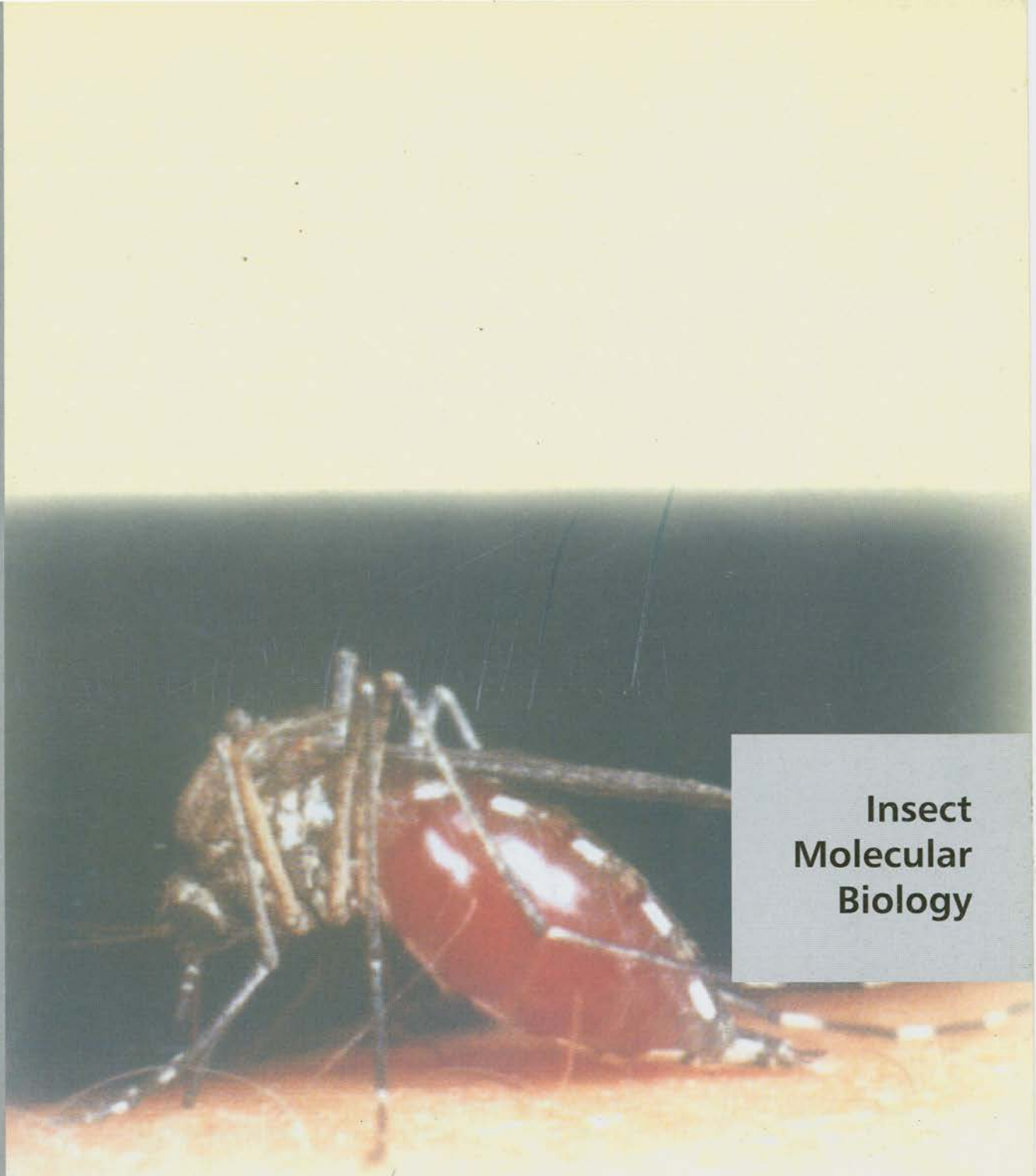
Identification and characterisation of functionally important determinants of viral homologs of complement control proteins

Previous *in vivo* studies have established that VCP is a virulence determinant of the virus that helps protect the virus from the host's complement attack. Thus, identification of vital structural determinants would not only allow us to better understand the structural features of viral CCPHs that are important in its biology but would also identify a significant control point in the molecule that would direct the development of pharmacological ligand molecules to neutralize these viruses. In order to characterize the functional determinants of CCPHs a series of mutants will be generated. We will generate deletion as well as chimeric mutants where the domains of viral CCPHs will be either deleted or shuffled with human complement control proteins. Such studies are possible since CCPHs are composed of short consensus repeats (SCRs) and it has been shown before that each SCR fold independently into a compact bead like structure. Currently we have initiated the generation of deletion mutants of VCP.

Monoclonal antibody that can block the function of a protein is another tool that can be utilized for the identification of functionally important determinants. Advantage of using this approach is that no bias is involved in this methodology. To this end, we have generated several hybridoma clones. Currently we have sub-cloned five of them and have also obtained ascites for them. Experiments are underway to functionally characterize these monoclonal antibodies. These mAbs will also be useful for characterisation of *in vivo* function of VCP.

Future plan

1. Detailed functional characterisation of VCP and CCPHs of HVS and HHV-8.
2. Characterisation of functionally important determinants of VCP and CCPHs of HVS and HHV-8.



Insect Molecular Biology

Milind S. Patole
patole@nccs.res.in

P. Cyril Jayakumar
Vijay Musande

Yogesh S. Shouche

When the expression of two isoenzymes namely DM1 and DM2 was studied in cells, it was observed that DM1 is expressed (as seen by Northern analysis) while DM2 was not expressed in cell line.

Two upstream fragments of 1600 and 600 bp have been obtained for DM1 and cloned in pGL3-Basic vector having luciferase enzyme reportor system. The cloned vector was transfected in D.Mel.2 cells and transient expression of luciferase was studied.

CLONING AND CHARACTERISATION OF INSECT HEXOKINASE GENES.

