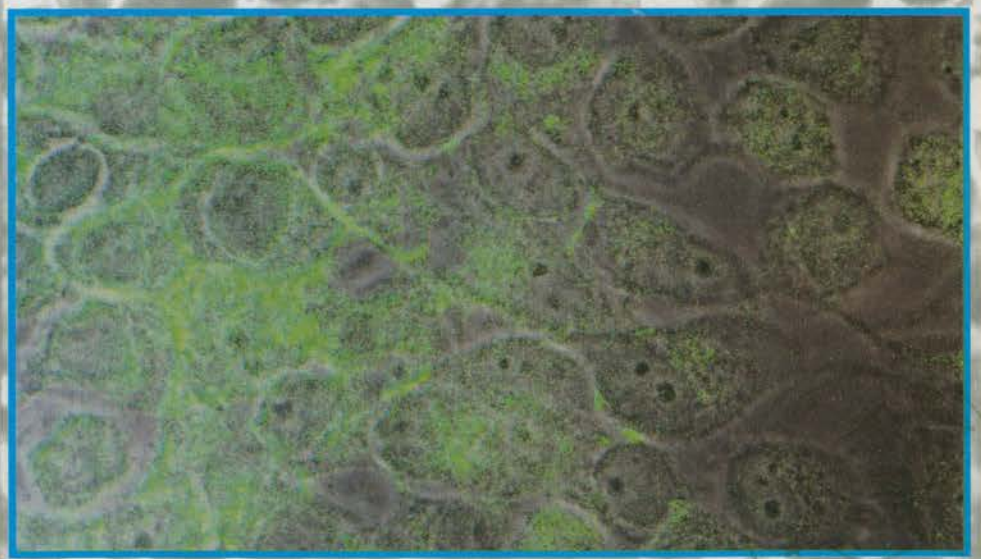
National Centre for Cell Science



Annual Report
1999-2000

National Centre for Cell Science

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

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NCCS Complex, Ganeshkhind, Pune 411 007

Contents

Terms of Reference

- To receive, identify, maintain, store, grow and supply: Animal and human cells/cell cultures, 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 organizations.
- Research and development in the above and cell culture related materials and products.
- To establish and conduct post graduate courses, workshops, seminars, symposia and training programmes in the related fields.
- 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 organizations including industries working in the country.
- To participate in such programmes 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 organizations in the areas relevant to the objectives of the facility.

From the Director's Desk

It is my pleasure and privilege to present you, once again, the recent achievements of National Centre for Cell Science (NCCS). The mandate of our organization has been research and development, teaching and training, and providing specific service as a national repository for cell lines/hybridomas etc., in the country.

The productivity of any organization lies at its critical mass which is necessary to sustain the quality of output, as well as enables branching out into supplementary areas of direct relevance to the main focus. Keeping this in mind, we have initiated several changes that will facilitate achievements of our goals. During the period under review, we have recruited several accomplished young scientists to strengthen our interests in the areas of cancer biology, cell biology, immunology, gene therapy and proteomics. As a result of this, we now have nine fully functional laboratories in comparison with four in 1997. Currently, twenty three scientists are engaged in research in frontier areas of modern biology. This intensified research activity is reflected in the fact that NCCS has, in the last year, published fifty six papers in National and International journals. In addition to this, we have also attracted peer-reviewed extramural funding from both National and International Agencies. Some of the other achievements of the institute are summarized below.

In the area of cancer biology, we have identified a new gene whose product has been implicated in tumorogenesis. Importantly, this gene does not share any sequence homology with the existing library of known oncogenes. We are currently focussing on understanding the mechanism of its action. In a parallel project we are also exploring apoptosis induction as a possible strategy for cancer therapy. Neuroblastoma cells are being used as a model, and we have shown that protein kinase C inhibitors induce programmed cell death in these cells. The biochemical machinery that is disrupted by such inhibitors is presently being delineated. We have also recently constructed new fusion proteins that can impair signaling pathways initiated by epidermal growth factor receptor. The aberrant expression of this receptor has been linked to cancer in humans. In addition, our preliminary data of a bone derived factor which induces cell migration is encouraging and in depth studies are being pursued. We have also initiated work on the effect of exogenous p53 (a tumor suppressor protein) expression in tumor cell lines which express abnormal p53 protein.

Diabetes mellitus is a silent yet dreaded disease that affects not only the developed, but also the developing countries. Recent statistics indicate a significant rise in the incidence in our country, probably due to shifting life-styles. The NCCS has long since recognized this problem with an active programme dealing with its many facets. Our approach has been to blend the traditional with the modern, fully recognizing the intrinsic value of both approaches. Thus, while on the one hand we explore the efficacy of "naturally derived" medicines such as the soluble extract from bittergourd, we also focus on "state of the art" strategies such as islet cell transplantation, and development of biocompatible materials to achieve the same. In connection with the latter, encapsulation of islet cells within a chitosan-alginate membrane was found to render them resistant to injury during cyropreservation. This brings the possibility of islet banking closer to reality.

Our research activities are also complemented by the development of technologies that are of immediate applicability. One such development has been in the area of transplantation. We have primarily focussed on the skin and bone marrow transplantation and the technologies that facilitate these were transferred to hospitals last year. It is my pleasure to put on record here that two centres have been established as skin banks for transplantation purpose. These are located at New Delhi and Mumbai. The technology for cryopreservation of bone marrow and its subsequent revival has also been transferred to Armed Forces Medical College (AFMC), Pune. We have facilitated procurement of all the necessary equipment, as well as imparted training to the staff at these centres. We are happy to note that a neuroblastoma patient has already benefited from this latter technology, through a successful bone marrow transplantation at the AFMC.

Sustenance of technologies requires continued research. In awareness of this we are also examining issues related to the biology for efficient transplantation. In this context, we have found that TGFβ1, secreted by bone marrow derived mononuclear cells, activates stromal cells. This promotes intercellular communication between the stromal cells and the closely associated mononuclear cells from the marrow. In our continued efforts to understand eukaryotic cell structure-function relationships, we have developed an *in vitro* model to study the cell migration in wounds. The results indicate a down regulation of integrin β1 in migrating cells. In the interests of economizing burgeoning costs of biological research, we have successfully substituted the expensive foetal calf serum with goat serum supplemented with soybean-derived lipids. We have shown this to be equally effective for growing hybridoma cell lines. Its efficacy for other cell types is currently being evaluated.

The looming pandemic of AIDS in India has prompted us to initiate a research programme on the pathogenesis of its etiologic agent, HIV-1. Our current emphasis is to understand the role of two vital proteins viz. Tat and Nef, in disease pathogenesis. We have also identified a novel protein, SMAR1, which appears to be critical for maintenance of chromatin structure during cellular transformation.

Mosquitoes are vectors for many diseases that affect developing nations. Despite the best efforts resistant strains frequently appear, frustrating all control strategies. The approach we have adopted is to manipulate vector microflora as an interventional methodology. In this connection, we have recently identified a new bacterial strain from the mosquito midgut which appears to be important in virus-vector interaction.

The outcome of immune response, vital to our protection against disease, is regulated by an array of cell surface molecules. We had previously discovered two such molecules and termed them as M150 and B3. In recent studies we find that the B3 protein is homologous to the M2 isoform of pyruvate kinase. It is likely that posttranslational modifications permit it to function as a membrane protein. Its role in modulating T lymphocyte function is the subject of our current investigations. We have also demonstrated that the interaction between neutrophils and macrophages results in regulation of CD28 dependent interferon-γ secretion. This, in turn, is implicated in the control of intra-macrophage parasite load during leishmania infection.

Our support services continue to expand in order to meet the increasing demand from researchers of this country. The repository for cell lines and cell-derived products has increased both its collection, as well as improved the efficiency of supply. Over four hundred and ninety cell lines have been provided to various institutions and investigators this year. Our training programmes have also been refurbished with increased technical content. We have conducted two courses in cell culture maintenance and media preparations for college teachers, research scholars and scientists. In addition, fifteen investigators from various institutions and industries have been given hands on experience in tissue and cell culture.

Our library and documentation services have also been reinforced with an addition of over six hundred and sixty five documents including books. The library subscribes to seventy four scientific journals and sixteen periodicals and it continues to actively participate in the Pune library network.

Although I am proud of our achievements in the last year, I must acknowledge that this is largely due to the commitment and dedication of all my colleagues at the institute. Our newly acquired staff have also enthusiastically joined in our collective efforts to make NCCS a centre of excellence in this millennium.

G.C. Mishra
Director

Repository

Tissue culture facility

The NCCS is the only tissue culture facility of its kind present in India catering the needs of researchers dependent on the usage of human/animal cells/cell lines. In the short span of its existence, which began as tissue culture facility, has grown in all aspects i.e. acquisition and supply. The achievements of cell repository have grown from a 100 odd cell lines to over a thousand from a broad spectrum of species. During the current year the centre has supplied 490 cell lines to various institutions across the country.

Tissue Banking

We have indigenously developed a microcapsule generator assembly for islet encapsulation. Using this setup we have encapsulated islets in chitosan-alginate microcapsules. Cryopreservation of islets in these microcapsules has resulted in significantly increased viable recovery of islets over non-encapsulated islets after thawing. Cryopreserved encapsulated islets have maintained their morphology as judged by digital image analysis and their functionality by insulin secretion in basal and glucose stimulated environments.

Technology transfers

Skin regeneration

The NCCS is one of the prime institutes in the country which possess the skin culture technology. The technology has in fact benefited patients with severe burns. Currently the work is being initiated to improve the growth rates of keratinocytes so as to cover the burn wounds faster than what has been achieved. In addition to this, the centre also focuses on pigmentary disorders in humans (which attach a social stigma and physiological trauma to an affected individual). In a nutshell the studies are being done to understand the role of melanocyte specific gene 1 and g-glutamyl transpeptidase in melanogenesis. The centre has already established two skin culture and production centres at:

Lok Nayak Jai Prakash Narayan Hospital, New Delhi
Lokmanya Tilak Municipal Corporation Hospital, Mumbai

Bone marrow stem cell differentiation & cord blood cryospreservation research

The stem cells currently occupy a very crucial place in biology because these cells can be used in gene therapy and cancer. However, these cells are very difficult to preserve and revive them to their full functionality. Hence, the centre undertook the research to provide methodologies involved in cryospreservation and revival of bone marrow and cord blood cells. In future it is possible to set up storage banks of bone marrow or cord blood which might be useful to needy individuals suffering due to cancer or other diseases. The technological know-how has been transferred to

Armed Forces Medical College, Pune.