Among all animals, flying insects have high body-mass specific rates of aerobic metabolism. The need for flight energy in insect is supplied by aerobic oxidation of hexoses. Hexokinase is the first regulatory enzyme in oxidation of hexoses and in insects, it functions very close to V_{max} as compared to its mammalian counterpart. This indicate that hexokinase play an important role in energy metabolism of insects. Although the purification of enzyme and mapping of genetic loci for hexokinase has been accomplished two decades ago, the exact number of genes were not known for any given insect species. Earlier, we have cloned two complete isoenzyme sequences for hexokinase from *Drosophila melanogaster* and studied their expression in adult insects and *in vitro* in cell line D.Mel.2. When the expression of two isoenzymes namely DM1 and DM2 was studied in cells, it was observed that DM1 is expressed (as seen by Northern analysis) while DM2 was not expressed in cell line. This differential expression indicated that the nature of 5' flanking sequences for these two ORFs which are regulating their expression is different.

Aim

- To investigate the nature of promotor elements for the two differentially expressed isozymes DM1 and DM2.

Work achieved

Cloning of 5' flanking sequences for DM1 and DM2 isoenzymes was performed. For DM1 promotor this was aided by mapping the exact transcription start site using different primers and 5'RACE protocol. Two upstream fragments of 1600 and 600 bp have been obtained for DM1 and cloned in pGL3-Basic vector having luciferase enzyme reportor system. The cloned vector was transfected in D.Mel.2 cells and transient expression of luciferase was studied. The expression was found to be more in case of vector having 1600 bp than with 600 bp insert. To locate the exact core promotor elements and cis-acting elements, deletion constructs were made using Exonuclease-III. The sequence of the 1600 bp upstream element when analyzed showed the absence of conventional TATA box like elements. The deletion clones are being sequenced and characterised using luciferase reportor system. Partial clones for hexokinase have been isolated from *Spodoptera, Culex* and leishmanial parasite.

Future Work

We plan to complete cloning of hexokinases from insects and parasite and characterize the transcription factors involved in regulation of hexokinases in Drososphila.

In an interesting application, mitochondrial 12S rRNA sequence analysis was used for the determination of species origin of skin sample received at Forensic Laboratory. Sequence analysis revealed that the skin that was thought to be from Tiger was actually of bovine origin.

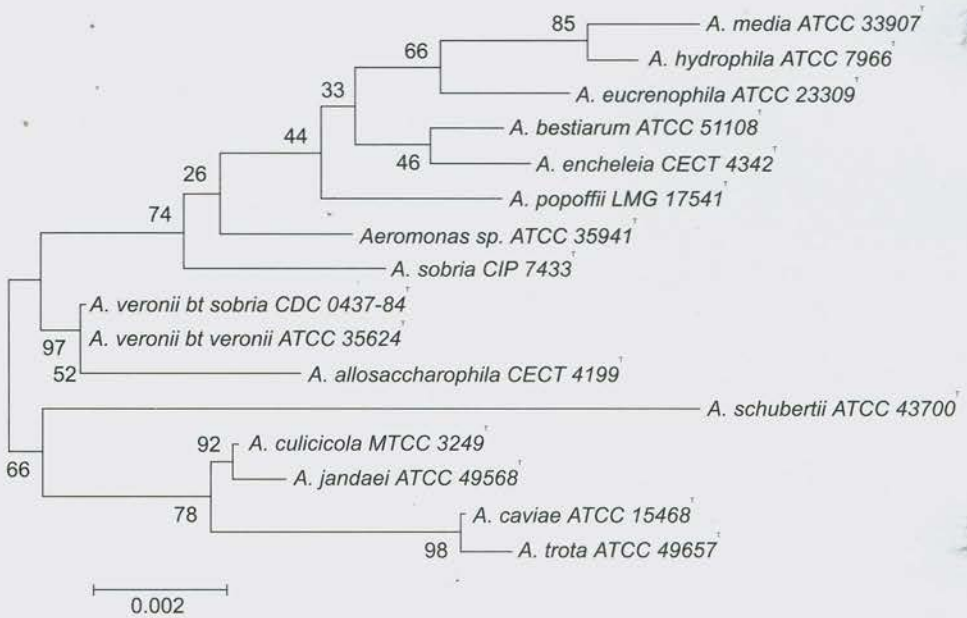


Fig 1. 16S rRNA gene sequence based phylogenetic relationship of Aeromonas culicicola sp. nov. MTCC 3249 with reerence strains (HGs) of the genus Aeromonas. The unrooted phylogenetic tree was drawn by using 1,336 nucleotides of 16S rRNA gene sequence using neighbour-joining (NJ) method in MEGA software with bootstrap value of 500.

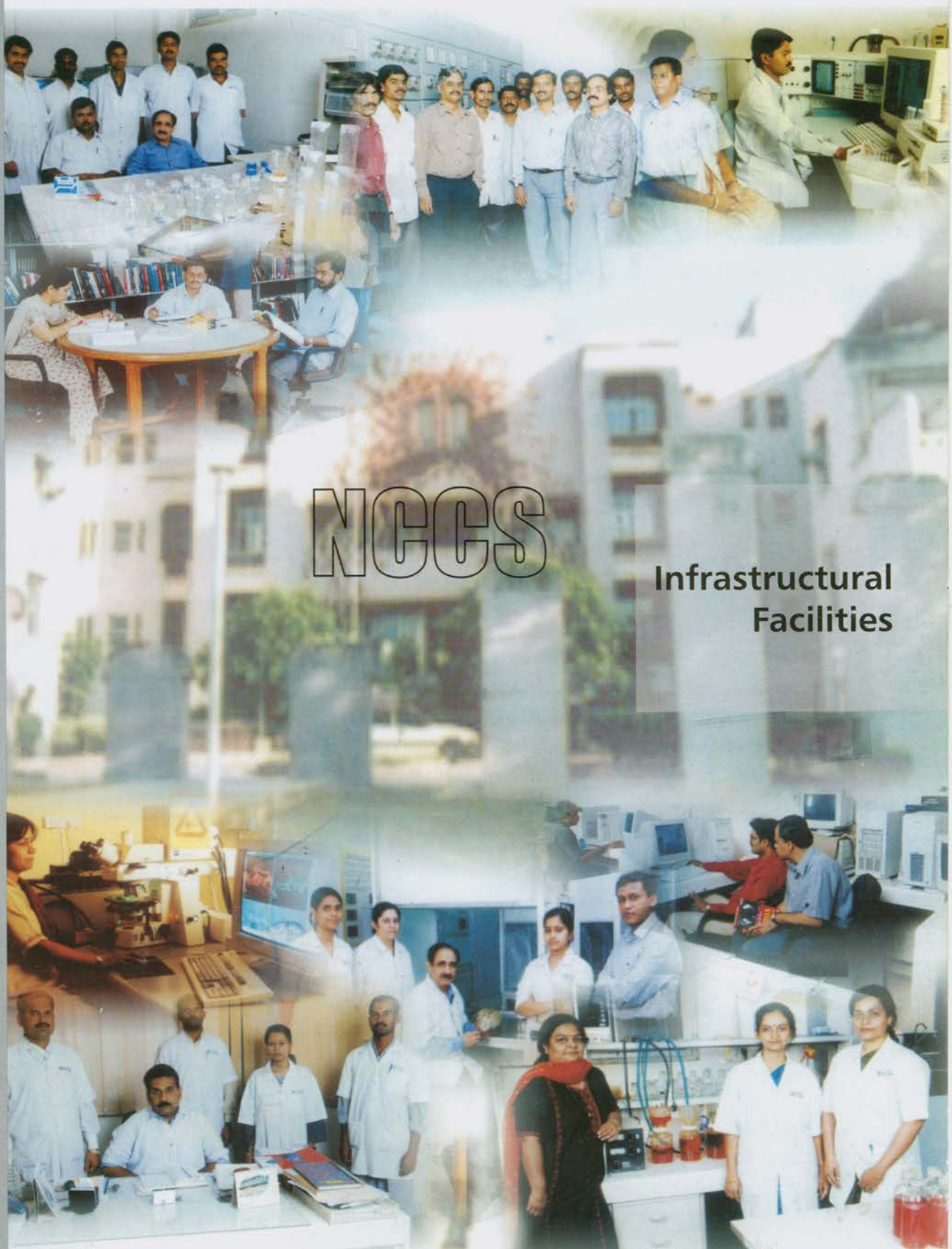
authenticated. Also the work on the phylogenetic studies on wasps was initiated in collaboration with Prof. Raghavendra Gadagkar (Centre for Ecological Sciences, Indian Institute of Science, Bangalore).

In an interesting application, mitochondrial 12S rRNA sequence analysis was used for the determination of species origin of skin sample received at Forensic Laboratory. Sequence analysis revealed that the skin that was thought to be from Tiger was actually of bovine origin.

Future work

Complete characterisation of libraries of PCR amplified 16S rRNA genes from mosquito mid gut and Antarctic soil will be done.

Experiments will be done to elucidate the role of gut flora of the mosquito.


NCCS
Infrastructural
Facilities

Experimental Animal Facility

The Experimental Animal Facility is an infrastructural service department of the Institute. It is a barrier-maintained facility for the breeding, maintenance and supply of high quality and standardised laboratory animals viz. inbred mice, rats, rabbits etc. for the ongoing research projects of the Institute. The following is the list of various laboratory animals maintained at the facility:

Mice strains	BALB/CJ, C57BL/6J, DBA/2J, SWISS, BALB/c*, Nude mice
Rat strains	WISTAR, LEWIS
Rabbit strains	NEWZEALAND WHITE
Mastomys	MASTOMYS COUCH

Defined barrier practices are followed scrupulously without any exception or allowance, with access to a select few personnel, to minimize the risk of microbial infection to the animals housed in the facility. The breeding program for the propagation of the three different inbred lines viz. BALB/c, C57BL/6, and DBA/2 is structured in a two-tier format, i.e. the Foundation colonies (FC) and the Production colonies (PC). Strict full-sib pairing only propagates the animals in the FC. The three different strains of mice namely BALB/c, C57BL/6 and DBA/2 are currently at F11, F9 and F8 respectively. These colonies are the nuclear colonies for the long-term propagation of an inbred strain.

* A single male mouse with spontaneous congenital cataract was detected in a production colony of BALB/c strain of mice. Attempts are being made to separate and propagate the line as a true breeding mutant strain. The mutant colony is currently at **F14** level of inbreeding.

The complete technical support and advice has been extended regularly to Scientists/ Research Scholars in the various aspects of animal experimentation namely, handling of laboratory animals, collection of blood and other samples, immunizations, surgical procedures etc. and the procurement of animals. As a part of our efforts to develop expertise in the area of transgenic animal production, several important techniques like Superovulation, Collection of day old embryos from the oviducts of mice, Collection of morulas and blastocysts from the oviducts/uteri, and Vasectomies were standardised in the various strains of mice available at the facility.

S. No	Strains species	Animals Procured	Animals bred	Animals Supplied
1	Rats			
	Wistar	--	71	51
	Lewis	--	58	6
2	Mice			
	BALB/C	325	4478	2756
	C57bl/6	--	1929	1115
	Swiss	--	2099	847
	Nude (nu/nu)	93	--	93
	BALB/C*	--	261	--
3	Mastomys Coucha	--	88	--
4	Rabbit (NZW)	4	9	5

The Library:

The NCCS Library has collection in the frontier areas of biotechnology having relevance to NCCS research activities. The Library holds more than three thousand bound journals, fifteen hundred books, and subscribes to 75 scientific journals and 30 other periodicals.

In the development of its collections, the Library's priority is to support NCCS research activities. The Library collection is expanded in consultation with NCCS scientists. The Library's print collections are growing by approximately 750 volumes per year. During the period of 2000-2001, the Library has added 130 books and 655 journals to its collection. In order to provide faster access to research information, the Library also subscribes for limited full text material online.

Additional documentation facilities include local area network confined to library activities, a number of CD ROM databases including, full text and factual databases. The library continues to be a part of the Pune Library Network.

The Fluorescence Activated Cell Sorter (FACS): The FACS has been upgraded to dual split 488 nm laser. A total of approximately 4500 samples were analyzed for surface staining of cell surface makers on FACS till date. Out of these, 22 samples were from National Chemical Laboratory.