Follow up of the transferred technologies

The NCCS took an active part in procurement and installation of necessary equipments for the two technologies transferred. Preservation and maintenance of bone marrow has been initiated at Armed Forces Medical College. The staff involved in the utilization of the two technologies at their respective user hospitals have been provided hands on training. In this regard we are very glad to announce that one successful transplant of bone marrow is achieved and hope that many more will follow in the future.

Human Resource Development

The centre with the help of Department of Biotechnology recognized the need for creation of quality human resources across the nation in the areas of cell/tissue culture. The centre conducts training courses and workshops for wide spectrum of people such as technicians, scientists, doctors, from both academic and professional institutions including industry. The main objective of the centre is to provide expertise to these individuals on various aspects of cell culture, creation of hybridomas and molecular techniques on a periodic basis. The centre's courses and workshops are tailor-made to suit individual needs and expertise. Till date the centre has provided training to more than two hundred researchers across the country. The hallmark of the progress is the increase in number of scientists from sixteen to twenty three and research fellows from five to forty six, who are currently working at the centre for their Ph.D. In addition, the centre invites young graduate students from all over the country for training and exposure during summer months for enhanced understanding towards research in the areas of modern biology.

Cell Biology

Vaijayanti P. Kale

George Fernandes
Arundhati Khedkar

L.C. Padhy
Tata Institute of Fundamental Research, Mumbai

... Blood cell formation or haematopoiesis is a complex process. The overall process involves the progress of primitive stem cells, through distinct stages, in an increasingly committed manner to reach a final destination of mature cells...

Identification of the factors triggering cell-cell interactions in the haematopoietic cells

Although the cell differentiation pathways originating from the pluripotent stem cell and culminating in fully differentiated end products are broadly understood, several aspects of this process still remain elusive.

Stem cells are found in intimate association with stromal cells in the haematopoietic micro environment *in vivo* as well as *in vitro*. Several experiments have documented the importance of haematopoietic environment, which is defined by the stromal cells and the extra cellular matrix components surrounding them. Experiments have also underscored the need for cell-cell interactions that allow effective interactions or cross talk between the differentiating haematopoietic cells and the supportive stromal cells. The central question is: what determines or triggers the cellular cross talk in the haematopoietic compartment? We believe that identification of the factors modulating the specific cell to cell contact may pave the way for modulation of haematopoiesis in a predetermined manner, at least in cell culture. In addition, it may aid in the identification of defective steps in human disease process.

Aims

- Identification of factors responsible for initiating the cross talk in the haematopoietic compartment.

Work accomplished

Identification of cell type responsible for transforming growth factor β1 release: We have reported earlier that TGFβ released by bone marrow derived mononuclear cells (BM-MNC) in response to erythropoietin, confers an adhesive phenotype on the stromal cells leading to both homotypic as well as heterotypic interactions. The heterotypic interaction of stromal cells and the CD 34^+ cells resulted in amplification of stem cells in a protein kinase C and Calcium dependent pathway. We also demonstrated that the competency of stromal cells play a decisive role in the stem cell behavior.

In order to identify the cellular target of erythropoietin action we then performed 'depletion' experiments. The bone marrow mononuclear cells were depleted of specific cell population using magnetic beads (Dynal) tagged to lineage markers namely CD2, CD14, CD15 and CD19. The cells were then incubated with or without erythropoietin and level of TGFβ1 was determined. Undepleted cells were used as positive control. It was observed that depletion of $CD2^+$ population completely abrogated the TGFβ1 release indicating a role of T lymphocytes in the process. These experiments were continued further to determine whether the T lymphocytes belonging to $CD4^+$ or $CD8^+$ phenotypes had any differential role to play in the process. It was observed that when the $CD4^+$ T cells were removed there was a marginal decrease in the

levels of TGFβ1 secreted from the bone marrow nuclear cells, but when the CD 8^+ cells were removed then there was an enhancement in the levels of secreted TGFβ1. The results indicate that the process is negatively modulated by $CD8^+$ T cells. Similarly, when $CD14^+$ monocytes and $CD19^+$ B cells were individually removed there was a decrease in the levels of secreted TGFβ1 but when the $CD15^+$ granulocytes were depleted there was an enhancement of TGFβ1 levels, indicating that the granulocytes also had a negative influence on the TGFβ1 secretion by bone marrow mononuclear cells.

RT-PCR experiments were also carried out with mRNA isolated from control and erythropoietin treated bone marrow mononuclear cells using specific primers. There was about 5 fold increase in the levels of TGFβ1 mRNA in the erythropoietin treated cells as against untreated ones.

Stromal cell mediated signals: Earlier we have reported that exposure of BM-MNC to competent stromal cells resulted in an enhancement of colony forming unit formation. We initiated low density cultures to specifically identify the colonies of various types (burst forming unit (erythroid), colony forming unit (granulocyte monocyte), granulocyte erythroid monocyte megakaryocyte) formed in response to stromal cell mediated signals. The effect of TGFβ1 and basic fibroblast growth factor treated stromal cells on colony formation from bone marrow mononuclear cells was examined to assess lineage specific effect, if any, using semisolid assay system. It was observed that both TGFβ1 and basic fibroblast growth factor treated stromal cells enhanced the colony formation towards various lineages. The effect appeared to be of a general nature as both erythroid as well as myeloid colonies were stimulated to similar extent.

In the next set of experiments we examined the effect of modulators of signal transduction pathways down stream of calcium mediated signals. In the earlier experiments intracellular calcium was found to play an important role in the colony formation. We observed that treatment of stromal cells with calmodulin and calcineurin inhibitors prior to TGFβ1 exposure, had a dose dependent inhibitory effect on the colony formation from mononuclear cells exposed to treated stromal cells as compared to the mononuclear cells exposed to transforming growth factor β1 treated stromal cells. The results indicate the involvement of calmodulin and calcineurin in the stromal cell mediated signaling process.

Identification of specific cell types exerting positive and negative modulatory roles and the finding that T cells serve an essential function critical to this process, may suggest novel strategies by which the levels of secreted TGFβ1 may be manipulated during the expansion of stem cells *ex vivo,* by converting the well known inhibitory effects of TGFβ1 to a more desirable positive modulatory role. We have established a system for molecular foot printing of signaling pathways involved in the development of the hematopoietic cells.

Lalita S. Limaye

S.G.A. Rao
Cancer Research Institute, Mumbai
R.L. Marathe
Jehangir Hospital, Pune

... Stem cells are immunologically naive cells. These are the cells that get destroyed during diseases like leukemia and are very difficult to preserve. These are the cells people normally seek from compatible donors in desperate situations to save an individual's life...

Studies on cryopreservation and revival of haematopoietic stem cells

The haematopoietic stem cells occupy a very crucial place in biology because these cells can be used for transplantation in high risk population like people employed in nuclear reactor plants and people with family history of leukemia. However, these cells are very difficult to preserve and revive them to full functionality. Hence, we undertook the research to provide methodologies involved in cryopreservation and revival of bone marrow and cord blood cells. In future it will be possible to set up storage banks for bone marrow or cord blood in such a way that these might be useful not only to same individuals, but also to others who are in need of bone marrow in extreme situations of suffering due to cancer or other diseases. The technological know-how has already been transferred to the medical institutions.

Aims

- Improvement of the technology of bone marrow cryopreservation.
- Research in the area of haematopoietic stem cell cryobiology.

Work accomplished

Previously we have standardized the method for preservation of small size samples in cryovials. During the review period we have extended the same method to preserve large volumes of samples. So far we have stored six cord blood samples and five were stored in liquid nitrogen and one was stored at –80°C. The cryopreserved samples showed good post thaw colony forming unit recovery (5 out of 6). The sample stored at –80°C showed poor colony forming unit recovery post freezing. We are in active collaboration with Sasoon Hospital, Armed Forces Medical College, and Ruby Hall Clinic to obtain samples as and when they are available.

We have also extended our study on normal human bone marrow, which was a cadaveric sample provided by Armed Forces Medical College. The sample was frozen by controlled rate freezing in Baxter cryobag and was plunged in liquid nitrogen and stored short term for 15 days. Parameters tested after revival included viability by dye exclusion, MNC recovery, fluorescence activated cell sorting analysis of surface markers and colony forming unit assay. All parameters showed satisfactory post thaw revival (Table 1). The long term bone marrow culture was set up in Eaves medium after revival. This culture could be successfully grown for 10 weeks (Fig. 1).

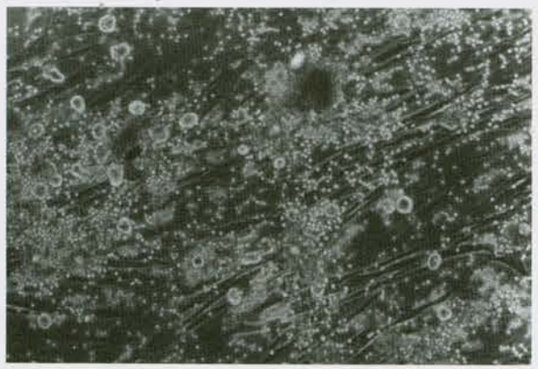

Fig. 1: Long term bone marrow cells in culture at three weeks.

Experiments on mouse bone marrow: Stem cell factor responsiveness of fresh and frozen marrow is being studied by both tritiated thymidine uptake and by colony forming unit assay. The preliminary results are encouraging. Engraftment potential of frozen marrow is being assessed by iv infusion of fresh vs frozen marrow in syngenic mice. The neutrophil and platelet count are monitored to assess engraftment potential and these will be examined further.

Experiments on human haematopoietic cells: 12 cord blood samples were frozen with or without additives in the conventional freezing medium. Their revival efficacy was tested by a differential colony count to determine whether there is any bias towards protection of a particular lineage in presence of additive during freezing. The parameters being assesed include colony forming unit (erythroid), burst forming unit (erythroid), colony forming unit (granulocyte, monocyte), granulocyte erythroid monocyte megakaryocyte.

Surface antibody profile	**Pre freezing (% +ve cells)**	**Post freezing (% +ve cells)**
CD34 (Early progenitor marker)	21.82	15.48
CD15 (Myeloid marker)	14.20	5.04
CD45 (Total lymphocyte marker)	70.28	60.18
CFU assay		
CFU-E	0	0
BFU-E	99 + 20	69 + 9
GM	106 + 14	138 + 5.1
GEMM	17 + 10	1.5 + .7
Total	226.6 + 36	209+4.2

Padma Shastry

Jayshree Jagtap
Prachee P. Gokhale

Medha S. Rajadhyaksha
Radhika Kelkar
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... Cells go through various stages before they become functional. Understanding the mechanisms involved in growth and differentiation would help in preventing the cells from becoming defective as in disease states...

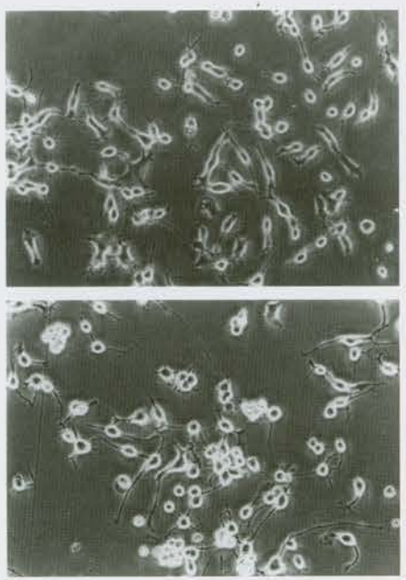

Fig. 1: Neuritogenesis in Neuro 2a cells A. Control B. Lithium (5mM)

Lithium induces morphological and functional differentiation in Neuro 2a cells

Lithium is a monovalent cation, clinically used to treat manic disorders. Lithium has been reported to affect signaling pathways of neurotransmitters, hormones, trophic agents and growth factors. Though earlier studies had demonstrated the influence of lithium on growth and differentiation of hematopoietic cells in primary cultures and cell lines, very few investigations have been carried out in neural cells. In neural precursor cell line NT3, lithium is reported to inhibit growth and arrest cells in S phase. However, the effect of lithium on differentiation of neural cells has not been investigated.

Aim

- To study the effect of lithium on differentiation of neural cells.

Work accomplished

We have used the mouse neuroblastoma cell line Neuro 2a as a model and differentiation was evaluated by morphological parameters, cell cycle analysis and by estimating the functional assays.

Neuro 2a cells were treated with serial concentrations in the range 0.62-10 mM of lithium chloride for different time periods and differentiation was evaluated. Viability was assessed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay at 24 hr and 48 hr time points. Cell cycle analysis was done by flow cytometry and neuronal marker acetylcholine esterase (AchE). Differentiation in Neuro 2a cells was assessed by morphological and functional parameters. In comparison with controls, lithium (2.5 and 5 mM) treated cells were seen to be flattened, became adherent and developed neurites. Majority of cells had bipolar projections with variable neurite length. While the neurite number and length were not significantly different from the control cells, the proportion of cells bearing neurites was significantly higher in cells treated with 2.5 mM lithium as compared to control. Cells treated with 5 mM lithium had significantly higher percent of cells undergoing neuritogenesis than cells treated with 2.5 mM lithium (Fig. 1).

Functional differentiation of Neuro 2a cells was assessed by monitoring AchE activity. A marker enzyme for Neuro 2a differentiation, AchE is known to have non-cholinergic function in developing nervous system and no change in AchE activity was observed at 24 hr of lithium treatment though neurite growth was initiated. There was a significant increase in AchE activity with 5 mM lithium treatment at 48 hr. However, no such increase was observed in cells treated with 2.5 mM lithium. At 72 hr of lithium treatment AchE activity was seen to be enhanced in cells treated with 2.5 mM as well as 5 mM lithium. Functional differentiation in Neuro 2a cells was observed 48 hr after morphological differentiation in lithium treated cells. There was no significant difference in the proportion of cells in the various phases of the cell cycle at 4 hr or 8 hr with lithium treatment. At 24 hr significantly higher proportion of cells were in G2/M phase following

lithium (2.5 mM). Growth arrest with higher proportion of cells in G2/M suggested a block in these phases of the cell cycle. Cell cycle block in G0/G1 is known to precede differentiation. Lithium has been reported to affect several signaling pathways, including those involved in transition of cells through various phases of the cell cycle. In the present study, lithium appears to inhibit transition from G2 /M to G0/G1 phases in Neuro2a cells. The molecular basis of lithium induced differentiation in Neuro 2a is under investigation.

J.M. Chiplonkar

Madhura Vipra

... Cell migration plays a central role in many physiological processes such as tissue formation and remodelling in embryogenesis, angeogenesis and neovascularization, wound healing, tumour invasion and metastasis...

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. Epithelial-mesenchymal transition (EMT) provides one of the most striking examples of cellular transformations leading to cell motility. It is characterized by the loss of epithelial and acquisition of mesenchymal features. Cells lose their cohesive nature and are converted into individual motile fibroblastic cells. The formation of EMT is a complex process and can result from the interaction of more than one regulator. It has been reported that a scatter factor may promote EMT-like changes *in vitro*: *e.g.*, haematopoietic growth factor influences both cell growth and motility. Each factor induces a particular transduction system and affect motility of cells but the net effect seen is a consequence of interaction of various transduction systems. It is important to understand the mechanism that triggers the specificity of the cellular response to the growth factor.

There is a growing body of evidence indicating that signals generated by a given growth factor can follow multiple pathways. The choice of pathways that are activated may be dictated in part by the stimulus and in part by the cell itself. Using *in vitro* wound healing models, it has been shown that repopulation of the denuded areas occurred, by and large, 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 mitogenic or motogenic response. Therefore, it is important to understand the mechanisms that evoke the specificity of the cellular response to the growth factor.

Aims

- Characterization of epithelial cell line(s) for growth factor responsiveness, cell cycle time/doubling time, duration of G1, S and G2+M phases.
- Analysis of 'early' and 'late' responses of the cell during wound healing and time kinetics.
- Response of synchronized cells to wound and effect of growth factors on wound healing.

Work accomplished

Assessment of migratory response of cells committed to cell division: As reported previously, an *in vitro* wound healing model has been developed to study the process of epithelial-mesenchymal transition, induced by external stimuli such as growth factors, etc. SiHa and MDBK cell lines were used for further experimental studies. We have earlier shown that cells synchronized at R in G1, could be induced to

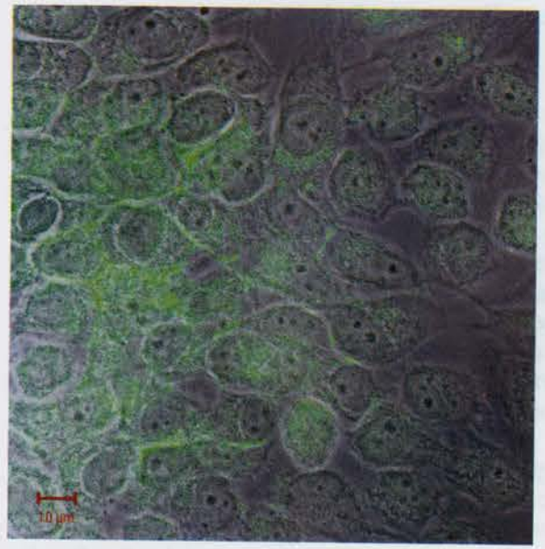

Fig. 1: Migration of SiHa cells into wounded area (right side).

migrate. These migrating cells showed delayed time to reach G1/S phase as compared to the non-migrating cells in the monolayers. Further experiments have been undertaken to analyze the response of the cells in S and G2 phases, which have already been committed to the progress of cell division.

The protocols for synchronization of the cells at G1/S were standardized for both SiHa and MDBK cells treated with known cell cycle blocking agents such as hydroxyurea, thymidine, etc. These protocols employed serum starvation followed by hydroxyurea, or thymidine. Alternatively, double thymidine block was also employed for arresting the cells at G1/S stage.

The cells brought to confluency were arrested at G1/S and wounds were made as described earlier. The cells lining the wounds were induced to migrate in the presence of fetal calf serum. The migration of these cells was assessed by subsequent incubation. Simultaneously, progress of S phase were studied by pulse labeling with BrdU at every 1 hour interval after making the wounds. The incorporated BrdU in the replicating DNA was detected by indirect immunofluorescence using a mouse monoclonal anti-BrdU antibody and FITC- or TRITC-tagged anti-mouse IgG secondary antibody. Proportion of BrdU positive nuclei was estimated in the migrating cells migrating into the wounded areas and compared with the non-migrating cells away from the wounds. It has been observed that the migrating cells undergo S phase and eventually come to S/G2 phase, within comparable time frame estimated for the non-migrating cells. Thus, it appears that cells committed to cell division in S phase did not alter their cell division progression and also undertook migration simultaneously. Further, absence of fetal calf serum in control wounded cultures showed similar progression through S phase. However, these cells showed absence of cell migration to repopulate the denuded areas of the wounds within the same time frame.

Comparative studies of F-actin, vimentin, fibronection, β-1 integrin etc. revealed differences in migrating and non-migrating cells. The expression of β-1 integrin was downregulated in the migrating cell population (absence of fluorescence; Fig. 1).

From these experiments the cells in S phase (I) could be stimulated with fetal calf serum to undertake migration; (ii) could not be stimulated to undertake migration in absence of fetal calf serum; (iii) could continue through S phase at the same rate in migrating as well as non-migrating cells, in the presence or absence of fetal calf serum.

P.B. Parab

Manisha Deshpande

... Fetal calf serum is a major component of tissue culture medium for cell growth but it is highly expensive. There is a need to look for cost effective alternatives...

Effect of supplementation with soybean lipids on goat serum induced apoptosis in the hybridoma cell line CC9C10

Cell death in cultures has been thought to occur by apoptosis. We have earlier showed that during adaptation of hybridoma cells cultured in medium supplemented with goat serum instead of fetal calf serum cell viability is lost by apoptosis to a significant extent. Further studies demonstrated that the CC9C10 hybridoma retains its functional characteristics when adapted to growth in medium with goat serum supplemented with soybean lipids

Aim

- To analyze the phenomenon of apoptosis in the anti-insulin antibody producing cell line CC9C10 in the presence of goat serum supplemented with soybean lipids.