The Confocal Laser Scanning Microscope (CLSM): The Confocal Laser Scanning Microscope (CLSM) is a state of the art laser scanning microscope equipped with 4 lasers covering the UV, 488 nm and 590 nm ranges. This presence of the four lasers has enhanced the choice of fluorophores for real time observation. The CLSM facility has analyzed about 60 samples belonging to various users till date.

The Computer Centre: The computer centre has two servers which provide local area network, internet browsing for fast access to the scientific literature and for easy transfer of data between laboratories and other institutions.

PUBLICATIONS (2000-2001)

1. Deshpande, M., Mitra, D., and Parab, P. (2000). Polyacrylamide gel as a matrix for the delivery of a layer or coat of other molecules, *Biotechnique,* 30, 258-262

2. Hardikar, A. A., Risbud, M.V., Remacle, C.R., Reusens, B, Hoet, J. J., Bhonde, R.R. (2001) Islet Cryopreservation: Improved recovery following taurine pretreatment, *Cell Transplantation (in press)*

3. Jacob, S. S., Shastry, P., and Sudhakaran, P. R. (2001) Influence of nonenzymatically glycated collagen on monocyte-macrophage differentiation, *Atherosclerosis (in press)*

4. Jaykumar, P. C., Souche, Y.S. and Patole, M. S. (2001) Cloning of two hexokinase isozyme sequences from Drosophila melanogaster, *Insect Biochemistry and Molecular Biology (in press)*

5. Kurup, S., Risbud, M.V. and Bhonde R.R. (2001) Selective cytotoxicity of Mia Paca-2 conditioned medium to acinar cells : A novel approach to reduce acinar cell contaminants in isolated islet preparations, *Transplant International (in press)*

6. Limaye, L. S. and Kale, V. P. (2001) Cryopreservation of human hematopoietic cells with membrane stabilizers and bio-antioxidants as additives in the conventional freezing medium. *J. Hematotherapy and Stem Cell Research* 10, 709 - 718

7. Prasanna, I, Lakra, W. S., Ogale, S.N. and Bhonde, R. R. (2000) Cell culture from fin explant of endangered mahasheer, Tor putitora (Hamilton), *Current Science,* 79, 93-95

8. Risbud, M.V., Saheb, N. D, Jyoti, J. and Bhonde, R.R. (2001) Preparation, characterisation and in vitro biocompatibility evaluation of poly (butylene terephthalate)/Wollastonite composites, *Biomaterials,* 22, 1591-1597

9. Risbud, M.V., Bhonde, M.R. and Bhonde R. R. (2001) Chitosan-Polyvinyl pyrrolidone hydrogel does not activate macrophages: Potentials for transplantation applications, *Cell Transplant.,* 10, 195-202

10. Risbud, M.V. and Bhonde, R. R. (2001) Polyamide-6 composite membrances : Properties and in vitro biocompatibility evaluation, *J. Biomaterial Sc. Polymer Edition,* 12, 125-136

11. Risbud, M.V. and Bhonde, R.R. (2001) Suitability of cellulose molecular dialysis membrane for bioartificial pancreas: In vitro biocompatibility studies., *J. Biomed. Materials Res.,* 54, 436-444

12. Risbud, M.V., Ringe, J., Bhonde R. R. and Sittinger. M. (2001) In vitro expression of cartilage specific markers by chondrocytes on chitosan hydrogel : Implications in cartilage engineering, *Cell Transplantatiion (in press)*

13. Sahu, A. and Lambris, J. D. (2001) Structure and biology of complement protein C3, a connecting link between innate and acquired immunity, *Immunoogical Reviews,* 180, 35-48

14. Sandhya S. L., Yogita Shewade, and Ramesh Bhonde (2000) Role of Bittergourd Fruit Juice in STZ-induced diabetic state in vivo and in vitro, *J. Ethnopharmacology,* 73, 71-9

15. Sarrias, M.R., Franchini, S., Canziani, G., Moore, W.T., Sahu, A., Lambris, J.D. (2001) Kinetic analysis of the interactions of complement receptor 2 (CR2, CD21) with its ligands C3d, iC3b and the Epstein Barr virus glycoprotein gp350/220. *J. Immunol.,* 167,1490-1499.

16. Savita, K. and Bhonde, R. R. (2000) Combined effect of nicotinamide and streptozotocin on diabetic status in partially pancreatectomized adult BALB/c mice, *Hormone and Metabolic Research,* 32, 330-334

17. Shastry, P., Basu, A., Rajadhyaksha, M. S.(2001) Neuroblastoma cell lines - A versatile in vitro model in neurobiology (Review article), *International Journal. of Neurosciences* 108, 1-18

18. Shewade Y. M. and Bhonde R.R. (2001) Differential action of fatty acids against streptozotocin induced in vitro cytotoxicity in islets, *In vitro Cell and Develop. Biol., (in press)*

19. Shiras, A., Shepal, V., Bhonde, M. and Shastry, P. (2001) Expression Analyses of S100 in Primary and immortalised Mouse Fibroblast cultures, *In vitro Cell and Dev. Biol-Animal,* 37, 172-174

20. Shiras A. S., Sengupta A., Shepal V. S. (2001) Cloning and Expression analyses of a novel homeobox gene clone Dlxin-1 with tumour suppressor properties. *Mol. & Cell Biol. Res. Commun.* (in press)

21. Shouche, Y. S. and Patole, M. S. (2000) Mitochondrial rRNA sequence analysis of some mosquito species, *J. Biosci.,* 25, 361 -366

22. Shukla, M. S., Pidiyar, V., Bhave, N. A., Patole, M. S. and Shouche, Y. S. (2000) PCR amplification and sequencing of mitochondrial 12S rRNA gene fragment from Muntiacus muntjak (Indian Muntjac), *Current Science,* 80, 101 102

23. Sitasawad, S.L, Shewde, Y.M and Bhonde R.R. (2000) Role of Bittergourd Fruit Juice in STZ-Induced Diabetic State in vivo and in vitro. *J. Ethenopharmacol.,* 73, 71-79

24. Sitasawad, S.L, Deshpande, M., Katdare, M., Tirth, S. and Parab P. (2001) Beneficial effect of supplementation with copper sulfate on STZ-diabetic mice (IDDM), *Diabetes Research and Clinical Practice,* 52, 77-84