Work accomplished

Mouse hybridoma cell line CC9C10 was maintained in DMEM/10% fetal calf serum, DMEM/10% goat serum and in DMEM/10% goat serum with 20 μg/ml soybean lipids. Presence of DNA fragmentation by ladder formation was used to investigate apoptosis under the three conditions. Cell death detection ELISA system was used to quantitatively measure the extent of apoptosis occurring under these different culture conditions. The results indicated that the constituents of goat serum induced apoptosis in the CCC9C10 hybridoma cells. The presence of soybean lipids in cell culture was able to protect the cells from under going apoptosis.

Skin Biology

Manoj Mojamdar

Pallavi Kulkarni,
Vaishali Chaubal,
Sujit Nair

... The skin of human body provides the first barrier of defense against pathogens in addition to providing beauty. But disorders like vitiligo or injury situations like burns, force the affected individual for an artificial skin as it attaches a strong social stigma to an affected individual...

Role and regulation of vital pathways involved in melanogenesis

Our laboratory has been focusing on development of technologies for artificial skin and fundamental studies on the role and regulation of melanogenesis.

Role of lawsone

The traditional Ayurvedic approaches indicated that lawsone, the principal compound of *Lawsonia innermis* (popularly known as 'mehendi') has been used as a drug for pigmentary disorders. The interaction of lawsone which interacts with tyrosinase has been studied by biophysical approaches. It is found that in the presence of L-dopa the activity of tyrosinase is upregulated.

Role of Melanocyte specific gene 1

Melanocyte specific gene 1 (MSG1) is expressed in pigmented but not in amelanotic B-16 melanoma cells. It's role in melanogenesis is not yet clear. MSG1 was shown to have a smad interacting domain and a transcription activating domain (CR2). The B16 melanoma cells were transfected with full length MSG1 and deletion mutants (MSG1-ΔSID, MSG1-ΔCR2). Following transfection, it was observed that stable lines over expressing MSG1 showed an increased piling up behavior in culture, a characteristic feature of aggressive tumors. When injected into C57 BL/6J mice, they gave rise to large tumors. Cells over-expressing MSG1 lacking the transcription activating CR2 domain showed an increase in Dopachrome tautomerase and melanin levels. Our preliminary experiments have shown an increase in eumelanin in cells over expressing MSG1 protein. Further experiments are being carried out in our laboratory to assign a role for MSG1 in melanogenesis and melanomagenesis.

Role of γ-Glutamyl transpeptidase in melanogenesis

Although γ-Glutamyl transpeptidase has been thought to play a role in melanogenesis its exact role remains to be elucidated. Work done in our laboratory has shown that γ-GT and tyrosinase, the key enzymes of melanogenesis are intricately linked. Further studies are being carried out to study the regulation of γ-GT at the molecular level in an attempt to elucidate a role for γ-GT in melanogenesis.

Culture of human skin keratinocytes, their 3-D epitheliation and grafting to burns, nevi, vitiligo and non-healing ulcer cases

The technology for the large scale expansion of cultured epithelia and melanocyte-bearing epithelia from a small stamp sized skin biopsy was clinically tried in a few patients as a prelude to large scale clinical trials. Comparison between currently used skin grafting and cultured epithelia grafting was done in this case. It has been seen that the cultured epithelia grafting results in normal skin color and no scarring is seen in routine skin grafting.

Composite dermis + epidermis culture

As mentioned earlier the cultured epithelia is fragile and difficult to handle. In order to provide tensile strength to this epithelia, a bio-artificial dermis preparation is being developed. Several collagen and collagen-chitosan membranes were prepared and cells were grown on them. During the course of our studies on wound covers, we had observed that bacterial cellulose pellicle has the excellent property of being a moisture absorbing wound cover property. Hence, a thick pellicle for the purpose of clinical trials as wound covers and a thin pellicles for growth of dermal and epidermal cells is being pursued.

Cancer Biology

Anjali Shiras

Varsha Shepal
Mandar Bhonde

L.C. Padhy
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... Cancer is probably one of the diseases that does not need any introduction. Despite the efforts that have poured in, the discovery of every new gene will raise the hopes higher for survival...

Identification and characterization of oncogenes implicated in melanoma

Melanoma, a cancer of the melanocytes, arises due to the uncontrolled perturbation in growth properties of melanocytes and disruption of the signal transduction cascade. Various oncogenes, cell adhesion molecules, and growth regulatory molecules of cdk family like p16 inactivation seem to be implicated in cell transformation, though an unequivocal association between oncogene expression and cell transformation have not been elucidated for melanoma.

Aims

- Identification of transforming genes in mouse melanomas using Cloudman Melanoma Clone M3 as an experimental cell system.
- Characterization of this cDNA and understanding its role in the signal transduction cascade in reference to cell transformation.

Work accomplished

We have cloned a 1.9 kb cDNA from Clone M3 with the automatic directional cloning approach which on expression induces transformation *in vivo* and *in vitro* and also displays transfectability in culture across human, mouse and rat cell-lines. The entire cDNA was sequenced using primer walking on an automated DNA sequencer. Nested primers were synthesized on the basis of this cDNA sequence for Northern and RT-PCR expression studies. The cDNA sequence did not reveal total homology to any known class of molecules using the BLAST Homology Searches. Experimental work is underway to understand the mechanism of transformation induced by this cDNA. The major emphasis of work this year was on complete sequencing of this cDNA and establishing its homology pattern with other growth regulatory molecules. The complete sequence information revealed about 45-55% homology with RNA binding proteins and hypothetical mouse open reading frames. The cDNA was found to express in mouse, rat and also homosapiens. The expression analyses of this molecule in different mouse primary tissues, and mouse and human cell-lines was investigated with RT-PCR and Northern hybridizations. The analyses implicated a specific distribution of this cDNA to melanomas, neuroblastoma and other neural crest derived tumors. Mouse primary tissues like embryos, brain and testis also expressed this cDNA. Work is underway to characterize this cDNA completely to decipher its role in the signal transduction cascade.

Padma Shastry

Anmol Chandele
Jayshree Jagtap

Prachee P. Gokhale
Medha S. Rajadhyaksha
Radhika Kelkar
Sophia College for Women, Mumbai

... Nature has designed how long a cell should remain in the body and when it should say goodbye. Nature's way of saying is 'apoptosis', a programmed cell death. If we exploit this phenomenon to eliminate tumor cells, we can prolong the lifespan...

Induction of apoptosis in human neuroblastoma cells with protein kinase inhibitors — study of apoptotic and antiapoptotic molecules

Apoptosis or programmed cell death is a genetically encoded cell elimination program that ensures a healthy equilibrium between cell proliferation and cell death. Induction of apoptosis in tumor cells is being explored as an important strategy in management of various types of cancers. Apoptosis induced by anticancer agents could occur by two intrinsic pathways. One involves receptor and its ligand interaction, leading to the transmission of apoptotic signals to the interior of the cell. The other involves the release of cytochrome c and the breakdown of the mitochondrial transmembrane potential which then triggers the apoptotic cascade. We have been attempting to study the pathways involved in apoptosis induced by staurosporin (STR) — a protein kinase inhibitor in neuroblastoma cells.

Aims

- To understand the role played by Bcl-2 family proteins, their expression, localization, translocation and the status of phosphorylation in NB cells on treatment with STR.
- To identify the functional role of the protein kinase C family in the Bcl-2 pathway and the subsequent resistance of these tumors to chemotherapy.

Work accomplished

Human NB cell lines SK-N-MC, SK-N-SH and IMR-32 were used to induce apoptosis with STR at concentrations ranging between 25-400 nM for 12, 24 and 48 hr time period. The three cell lines responded differently with respect to changes on treatment with STR. At 25 nM, with in 12 hr SK-N-SH and IMR-32 cell lines showed neurite extension. The effect of STR on proliferation in the three NB cell lines at 12, 24 and 48 hr treatment was studied by tritiated thymidine incorporation assay. Inhibition of proliferation was noted on treatment with STR (100 nM) for 24 hr in all three cell lines.

Since there was a decrease in proliferation we checked whether STR induced apoptosis. At 100 nM, apoptosis was induced in SK-N-SH cells within 12 hr. SK-N-MC and IMR-32 cells treated with 100 nM STR showed apoptotic features after 24 hr treatment with 40% in SK-N-MC cells, 63% in SK-N-SH cells and 10% in IMR-32 cells undergoing apoptosis on treatment with 100 nM STR for 24 hr (Fig. 1) SK-N-SH and IMR-32 cell lines showed a correlation between inhibition of proliferation and an increase in the per cent apoptotic population. Cell cycle analysis was done to examine the relation between inhibition of proliferation and induction of apoptosis. Accumulation of cells in the G2/M phase was observed with 100 nM of STR for 24 hr was 63%, 61% in SK-N-MC and IMR-32 cells respectively. Since the Bcl-2 protein has been shown to confer resistance against apoptosis, it was of interest to study the expression

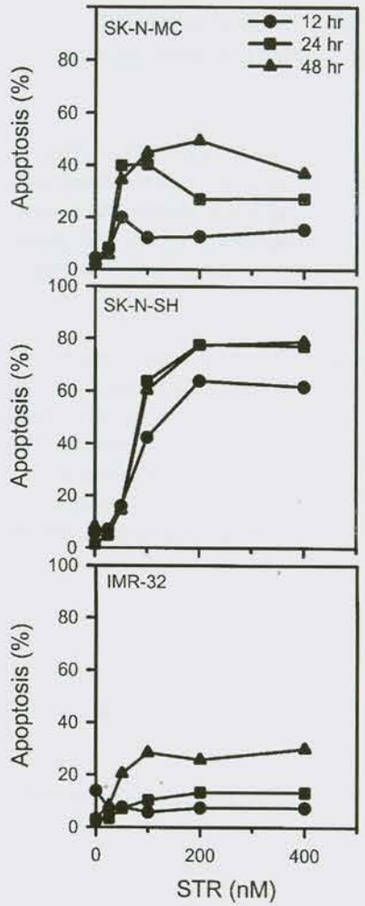

Fig. 1: Staurosporin induced apoptosis in neuroblastoma cell lines.

of Bcl-2 in the three NB cell lines. Constitutive expression of Bcl-2 was observed in the three NB cell lines as detected by western blots. A decrease in the Bcl-2 expression was observed on treatment with 25 nM STR and at 200 nM levels, Bcl-2 levels were increased in all three NB cell lines compared to the untreated cells. To confirm the results obtained at the protein level, RT-PCR is currently being used to study the status of the mRNA of the Bcl-2 gene. Further experiments are aimed at studying the localization of Bcl-2 protein and its correlation in apoptosis in the NB cells treated with STR.

Gopal C. Kundu

Neeraj Tiwari
Subha Phillip
Anuradha Bulbule

... The invasion of cancer including the breast and prostate is the first step in the complex multi-step process that leads to the formation of metastasis...

Identification and characterization of novel bone derived chemoattractant factor(s) that regulates breast and prostate cancer cell migration and bone metastasis

After local invasion of adjacent host tissue barriers, the cancer cells invade the vascular wall in order to disseminate. Cancer cells entering the circulation are able to evade host defenses, survive the mechanical trauma of the blood flow and arrest in the capillary bed of the target organs. Once arrested, the cancer cells again invade the vascular wall to enter the organ parenchyma. The extravasated cancer cell grows in a foreign environment different from the tissue of the origin and initiates a metastatic colony. The major cause of death in most human malignancies is metastasis.

Previous studies have shown that the inoculation of human breast cancer cells in the nude mice developed osteolytic bone metastasis. Moreover, the initial data also indicated that mineralized bone proteins induce the attachment of human prostate adenocarcinoma cells *in vitro* and suggest that bone protein may play a key role *in vivo* during the development of metastatic prostate lesions in bone. Therefore, the work began with the hypothesis that the factor(s) from bone or bone derived cells might show the induction of breast cancer cell migration and may play significant role(s) in the control of cell proliferation, invasion and bone metastasis of both the breast and prostate cancer cells possibly by interacting with its putative receptor.

Aims

- To study the molecular mechanism of cell motility and invasiveness of breast and prostate cancer cells.
- To identify and characterize the putative receptor of the factor and to study the structure function aspect between the ligand and the receptor.

Work accomplished

We have purified and obtained the amino terminal sequence of the factor from the bone extract as well as from conditioned medium of bone derived osteosarcoma cells. It appears that the sequence has 67% identity with human serum albumin.

Preliminary binding experiments show that the radio-labeled form of this factor interacts with its putative receptor in breast adenocarcinoma cells and this factor induces the cellular migration of progesterone receptor deficient human breast adenocarcinoma cells in a dose dependent manner (Fig. 1).

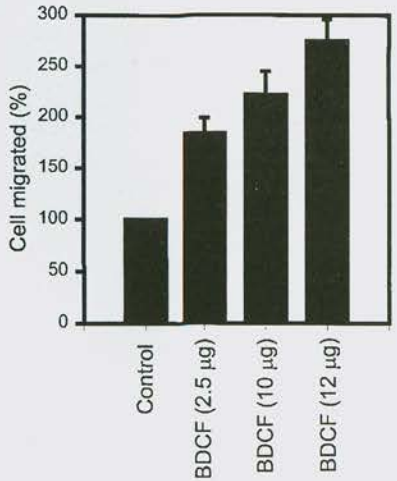

Fig. 1: Effect of human bone derived chemoattractant factor on human breast adenocarcinoma cell migration.

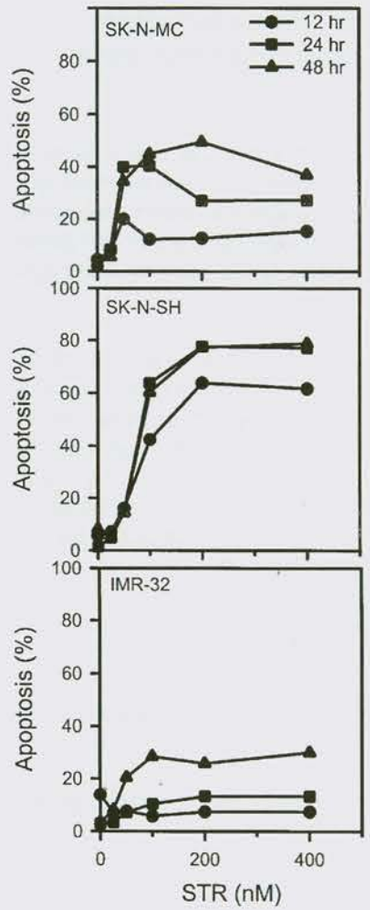

Fig. 1: Staurosporin induced apoptosis in neuroblastoma cell lines.

of Bcl-2 in the three NB cell lines. Constitutive expression of Bcl-2 was observed in the three NB cell lines as detected by western blots. A decrease in the Bcl-2 expression was observed on treatment with 25 nM STR and at 200 nM levels, Bcl-2 levels were increased in all three NB cell lines compared to the untreated cells. To confirm the results obtained at the protein level, RT-PCR is currently being used to study the status of the mRNA of the Bcl-2 gene. Further experiments are aimed at studying the localization of Bcl-2 protein and its correlation in apoptosis in the NB cells treated with STR.

Effect of osteopontin on the expression of matrix metalloproteinases (MMPs), and tissue inhibitor of matrix metalloproteinases (TIMPs)

Osteopontin (OPN) is a member of extracellular matrix protein family which plays significant roles in cell adhesion, proliferation and migration in various cell types. The cell adhesion, proliferation and migration properties might require its association with matrix metalloproteinases or tissue inhibitor of matrix metalloproteinases or integrins. The matrix metalloproteinases are zinc dependent proteinases which are active at neutral pH which are classified into at least four subgroups based on their substrate specificity, primary structures and cellular localization. Their physiological roles include degradation of several extra cellular matrix proteins and play critical role in tissue repair, embryonic development, tumor invasion and metastasis. The tissue inhibitor of matrix metalloproteinases (TIMPs) are the specific inhibitors of matrix metalloproteinases. Osteopontin (an extracellular matrix protein) may have some role to induce the expression of MMPs and to enhance the cellular migration in melanoma cells and there might be a functional link between the two processes.

Aim

- To examine the effect of purified osteopontin on the expressions of matrix metalloproteinases and tissue inhibitor of matrix metalloproteinases at mRNA and protein levels in melanoma cells.

Work accomplished

In the preliminary studies, we have purified osteopontin from human milk and showed that purified human osteopontin induces the expression of MMP-2 in a dose dependent manner in murine melanoma cells (Fig. 2). Treatment of the same cells with other extracellular matrix-proteins such as fibronectin, collagen, laminin and osteonectin have no such effect on the induction of matrix metalloproteinase-2 expression. Moreover, our data also demonstrated that treatment of the same cells with purified osteopontin enhances the cellular migration and extracellular matrix invasion by interacting with $\alpha v\beta 3$ integrin.

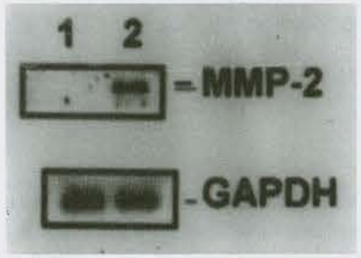

Fig. 2: Detection of expression of matrix metalloproteinase-2 mRNA in murine melanoma cells by RT-PCR. Lane 1: untreated cells and lane 2: cells treated with osteopontin.

Musti V. Krishnasastry

Navneet Sangha
Surinder Kaur
Ravi Vijayavargia
Devapriya N. Samanta
Anag A. Sahasrabuddhe
Anil Lotke

... Cellular communications are so precise that any malfunction can lead to life threatening proliferative disorders. Hence, any approach that can safely tackle such situations is of enormous importance for survival...

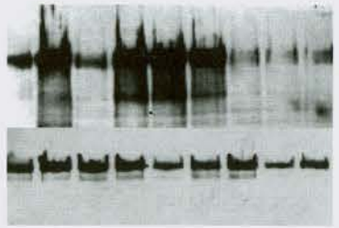

Fig. 1: Phosphorylation patterns of epidermal growth factor receptor in A431 cells.

Design and construction of immunolysins by protein engineering

Proliferative disorders are a result of malfunctioning communications of a cell, which the body eventually loses control resulting in a life threatening situations. Hence, the need to understand the cellular communication *i.e.* intra and inter cellular communications should be the first steps one needs to put for an effective therapeutic intervention. Incidentally, these will prove to be the most effective way of tackling the proliferative disorders. Our group primarily focuses on how the pathogens exploit the weakness in such communication pathways designed by nature which are stringent in design. We have employed a pore forming protein, α-hemolysin as the model to understand (i) how it functions by itself (ii) what is the mechanism of the action of α-hemolysin on nucleated cells at molecular level. By studying the mechanism of action of α-hemolysin at molecular level, we hope to redesign the molecule(s) in such a way that its lethal properties will be substituted with desirous ones. We hope to follow the smart path of a pathogen at the same time regulating its indiscriminate attacking capabilities.

Aim

- Design various fusion proteins of α-hemolysin, a pore forming protein from *Staphylococcus aureus* and transforming growth factorα, an epidermal growth factor receptor binding peptide, to study the functional ability of fusion proteins at molecular level.

Work accomplished

We have studied in depth how α-hemolysin function upon binding to nucleated cells. The first steps in the action of α-hemolysin are self assembly and killing of the cell by leakage of the essential contents. But a closer examination has revealed that α-hemolysin does one of the most important things i.e. it disengages the cellular communication pathway. The disengagement of signal generated by epidermal growth factor receptor is done by activating a cell associated protein tyrosine phosphatase which removes the signal associated with the epidermal growth factor receptor selectively. This feature is of enormous importance for various reasons *e.g.* (i) α-hemolysin is able to achieve the reversal of epidermal growth factor receptor signal just by staying outside of the cell unlike several kinase inhibitors that need to cross the cell membrane (ii) it appears to be very selective to epidermal growth factor receptor. This indicates that α-hemolysin takes the assistance a molecule that is present on certain target cells. (iii) In the presence of α-hemolysin, the epidermal growth factor receptor can not be activated at all even in the presence of its own ligand which can very effectively stimulate the receptor otherwise (Fig. 1).