25. Venuprasad, K., Parab, P., Prasad, D.V.R., Sharma, S., Banerjee, P., Deshpande, M., Mitra, D. K, Pal, S., Bhadra, R,. Mitra, D., and Saha, B. (2001) Immunobiology of CD28 expression on human neutrophils I : CD28 regulates neutrophil migration by modulating CXCR-1 expression, *Eur. J. Immunol.,* 31, 1536-1543

26. Nair, S. S., Chaubal, V. A., Shioda, T., Coser, K. R., Mojamdar, M. (2001) Over-expression of MSG1 transcriptional co-activator increases melanin in B16 melanoma cells: a possible role for MSG1 in melanogenesis. *Pigment Cell Research,* 14, 206-209

27. Chaubal, V. and Mojamdar, M. (2000) Inter-relationship between tyrosinase and TRP-1 with -Glutamyl transpeptidase in B16 melanoma cells. *Pigment Cell Research,* Suppl 7, 48

PAPERS PUBLISHED BY THE NCCS SCIENTISTS (WORK DONE ELSE WHERE)

28. Bachhawat, K., Thomas, C. J., Amutha, B., Krishnasastry, M. V., Khan, M. I., Surolia, A. (2001) On the stringent requirement of mannosyl substitution in mannooligo-saccharides for the recognition by garlic (Allium sativum) lectin: A surface plasmon resonance study. *J. Biol. Chem.* 276, 5541-5546.

29. Franchini, S., Zarkadis, I.K., Sfyroera, G., Sahu, A., Moore, W. T., Mastellos, D., Lapatra, S. E. and Lambris, J. D. (2001) Cloning and purification of the rainbow trout fifth component of complement (C5), *Dev. Comp. Immunol.*, 25, 419-430

30. Kundu, G. C., Zhang, Z., Mantile-Selvaggi, G., Mandal, A., Yuan, C-.J., Mukherjee, A.B. (2000) Uteroglobin binding proteins: regulation of cellular motility and invasion in normal and cancer cells, *Ann. N.Y. Acad. Sci.*, 923, 234-248

31. Rong Zheng, Eriko Matsui, Yulong Shen, Krishnasastry V. M., Yan Feng, Sophie Darnis, Yutaka Kawarabayasi, Hisasi Kikuchi, Kazuaki Harata, Ikuo Matsui: The novel function of a short region K253XRXXXD259 conserved in the exonuclease domain of hyperthermostable DNA polymerase I from Pyrococcus horikoshii, *Extremophiles (in press)*

32. Sahu, A. and Lambris, J.D. (2000). Complement inhibitors: a resurgent concept in anti-inflammatory therapeutics. *Immunopharmacology,* 49, 133-148.

33. Sahu, A., Soulika, A.M., Morikis, D., Spruce, L., Moore, W.T. and Lambris, J.D. (2000). Binding kinetics, structure-activity relationship and biotransformation of the complement inhibitor Compstatin. *J. Immunol.,* 165, 2491-2499.

34. Sahu, A., Morikis, D., and Lambris, J.D. (2000). Complement inhibitors targeting C3, C4, and C5. Chapter 4. In Therapeutic Interventions in the Complement System. J. D. Lambris and V. M. Holers, editors. *Humana Press Inc., Totowa, New Jersey, U.S.A. pp 75-112.*

35. Shen, Y., Krishnasastry, M. V., Hiramoto, M., Kikuchi, H., Kawarabayashi, Y., and Matsui, I. (2001) Invariant Asp1122 and Asp1124 are essential residues for polymerization catalysis of family D DNA polymerase from Pyrococcus horikoshii *J. Biol. Chem. (in press)*

36. Soulika, A.M., Khan, M., Hattori, T., Bowmen, F.W., Richardson, B.A., Sahu, A., Edmunds Jr, H.L. and Lambris, J.D. (2000). Inhibition of heparin/protamine complex-induced complement activation by Compstatin in baboons. *Clin. Immunol.,* 95, 212-221.

37. Zheng, F, Kundu, G. C., Zhang, Z. and Mukherjee, A. B. (2000) Identical glomerulopathy in two different mouse models of uteroglobin deficiency, *Am. J. Kid. Dis.*, 35, 362-363

38. Zheng, F, Kundu, G. C., Zheng, F., Yuan, C-.J., Lee, E., Westphal, H., Ward, J., DeMayo, F. and Mukherjee A. B. (2000) Insight into the physiological function(s) of uteroglobin by gene knockout and antisense-transgenic approaches, *Ann. N.Y. Acad. Sci.*, 923, 210-233

39. Zhu, X.Guang, Park, S. K., Kaneshige, M., Bhat, M. K., Zhu, Q., Mariash, C. N., McPhie, P. and Cheng, S-. Y. (2000) *Mol. Cell Biol.*, 20, 2604-2618.

PATENTS:

US patents filed

1. ***Mohan Wani, Pradeep Parab and Anil Chatterjee***
   An extract from the Indian green mussel (*Perna viridis*) inhibits osteoclast formation and bone resorption.

2. ***Pradeep Parab and Anil Chatterjee***
   A novel process of preparing an antidiabetic drug from the perivitalline fluid collected from the fertilized eggs of the Indian Horse Shoe Crab (*Tachypleus gigas*)

HONOURS CONFERRED ON NCCS SCIENTIST:

Ramesh Bhonde was selected as Member of National Academy of Sciences, India (MNASC).

EXTRAMURAL FUNDING RECEIVED BY THE NCCS SCIENTISTS

Debashis Mitra

Jai Vigyan Mission Project: immune response to HIV infection towards generation of DNA vaccine (DBT).

Identification and characterisation of anti-HIV compounds in Indian marine bivalves(DBT).

New natural products as HIV-1 reverse transcriptase inhibitor from the genus *Calophyllum* (ICMR).

Ramesh Bhonde and Pradeep Parab

Studies on the Induction of islet neogenesis in vivo and in vitro for its potential role in the treatment of diabetes (DBT).

Samit Chattopadhyay,
Debashis Mitra and Bhaskar Saha

Targeted modulation of gene regulation: Inhibition of HIV infection by gene therapy (DBT).