Extensive array of experiments conducted so far strongly indicate that these fusion molecules can be further engineered to selectively deliver the signal of apoptosis, a programmed elimination of cells without attracting any attention. Future experiments will mainly focus on the molecules that assist in the

process, their designated roles, and design of new molecules with capabilities to undo the uncontrollable signals.

In Fig. 1 the upper panel is probed with anti-phosphotyrosine antibody and the same panel is probed for anti-epidermal growth factor receptor antibody. It is clear that as the concentration of fusion protein increases (towards right) the phosphorylation signal diminishes. This shows the absence of signal but presence of receptor.

Manoj Kumar Bhat

Reena Ramesh
Pallavi Dhoot
Vijay Kumar

... Clinical studies have repeatedly shown that some cancers respond to chemo-therapeutic or irradiation treatment well, whereas others do not. Also, it has been reported that tumors, which initially respond to anticancer therapeutics, sometimes fail to do so during the course of treatment regimen...

Regulated expression of wild-type tumor suppressor 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 tumor suppressor protein. 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. After DNA damage, p53 levels increase and mediate multiple cellular responses: (a) G1 arrest via transcriptional induction of p21, a cyclin dependent kinase inhibitor; (b) DNA damage repair via transcriptional induction of GADD45; and (c) induction of apoptosis in some cell types, if the damage is excessive. Alteration of the p53 tumor gene is most commonly reported genetic abnormality in a number of cancers. Studies with mouse cells have provided evidence of drug resistance after p53 inactivation, but the extrapolation of these results to humans is far from straightforward. The influence of p53 on apoptosis in malignant tissues of non-hematological origin is not yet clear.

In order to delineate further in the area of cancer therapeutics, we are exploring the effect of wild-type p53 expression (sense/antisense) in human tumor cell lines. Inducible gene expression systems will be used to modulate such exogenous gene expression. These systems have applications in many areas of biology. By 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 by expression of p53 antisense mRNA. 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.

Aims

- Establishing tetracycline based regulated gene expression system and standardization of transfection protocols for various cancer cell lines, using different protocols.
- Role of p53 overexpression or deletion on anticancer therapeutic drugs.

These studies will hopefully provide us with knowledge for more effective cancer therapy and effectiveness of p53 gene therapy for the treatment and management of various types of cancers.

Work accomplished

Transient transfection experimental protocols were standardized and the feasibility of using tetracycline regulated inducible gene expression system was established. CV1 and 293T cells were transiently

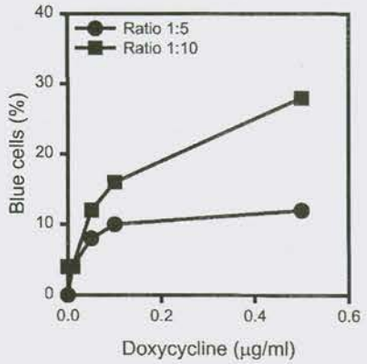

Fig. 1: Effect of doxycline on transiently transfected 293T cells.

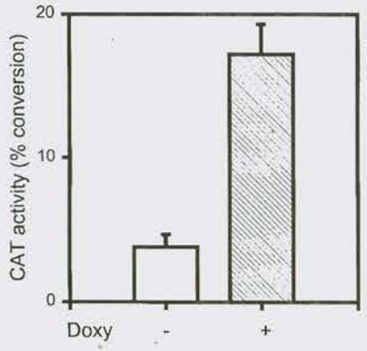

Fig. 2: Induction of p53 transcription in the presence of doxycycline.

transfected with the inducible plasmid system, including the β-gal reporter. Following treatment of cells with doxycycline, the cells were processed for *in vivo* X-gal staining and the blue stained β-gal positive cells were scored. Fig. 1 shows the result of one such experiment carried out with 293T cells. As seen in this Fig., the system works efficiently and responds well to the concentrations of the inducer in a dose dependent manner.

The cDNA for human wild-type p53 was excised from the plasmid pC53SN3 and sub cloned into pTRE plasmid vector. We were able to obtain pTRE containing p53 cDNA in the sense orientation (pTRE p53) and that containing p53 cDNA in antisense direction (pTRErevp53).

The functional ability of pTRE plasmid to express wild-type p53 in response to the inducer doxycycline was tested in the human leukemic cell line K562 (null for endogenous expression of p53). The K562 cells were transfected with plasmids pTet-On; pTREp53 and pG13-CAT (CAT reporter for wild-type p53). The cells were then grown in the presence or absence of doxycycline. The cell lysate were assayed for CAT reporter activity by TLC. Fig. 2 shows the results of one such experiment. As indicated, in the presence of doxycycline, p53 expressed is transcriptionally active.

Molecular Biology

Yogesh S. Shouche

Milind S. Patole
Vyankatesh Pidiya
P. Cyril Jaykumar
S. Prakash
Anagha Matapurkar

... Mosquitoes harbour a large number of bacteria in their midgut. As this is the first site any pathogen enters after the mosquito's blood meal, this becomes a promising site for disease control. Members of gut flora can be genetically modified to express the factors that will block the development of pathogen in mosquito...

Studies on midgut flora of mosquitoes

Mosquitoes are responsible for transmission of several human diseases including malaria, filaria, Japanese encephalitis, dengue and yellow fever. Development of insecticide resistance strains has resulted in reappearance of these diseases in worrying proportions and genetic methods are being tried to control mosquito populations.

Aim

- Characterisation of midgut flora of mosquitoes.

Work accomplished

Previously we have studied the midgut micro flora of both male and female mosquitoes before and after blood meal and have identified thirty two different types of bacteria based on their colony morphologies and gram characteristics. However this method has limitations. It is proposed that as little as 10% of the total flora is unable to grow under the controlled laboratory conditions. In order to have a complete picture of the gut we have performed ribosomal RNA (SSU rRNA/16 S rRNA) sequence analysis.

In our attempts to characterize the midgut flora, we came across the haemolytic isolate, MTCC 3249, which showed a very close homology to *Aeromonas jandaei* 16S r RNA sequence. We studied this isolate further. Since gyrase B sequence for the *Aeromonas jandaei* ATCC 49658 was not available in the database, we procured this culture and sequenced the gyr B gene. On comparison, the sequence of our isolate MTCC 3249 was found to be very different from *Aeromonas jandaei* and was closer to *Aeromonas hydrophila.* Taken together with the biochemical characteristics and the fact that 16S rRNA sequences of genus *Aeromonas* are highly homologous indicated that our isolate is a new distinct species of this genus. We have named it as *Aeromonas culicicola.*

We also analysed rRNA operon of this isolate. The PCR product obtained with the primers that amplify the entire operon was cloned and screened by restriction enzyme digestion to identify the unique clones. Preliminary results indicated that this isolate has minimum 10 operons for rRNA.

Library of PCR amplified 16S rRNA genes from total midgut DNA was prepared in pGEM T-Easy vector. A total of 441 white clones were selected for screening of which 175 were found to contain the expected 1.5 kb insert. Restriction fragment length polymorphism with tetra cutter restriction enzymes like *Rsa* I, *Hae* III, *Cfo* I and *Msp* I was done for these clones. Based on this, the clones were grouped into 63 groups. These will now be examined in detail by DNA sequencing.

Studies were initiated to investigate the role of midgut flora in the physiology and nutrition of mosquitoes. Mosquito eggs were hatched in antibiotic containing water and then grown under aseptic conditions. It was observed that viability and development was affected.

Milind S. Patole

Yogesh S. Shouche
Vyankatesh Pidiyar
P. Cyril Jaykumar
S. Prakash
Anagha Matapurlar

... Mosquitoes are vectors for many diseases that affect the developing nations. Despite the best efforts, several resistant strains often appear complicating its control. Hence, understanding the physiology at the genetic level will help in their effective control...

Molecular biological studies of mosquitoes

Mosquitoes are responsible for transmission of several human diseases including malaria, filaria, Japanese encephalitis, dengue and yellow fever. Development of insecticide resistant strains has resulted in reappearance of these diseases in worrying proportions and genetic methods are being tried to control mosquito populations. This necessitates the need for complete understanding of molecular mechanisms and control circuits. With this goal in mind detailed molecular biological studies on mosquitoes are being undertaken. Initially some housekeeping genes and their control elements are being investigated. Gene specific primers have been designed for the amplification of two such enzymes namely Hexokinase and Chitinase genes. Using these primers gene fragments are being cloned by PCR or RT-PCR amplification.

Aims

- Cloning and characterization of promoter sequences of hexokinase.
- Studies on the developmental and tissue specific regulation of chitinase and hexokinase.

Work accomplished

Two full length coding sequences for *Drosophila melanogaster* hexokinase were cloned which will be coding protein of approximately 49 kD. These sequences have conserved motifs which are responsible for binding of ATP and glucose. Both the clones show 50% homology with human hexokinases. The copy number of both the sequences is at unity as seen by Southern analysis. Expression of the sequences was found to be differential in adults and cell cultures. Work is in progress to clone the promoters for both the loci and characterise them.

Degenerate primers were designed from the known chitianse sequences and a 360 bp PCR product was amplified from the genomic DNA of *Aedes albopictus* and *Culex bitaeniorhynchus*. The product from *Aedes albopictus* confirmed by the sequencing of PCR product was then cloned in pBluescript vector. Genomic library of *Aedes albopictus* DNA was prepared in EMBL4 vector and screened with this clone product. Additionally the same product was used to probe the total genomic digest of *Aedes albopictus*, and the range of DNA fragments (around 9 kb) that showed hybridization was eluted and cloned in EMBL 4. The positive clone that lighted up in this library was further purified and confirmed by hybridization with the probe. At present this clone is being sequenced to confirm the presence of chitinase gene.

For *Culex bitaeniorhynchus*, the PCR product was cloned in pGEM T-Easy vector and confirmed by sequencing. At present it is being used for hybridization in southerns to generate a "book shelf" library.

Diabetes

Ramesh R. Bhonde

Makarand Risbud
Savita Kurup
Yogita Shewade

... Diabetes affects millions of people around the world and the numbers are expected to be at alarming levels in the future. The fundamental process by which this occurs is more complex and investigations around the world have led to the identification of basic mechanistic process that control the development of diabetes mellitus...