Anjali Shiras and Padma Shastry

tudies on expression analyses of S-100 A2, A4, A6 and beta in neuroectodermal tumours (ICMR).

Lalita Limaye and Vaijayanti Kale

Bone marrow cryopreservation: Addition of membrane stabilizers and antioxidants in the conventional freezing mixture to conserve growth factor responsiveness and engraftment potential of hematopoietic stem cells (DRDO).

Gopal Kundu

Role of osteopontin on matrix metaloproteinase 2 expression, cell migration and ECM-invasion in melanoma cells (DST).

Development of breast cancer specific drugs (British council, UK).

Samit Chattopadhya

Identification and characterisation of a novel MAR binding protein that has a role in V(D)J recombination and autoimmunity (DST).

Samit Chattopadhya and Gyan Mishra

Construction of a safe extra chromosomal replicating vectors for gene therapy (ILTP, Indi-Russian collaborative programme in Medical Biotechnology).

Arvind Sahu

Structure-function analysis of viral homologues of complement control proteins. (Senior Research Fellowship, Wellcome Trust, UK).

Bhaskar Saha

Modulation of toxic shock syndrome by neonatal priming with staphylococcal enterotoxin-B (DRDO).

Neonatal vaccination against leishmania infection: characterisation of memory T-cell response (DST).

Role of co-stimulatory molecules in the regulation of anti-leishmanial immune response (Indo-UK).

Samit Chattopadhay	Satish Totey, Subeer Majumdar, Rajesh Anand, Neerja Gulati and Arundhati Mandal, National Institute of Immunology ***Construction of transgenic mice for SMAR1***
	Alexey Nikolave and Vyacheslav, Tarantul Institute of Molecular Genetics, RAS, Moscow ***Construction of a safe extra chrosomal replicating vectors for gene therapy,***
Bhaskar Saha	Simon Croft, London school of hygiene tropical medicine, Subhas Padhye, Dept. Chemistry, University of Pune. ***Screening compounds active against leishmania.***
	Paul Kaye, London school of hygiene tropical medicine ***Role of co-stimulatory molecules in regulation of anti-leishmanial immune response***
Musti Krishnasastry	Manoj Raje, IMTECH, Chandigarh ***Design and construction of Immunolysins.***
Mohan Wani	Anil Chatterjee National Institute of Oceonography, Dona Paula, Goa. ***Identification of anti-osteoporotic activity from marine bivalves.***
Lalita Limaye	S. G. A. Rao Cancer Research Institute, Mumbai. R. L. Marathe Jehangir Hospital, Pune. ***Studies on cryopreservation of haematopoietic cells.***

COLLABORATIVE RESEARCH PROJECTS-NATIONAL / INTERNATIONAL

Debashis Mitra	Anil Chatterji, Scientist, National Institute of Oceanography, Goa. ***Anti-HIV activity in marine bivalves.***
	M. K. Gurjar and S.P. Joshi, National Chemical Laboratory, Pune. ***Anti-HIV activity in Calophyllum.***
Gyan Mishra **Prakash Deshpande**	Pr.Pierre-Andre CAZENAVE Pasteur Institute, Paris, France Sylviane PIED, CR, CNRS. ***T Lymphocyte response in Human Malaria Pathogenesis.***
Gyan Mishra	Paul M. Kaye, London School of Hygiene Tropical Medicine. ***The role of co-stimulatory molecules in the regulation of anti leishmanial immune response***
Vaijayanti Kale	L. C. Padhy, Molecular Biology Group, TIFR, Mumbai. ***Stromal cell biology***
Gopal Kundu	Christopher J. Newton, Medical Research Laboratory, University of Hull, UK. Subhas Padhye, Department of Chemistry, University of Pune, Pune. ***Development of Breast Cancer Specific Drugs.***
	C. G. Naik, National Institute of Oceanography, Goa. ***Identification of Bioactive Compound from Marine Sources.***
Anjali Shiras	L. C. Padhy, Molecular Biology Group, TIFR, Mumbai. ***Transforming genes in melanoma***
Ramesh Bhonde	Anil Chatterjee, National Institute of Oceanography, Goa ***Production of Indian Horseshoe crab amoebocytes in vitro.***
Debashis Mitra **Ramesh Bhonde** **Pradeep Parab**	Anil Chatterjee National Institute of Oceonography, Dona Paula, Goa. ***Antiviral compounds from the Indian Marine bivalves and their potential role in curing diseases***

INVITED TALKS / SEMINARS

Ramesh Bhonde	**Tissue Engineering** National seminar on zoology for 21st century, dept of zoology, Goa university, Goa.
	Human Health in 21st Century Dreams & Challenges of 21st Century, S. P. College, Pune.
Jayant Chiplonkar	**Animal Tissue culture** Ahmednagar College, Ahmednagar.
Samit Chattopadhyay	**Use of knock-out techniques and understanding of immune system in mice** Workshop on production of transgenic and gene knock-out animals, NII, New Delhi.
Bhaskar Saha	**Immunobiology of CD28 expression on human neutrophils** University of Hull, Hull, UK.
Yogesh Shouche	**PCR strategies** 3rd National Molecular Biology workshop, AFMC, Pune.
Gopal Kundu	**Uteroglobin binding proteins: Regulation of cellular motility and invasion in normal and cancer cells** The NY Academy of Sciences, Conference on the Uteroglobin / Clara Cell Protein family.

SEMINARS AT NCCS BY VISITING SCIENTISTS

Jay A. Levey Prof. Medicine University of California San Francisco, USA	Host Immune response can prevent HIV infection and disease
Devinder Sehgal Molecular Immunogenetics section National Institutes of Health Bethesda, USA	Gene conversion in B cell ontogeny during immune responses within germinal centre
Chinmoy Mukhopadhyay Department of Cell Biology The Lerner Research Institute Cleveland Clinic Foundation Cleveland, USA	Ceruloplasmin: A tale of two functions
Chris Newton University of Hull Hull, UK	Apoptosis: A target for cancer drug development
Madhulika Shrivastava National Institutes of Health Bethesda, USA	Regulation of monoallelic expression by genomic imprinting

PARTICIPATION IN NATIONAL / INTERNATIONAL CONFERENCES

XXIV All India Cell Biology Conference held at Dept. of Molecular Medicine Jawaharlal Nehru University, New Delhi in Nov.2000.