Diabetes and regeneration, design and development of biocompatible materials for use in transplantation, tissue regeneration and controlled delivery

The approaches to the study of Diabetes mellitus by various parameters such as islet isolation, stabilization, study of nutrient requirements for viability, zymogen fragility, nicotinamide and streptozotocin (STZ) on diabetic status, effect of nutritive substances on the islet, survival under diabetogenic influences, design and development of biocompatible materials for islet transplantation have been studied.

Zymogen fragility

Despite variation in the dissociation protocols, culture medium and nutrient supplements it has not been possible to maintain primary cultures of pancreatic acinar cells for more than 4 days. In order to investigate the possible mechanism of such short-life of acinar cells *in vitro* we studied their zymogen granule fragility under different set of conditions with reference to pH, glucose concentration and salt concentrations. Zymogen granule fragility is known to increase under *in vivo* conditions by chronic ethanol consumption alone or in combination with protein deficiency leading to pancreatic injury and thereby pancreatitis.

Work accomplished

The present studies delineate on susceptibility of zymogen granules to pH, requirement of glucose in culture conditions, salt and nutrient conditions which play an important role in the zymogen granule formation and stability. It can be inferred from the studies that maintenance of functional state of acinar cells *in vitro* is controlled by the composition of the nutrient media, which may differ from their *in vivo* counterparts. Moreover, these studies further ascertain the importance of zymogen granule fragility against viability of the cells, which is normally overlooked during *in vitro* studies. Our results indicate that either at high or at low pH, the zymogen granules are fragile resulting in lysis of cells as indicated by decreases in viability (Fig. 1). Similarly it is clear from Fig. 2 that omission of glucose led to total loss of pancreatic acinar cell viability.

It is interesting to note that omission of glucose from culture medium for 24 hr did not lead to the drastic drop in the latency although it was expected from the viability studies. In summary, the pancreatic acinar cells appear to have high energy requirements in culture that can be met by a variety of energy sources, not just by glucose or its metabolites.

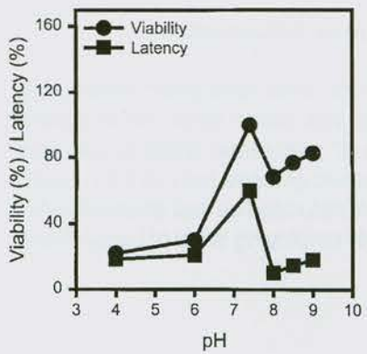

Fig. 1: Effect of pH on viability and latency of pancreatic acinar cells.

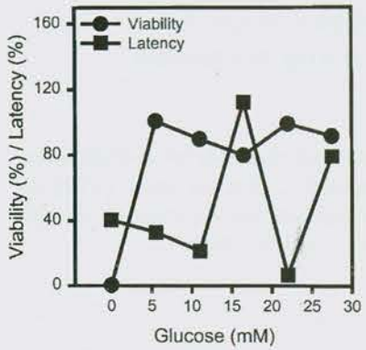

Fig. 2: Effect of glucose on viability and latency of pancreatic acinar cells.

An alternate model for experimental diabetes

We have recently reported that Streptozotocin diabetic animals operated for partial panceatectomy showed normoglycemic status after the operation as compared to uncontrolled hyperglycemia and even death in the diabetic sham operated animals. In drug and virus induced experimental diabetic models there is a high mortality of animals due to uncontrolled destruction of the β-cells. In order to destroy sufficient β-cell mass so as to induce diabetes but prevent mortality we designed present studies to investigate the combined effect of pancreatectomy, nicotinamide and STZ on diabetic status of Balb/c mice.

Work accomplished

Balb/c mice of either sex were subjected to 50% pancreatectomy and were then treated with nicotinamide (350 mg/kg body weight) before and after steptozotocin treatment (200 mg/kg body weight) administration. The changes in body weight, blood glucose levels, serum and pancreatic insulin contents of these animals were monitored in experimental and control group for 12 weeks and did follow up studies of these animals for further 12 weeks. The results indicate that partial pancreatectomy followed by nicotinamide and streptozotocin treatment leads to long lasting hyperglycemic state depicting the clinical symptom of NIDDM without mortality.

The data taken together indicate that combined administration of nicotinamide and STZ in partially pancreatectomized adult Balb/c mice led to development of a sustained, low level hyperglycemia concomitant with depleted insulin in serum and pancreas coupled with impaired glucose tolerance. This mouse model certainly shares some of the characteristic features of human NIDDM such as loss in body weight, persistent hyperglycemia, impaired GTT which is noteworthy.

Effect of nutritive substances on the islet survival under diabetogenic influences

The role of dietary fats, oils and nutrients such as naturally occurring amino acids have been investigated with respect to the islet survival under diabetic conditions. Fatty acids and amino acids are important metabolic substrates for pancreatic β-cells and long term exposure of pancreatic islets to elevated concentrations of fatty acids and certain amino acids can result in lowered glucose set point for insulin secretion. We therefore undertook the present study to ascertain the role of saturated as well as unsaturated fatty acids in either prevention or promotion of the STZ induced cytotoxicity employing islets of Langerhans.

Work accomplished

We have examined the effect of fatty acids and amino acids on the islets of Langerhans isolated from mouse islets. The fatty acids used were of two categories viz. saturated fatty acids and unsaturated fatty acids and the amino acids were alanine, arginine, leucine, methionine, glycine, cystine and glutamate. The parameters tested were viability using trypan blue and dithiozone. Lipid peroxidation is measured in terms of the MDA formed and superoxide dismutase activity. While palmitate and stearate were ineffective, Linoleate and DHA were very effective in protecting the islets from cytotoxic effect of STZ. Lipid peroxidation levels after treatment with palmitate and stearate had produced insignificant decrease. Superoxide dismutase activity was highly increased with palmitate while moderate increase is observed with DHA. In short, results obtained were in accordance with those reported for *in vivo*. Saturated fatty acids were not very useful in protecting the islets against cytotoxic effect of the STZ, whereas unsaturated and ω-3, ω-6 fatty acids were very useful in reducing oxidative stress. Invariably almost all the amino acids tested were found to protect the islets against the cytotoxic effect of the STZ with slight variation in the degree of survival. Except for arginine all other amino acids also showed the reduction in the lipid peroxidation. Superoxide dismutase activity is highly increased with methionine which is more or less closer to the one obtained with STZ.

Design and characterization of biocompatible materials

The semi-IPN hydrogel membranes of various proportions of polyvinyl pyrrolidone (PVP) and chitosan were synthesized by crosslinking chitosan with glutaraldehyde. The hydrogel membranes were characterized by spectroscopic, swelling, thermal and mechanical properties. These hydrogels are hydrophilic and possess high tensile strength. The *in vitro* haemorheological studies indicated the biocompatible nature of membranes with no significant changes in whole blood and plasma viscosity and red blood cell rigidity. These hydrogels can find significant applications in the area of tissue regeneration, controlled drug delivery and islet immunoisolation.

The other salient features of these hydrogels are:

- Hydrophilic nature by octane contact angle, protein adsorption, diffusion properties and cytotoxicity
- Allowed regulated transport of insulin and did not allow anti-insulin antibodies to pass through with no cytotoxic effects.
- Retainment of islet functionality when cultured on hydrogels, as judged by insulin secretion in response to glucose challenge.

- Dual property of allowing growth of epithelial cells (SiHa) while selectively inhibiting fibroblast (NIH3T3) growth essentially due to their inability to attach on to the hydrogel.
- Controlled delivery of amoxicillin incorporated in the hydrogel matrix.

P.B. Parab

Sandhya Sitaswad
Manisha Deshpande

... Insulin dependent diabetes incidence is on rise in India like in developed nations. One of the complications in diabetes is known to be associated with abnormality in the immune system leading to the destruction of insulin producing cells. Therefore, understanding the role of immune system at fundamental level will help in...

Immune reactions in low dose streptozotocin induced autoimmune diabetes

Insulin dependent diabetes mellitus (IDDM) pathogenesis is invariably associated with T cell infiltration into the pancreatic islets (insulitis) and it is characterized by a progressive T cell mediated destruction of insulin producing beta cells. Low dose streptozotocin induced autoimmune diabetes model in mice simulates Type I (IDDM) diabetes with similar immunopathologic profile to the human disease.

Aim

- To understand the immunological aspects of insulin dependent diabetes mellitus

Work accomplished

Part I: In order to test the effect of islet cell culture supernatant (ICCS) on regeneration of beta cells in IDDM animals, we injected these animals with ICCS or RPMI-1640 as control. Initial results exhibited a presence of Th1 response in IDDM animals slowly shifting to Th2 response as evident by an increase in IgG_1 levels and reduction of IgG_{2a} levels. Attempts are being made to study mRNA expression of interferon-γ and interleukin-4 as well as inducible nitric oxide synthase in ICCS treated animals.

Part II: It has been reported that reactive oxygen species participate in the destruction of pancreatic beta cells in streptozotocin-induced type I diabetes as evidenced by increased levels of interferon-γ and inducible nitric oxide synthase *in vivo*. We investigated the potential of oxygen radical scavenger such as copper sulphate on the *in vivo* levels of interferon-γ and inducible nitric oxide synthase in streptozotocin induced type 1 diabetes. C57B6 female mice were treated with copper sulphate on every alternate day for 30 days followed by an intra peritoneal injection of streptozotocin for five consecutive days. Effects of copper sulphate treatment were evaluated one week after the last dose of streptozotocin. Animals treated with copper sulphate showed significant decrease in blood glucose and lipid peroxidation levels. mRNA expression of the enzyme inducible nitric oxide synthase, interferon-γ and interleukin-4 were found to be decreased as compared to the untreated group of animals.

Sandhya Sitasawad

R.R. Bhonde

... In the modern times, we often hear of stress related to humans. One of the biggest damages our body tries to cope up with is the oxidative stress: a means by which reactive species are generated in our body which can lead to...

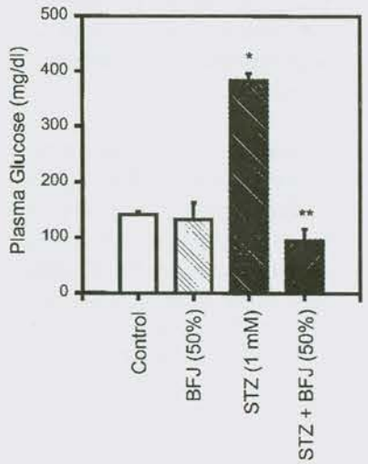

Fig. 1: Effect of bittergourd fruit juice on glucose levels in STZ induced diabetic Balb/c mice.