Anjali Shiras, Sengupta A. and Shepal V.S.
Identification and Tissue Specific expression studies of a novel Dlxin-1 clone from Melanoma cells.

Shepal V.S. Shiras A.S.
Cloning and characterisation of a 600 bp clone from a melanoma cells with transforming potential.

Savita Kurup & R. R. Bhonde
Zymogen granule fragility as parameter for ascertaining the functional state of pancreatic acinar cells *in vitro*

Ajith Mathew, J. Ladha and D. Mitra
Nef regulates HIV-1 LTR mediated gene expression in human cell lines.

Philip S; Tiwari N; Bulbule A and Kundu G.C.
Osteopontin induced expression of matrix metalloproteinase-2 correlates with invasiveness and tumour growth by using murine melanoma cells.

Samit Chattopadhyay
A putative proapoptotic role of a novel MAR binding protein in tumour cells.

Padma Shastry
Induction of apoptosis in leukemic cell lines by beta carotene- role of p27.

P. B. Parab
Beneficial effect of supplementation with copper sulfate on STZ induced diabetic mice (IDDM).

Pallavi Dhoot and Manoj Bhat
Regulated expression of p53 in human cancer cell lines: A tool to study functional effect of over expression of p53.

Reena Ramesh and Manoj Bhat

Overexpression of p53- Is it predictive of tumour cell death

Mohan Wani
Effect of interleukin-4 on stroma free population of bone marrow cells

Ruchika Kaul, Aniruddha Deshpande and Samit Chattopadhya
A novel lymphocyte specific protein SMAR1: Possible role in chromatin structure modulation and tumourogenesis

Royal Society conference on genomics of parasitic protozoa

Bhaskar Saha

XIX National Symposium of the Society for Reproductive Biology & comparative Endocrinology at M.S. University of Baroda, Baroda Jan.17-19, 2001.

R. R. Bhonde
Novel Approaches for the reversal of experimental diabetes mellitus: Role of pancreatic regeneration & islet transplantation

National Update on Hematopoietic Stem Cell-2000" held at AFMC, Pune on 10th December 2000.

Vaijayanti Kale
Strategies used in the stem cell expansion.

Lalita Limaye
Haematopoietic stem cells: Tissue culture techniques.

Mohan Wani

New York Academy of Sciences Conference, Bethesda, Maryland, USA, April 14-16, 2000

Kundu G.C., Zhang, Mantile-Selvaggi G., Mandal A., Yuan C-J. & Mukherjee A.B.

Uteroglobin binding proteins: regulation of cellular motility and invasion in normal and cancer cells.

20th Annual Convention of Indian Association for Cancer Research, The Gujarat Cancer and Research Institute, Ahmadabad, January 19-21, 2001.

Tiwari N, Philip S. and Kundu G.C.
Potential role of human bone derived chemoattractant factor (BDCF) in regulation of breast adenocarcinoma cell migration and bone metastasis.

Philip S. and Kundu G.C.
Role of osteopontin in regulation of matrix metalloproteinase-2 expression, extracellular matrix invasion and tumour growth in melanoma cells.

The "5th Annual Convention and National Symposium on Current Features of Specific Diseases of Animals and Poultry including Zoonoses" at ANGRAU, Hyderabad from 14-16 December 2000.

K.N. Kohale

Workshop on Production of Transgenic and Gene Knock-out animals held at NII, New Delhi between February 15 to March 01 2001.

B. Ramanamurthy

4th ADNAT Convention and Symposium on DNA Technologies in the management of biodiversity, Chennai, February 2000

Vyankatesh Pidiyar, M.S. Patole and Yogesh Shouche
16S rRNA sequence based studies on microbial diversity: a case of mosquito mid gut.

Patole M. S. and and Yogesh Shouche
Mitochondrial rRNA gene sequences in the studies of insect biodiversity: Studies on mosquitoes.

International Symposium of Developmental Biology, Pune, India February 2000.

P. Cyril Jaykumar, Yogesh Shouche and Milind Patole
Cloning of two hexokinase isozyme sequences from *Drosophila melanogaster.*

Preclinical Toxicology, National Institute of Nutrition, Hyderabad, February 5-10, 2001
Sandhya Sitaswad

Participation in Discussion groups

Jayant Chiplonkar
On confocal laser scanning microscope at National Institute of Immunology, New Delhi,.

Vaijayanti Kale
characterisation of Stem cells, Stem cell biology and Its Application at Task force meeting, Department of Biotechnology, New Delhi.

Yogesh S. Shouche
Discussion meeting on "Origin and Evolution of Life" at Indian Academy of Sciences, Bangalore 2000.

FELLOWSHIPS FOR FOREIGN TRAVEL.

Ramesh Bhonde	INSA / DFG exchange of scientist fellowship Medical Polyclinic University of Bonn, Germany.
Debashis Mitra	Invitee Laboratory for AIDS virus Research, Cornell University Medical College, 411 East 69th Street, New York, NY 10021, USA
Gopal Kundu	Invitee The New York Academy of Sciences, Conference on The Uteroglobin/Clara Cell Protein Family.
	British Council Higher Education programme University of Hull, Hull, U.K.
Musti Krishnasastry	Japan Industrial Technology Association Fellowship National Institute of Bioscience and Human Technology, Tsukuba, Japan
Bhaskar Saha	Indo-UK Council Fellowship London School of Hygiene & Tropical Medicine
	British Council Higher Education programme University of Hull, Hull, U.K.
Samit Chattopadhyay	Invitee Stem Cell Biology Division, Massachusetts General Hospital, Charlestown, USA.

MEMBERS OF NCCS SOCIETY

President

Prof. Murli Manohar Joshi
Hon'ble Minister for HRD
Science & Technology
Ocean Development
Department of Education
Shastri Bhavan
New Delhi - 110 001
Ph. (011)-378-2698

Members

Dr. (Smt.) Manju Sharma
Secretary, Department of Biotechnology
Ministry of Science & Technology
Block No.2, 7th floor
CGO Complex, Lodi Raod
New Delhi - 110 003
Ph: (011)-436-2950

Dr. V. K. Vinayak
Adviser, Department of Biotechnology
Block No. 2, 7th - 8th Floor
CGO Complex, Lodhi Road
New Delhi - 110 003
Ph: (011)-436-0718

Shri. Arun Sharma
Joint Secretary & Financial Adviser
Dept. Of Science & Technology
Technology Bhavan
New Mehrulli Road
New Delhi - 110 003
Ph: (011)-651-3883

Prof. Ashok S. Kolaskar
Vice Chancellor
University of Pune
Pune - 411 007
Ph: (020)-565-3868

Prof. G. Padmanaban
Ex-Director
Indian Institute of Science
Bangalore - 560 012
Ph: (080)-360-1492

Prof. N. K. Ganguly
Director General
Indian Council of Medical Research
Ansari Nagar, Post Box 4911
New Delhi - 110 029

Dr. A. N. Bhisey
Director, CRI,
7, Yugprabhat Road
Mahim, Mumbai - 400 016

Prof. D. N. Deobagkar
Head, Department of Zoology
University of Pune
Pune - 411 007

Dr. Lal Krishna
Asst. Director General (Animal Health)
Dept. Of Agricultural Research & Education
And ICAR, Krishi Bhavan,
Dr.Rajendra Prasad Road
New Delhi - 110 001

Dr. Padma Shastry
Scientist E
NCCS,
Pune - 411 007
Ph: (020)-569-0922

Member Secretary

Dr. G. C. Mishra
Director
National Centre for Cell Science
Pune - 411 007
Email: gcmishra@nccs.res.in

Special Invitees

Shri. S. L. Bhatt
Joint Secretary(Administration)
Department of Biotechnology
Block No.2,7th Floor
CGO Complex, Lodi Road
New Delhi - 110 00

Shri. Sukhdeb Sinha
Director
Department of Biotechnology
Block No.2, 7th Floor
CGO Complex, Lodi Road
New Delhi - 110 003

MEMBERS OF GOVERNING BODY

Chairperson

Dr. (Smt.) Manju Sharma
Secretary, Dept. of Biotechnology
Ministry of Science & Technology
Block No.2, 7th floor, CGO Complex, Lodi Raod
New Delhi - 110 003
Ph: (011)-436-2950

Members

Dr. V. K. Vinayak
Adviser, Dept. of Biotechnology,
Block No. 2, 7th - 8th Floor,
CGO Complex, Lodhi Road,
New Delhi -110 003
Ph: (011)-436-0718

Shri. Arun Sharma
Joint Secretary & Financial Adviser
Dept. Of Science & Technology
Technology Bhavan
New Mehrulli Road
New Delhi - 110 003
Ph: (011)-651-3883

Dr. Ashok S. Kolaskar
Vice Chancellor, University of Pune
Pune - 411 007
Ph: (020)-565-3868

Prof. G. Padmanaban
Ex-Director
Indian Institute of Science
Bangalore - 560 012
Ph: (080)-360-1492
Email: geepee@biochem.iisc.ernet.in

Dr. A. N. Bhisey
Director, CRI
7 Yugprabhat Road
Mahim, Mumbai - 400 016
Ph: (022)-444-3025

Prof. N. K. Ganguly
Director General,
Indian Council of Medical Research,
Ansari Nagar, Post Box 4911
New Delhi - 110 029
Ph: (011)-651-7204
Email: icmrhqds@sansad.nic.in

Prof . D. N. Deobagkar
Head, Dept. of Zoology
University of Pune
Pune - 411 007
Ph: (020)-565-4952
Email: dndeb@unipune.ernet.in

MEMBERS OF SCIENTIFIC ADVISORY COMMITTEE

Chairman

Prof. G. Padmanabhan
Emeritus Scientist
Department of Biochemistry
Indian Institute of Science
Bangalore - 560 012
Ph: (080)-360-1492
Email: geepee@biochem.iisc.ernet.in

Members

Dr. Sandip K. Basu
Director, National Institute of Immunology
Aruna Asaf Ali Marg
New Delhi - 110 067
Ph: (011)-617-5002
Email: sandip@nii.res.in

Dr. A. N. Bhisey
Yugprabhat Co-Op. Hsg. Soc
Sitaladevi Temple Road
Mahim, Mumbai - 400 016
Ph: (022)-444-30256

Prof. Vijay K. Choudhary
Department of Biochemistry
Delhi University (South Campus)
New Delhi - 110 018
Ph: (011)-688-2231
Email: VKC@DUSC.ernet.in

Dr. Pradeep Seth
All India Institute of Medical Science
Ansari Nagar
New Delhi - 110 029
Ph: (011)- 659-3288
Email: pseth@medinst.ernet.in

Prof. N. K. Ganguly
Director General
Indian Council of Medical Research
Ansari Nagar
New Delhi - 110 029
Ph: 011 653980
Email: icmrhqds@sansad.nic.in

Dr. C. M. Gupta
Director, Central Drug Research Institute,
Chattar Manzil, Lucknow - 226 001
Ph: (0522)-223286
Email: cmg@gateway.cdri.res.in

Dr. Kanuri Rao
International Centre for Genetic
Engineering & Biotechnology
NII Campus, Aruna Asaf Ali Marg
New Delhi - 0 067
Ph: (011)-617-6680
Email: kanury@icgeb.res.in

Prof. N. Kochupillai
Head, Department of Endocrinology and Metabolism
All India Institute of Medical Sciences
Ansari Nagar, New Delhi - 0 029
Ph: (011)-696-1612

Dr. Jayant Udgaonkar
National Centre forBiological Sciences
UAS-GKVK Campus, Bellary Road
Bangalore - 560 065
Ph: (80)-363-6421
Email: jayant@ncbs.res.in

Prof. Avadhesha Surolia
Molecular Biophysics Unit
Indian Institute of Science
Bangalore - 560 012
Ph: (80)-309-2459
Email: surolia@mbu.iisc.ernet.in

Dr. V. K. Vinayak
Advisor
Department of Biotechnology
11, Lodi Road, Block No. 2
7th Floor, CGO Complex
New Delhi - 0 003
Ph: (011)-436-0718

We and us at NCCS