Oxidative stress in diabetes mellitus and development of therapeutic strategies

A large body of evidence indicates that free radicals are increasingly being implicated in the development and complications that arise out of Diabetes mellitus and this oxidative stress may act as a common pathway to diabetes, as well as to its associated complications. In patients suffering from Diabetes mellitus various hypoglycemic drugs are routinely used to control hyperglycemia and keep blood sugar within normal limits. Aqueous juice of bittergourd fruit (*Momordica charantia* Linn) of Cucurbitaceae family has been shown to possess hypoglycemic activity. However, the mechanism of its action is not known. Hence *in vitro* and *in vivo* experiments will identify the role of bittergourd fruit juice on the diabetic status. The activity of bittergourd fruit juice was tested *in vivo* using Balb/c mice, and *in vitro* using rat insulinoma (RIN) cells and isolated islets.

Aim

- Determination of hypoglycemic activity of bittergourd fruit juice in Balb/c male mice and its antidiabetic activity on streptozotocin- induced lipid peroxidation, DNA and protein damage using the rat insulinoma cells (RINm5F) and islets from mouse.

Work accomplished

Streptozotocin is a diabetogenic agent known to cause oxidative stress and exert its cytotoxic effect by generating free radicals which lead to lipid peroxidation and thereby diabetes. In the diabetic state of a patient, it is necessary to keep the blood sugar within normal limits by means of hypoglycemic drugs. Hence, the ability of aqueous juice of bittergourd fruit is being tested for hypoglycemic activity on streptozotocin treated RIN cells and isolated islets *in vitro*. It was found that feeding the mice with bittergourd fruit juice caused a significant reduction in streptozotocin induced hyperglycemia in mice (Fig. 1). It markedly reduced the streptozotocin induced lipid peroxidation as in pancreas of mice, RIN cells and islets. Further it also reduced the streptozotocin induced apoptosis in RIN cells indicating the mode of protection of bittergourd fruit juice on RIN cells, islets and pancreatic β-cells. Present study thus confirms hypoglycemic effect of bittergourd fruit juice and provides sufficient documentation to define its role and action for its potential and promising use in treating diabetes.

Infection and Immunity

Debashis Mitra

Ajith Mathews
Jayashree Ladha
Sujata Bhade
Bhaskar Saha
G.C. Mishra

... Acquired Immuno Deficiency Syndrome caused by HIV is growing at an alarming rate in India. The need of the hour is to understand basic aspects of host-virus interactions at molecular level which may lead to the development of new antiviral strategies...

Molecular and cellular basis of HIV pathogenesis

Human immunodeficiency virus is the causative agent of acquired immunodeficiency syndrome (AIDS), which is defined by a reduction in the number of $CD4^+$ T cells and the onset of opportunistic infections. The incidence of HIV infection has reached an alarmingly high levels worldwide including India. The therapeutic strategies being used at present can reduce the viral load remarkably but is too expensive a regimen to be used in the Indian context. Thus it is very important to understand the pathogenesis of the virus and to 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 objective is to gain more understanding of the virus and its interaction with the host cell, which may lead us to new antiviral strategies.

Aims

- Differential gene expression studies in HIV infected and bystander T cells, to understand molecular mechanism of HIV induced T cell depletion.
- Role of viral regulatory proteins Tat and Nef in HIV induced neuropathogenesis and their interaction with host cell factors.
- Immune response to HIV infection towards generation of DNA vaccine.

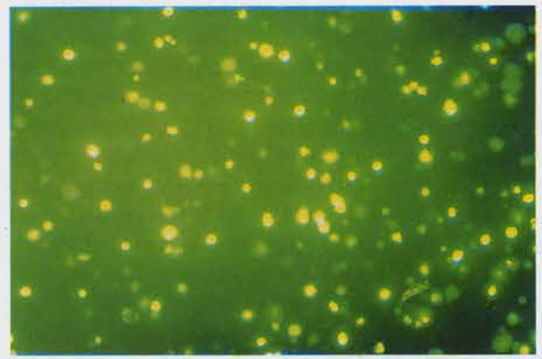

Fig.1: Jurkat-GFP cells transfected with pCDNA-Tat. Transfection was done using Fugene-6 reagent and the photograph was taken at 48 hours post transfection using Olympus inverted microscope with fluorescence attachment.

Work accomplished

Differential gene expression in HIV infected and bystander T cells

The study focuses on one of the hallmark of HIV infection, i.e., $CD4^+$ T cell depletion during the course of infection. 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 main objective of this proposal will be to identify differentially expressed molecules in HIV infected and uninfected bystander cells and to elucidate the interaction of those molecules in the signalling cascade leading to cell death. In order to distinguish HIV infected cells from uninfected cells, we have planned to utilize the reporter protein, green fluorescent protein (GFP). We have cloned the HIV-1 LTR upstream of GFP in eukaryotic expression vector. The vector has been stably transfected in Jurkat T cell line, and is able to express GFP at low levels. Transfection of Jurkat-GFP with Tat expression vector, pCDNA-Tat induces high level of GFP expression (Fig. 1). The limiting dilution cloning is in progress. Using these cells, it will be possible to distinguish between infected and uninfected cells based on GFP fluorescence. This cell line will also be useful in preliminary screening of anti-HIV compounds without any expensive assays.

Role of viral regulatory proteins Tat and Nef in HIV induced neuropathogenesis

HIV dementia is a central nervous system complication that affects about 30% of individuals infected with HIV and is a defining condition for AIDS. The underlying cause of the dementia is unknown but it is thought to be caused by neuronal dysfunction resulting from the release of putative neurotoxic products by infected microglia or, alternately, by neuronal interaction with viral proteins released or expressed by the infected cells. Among the viral proteins, Tat and Nef have been directly implicated in contributing to neuronal damage, however, the mechanism remains to be elucidated. The present study intends to see the effect of Tat and Nef in cell lines of neuronal and microglial origin. We also plan to identify Tat and Nef interacting cellular factors in the brain. We have been successful in cloning the HIV-1 Tat and Nef sequences in the expression vector pCDNA using HIV-1 genome in λHXB3 clone (Fig. 1). We have confirmed the sequence of Tat and Nef in the vector pCDNA by DNA sequencing. We have also been able to confirm the expression of Tat by transactivation assay and immunoblotting. Using coupled in *vitro* transcription-translation reaction we have been able to visualize a 27 KD protein band with pCDNA-Nef. These two vectors will be used not only for understanding the role of Tat and Nef in neuropathogenesis but also to study the interaction between these two proteins, if any. Co-transfection and co-immunoprecipitation studies are in progress to elucidate the interaction.

Immune response to HIV infection towards generation of DNA vaccine

HIV-1 is a uniquely difficult target to develop immunological intervention against it. In this regard, one of the main hindrances has been the lack of understanding as to what constitutes protective immunity to the virus. The high rate of replication, mutation and recombination of HIV enable the virus to evolve rapidly in the host and so outsmart immune response evoked by natural immunity or a vaccine. At present, our knowledge of CTL 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 murine model and eukaryotic expression vectors encoding different HIV proteins like Tat, Rev, Nef, gp120, etc. In order to define the requirement for the first and second signals leading to T cell activation and also to understand the effector system, a variety of genetically deficient mice will be primed with the construct carrying genes for different HIV proteins. Thereafter, the response will be analysed using CTL assays and T helper cell analysis. We have started DNA immunization studies in mice using the pCDNA-Tat vector mentioned earlier. In addition to Tat, we are also trying to clone the gp120 gene of subtype C of an Indian isolate in expression vector and use it in DNA immunization studies.

Bhaskar Saha

K. Venuprasad
Meenakshi Jadhav

... Leishmaniasis (popularly known as 'Kala azar)' is a disease that affects substantial numbers in India. Understanding of mechanism for the generation of protective immunity against this parasite will help in designing the therapeutic agents and/or vaccines...

T cells and non-T cells in determining the susceptibility to Leishmania infection

The cells of innate immune systems (e.g., neutrophils, macrophages, mast cells, etc) and specific immune systems (T and B cells) go hand in hand to initiate and maintain an immune response. The cell surface molecules play a big role in regulating the activity of all these important 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

- Whether neutrophil expressed CD28 interacts with macrophage expressed CD80/CD86.
- If it does then what is the effect of CD28 signaling on the cytokine secretion by neutrophils? Does it contribute to the regulation of Leishmania growth?
- What role does CD40-CD154 play in *Leishmania* infection?

Work accomplished

We observed that neutrophils interact with macrophages to regulate CD28 dependent interferon-γ secretion. The neutrophil secreted interferon-γ activates macrophages to clear the intracellular infection of Leishmania. CTLA4-Ig, a fusion protein which blocks the CD28-B7 interaction, has been shown to block the leishmania killing in the neutrophil-macrophage co-culture *in vitro* suggesting that the neutrophils may play important role in the regulation of anti-leishmanial function of macrophages. It is observed that treatment of macrophages with an agonistic anti-CD40 antibody *in vitro* leads to restriction of the parasite growth and secretion of IL-12. The administration of same antibody in mice controls *Leishmania major* infection *in vivo*. Similarly, use of an anti-CD154 antibody, which blocks the interaction between CD40 and CD154, results in decreased IL-12 and interferon-γ secretion in vitro by the LN cells from *L. major* infected mice. Administration of the same antibody in mice exaggerates the infection *in vivo*, which is associated with decreased interferon-γ secreting T cells and increased IL-4 secreting T cells. All these observation together suggests an important role for CD40-CD154 interaction in the control of anti-leishmanial immune response.

G.C. Mishra

Chayya Iyengar
D.V.R. Prasad
Pinaki P. Banerjee
Vrajesh Parekh
Satyan Sharma
Musti V. Krishnasastry
P.B. Parab

... The front line defense for humans revolves around the stimulatory signals generated by T-cells and B-Cells. The signals are further fine tuned in terms of co-stimulatory signals which play a vital role in the type of immune response an individual should generates. Hence, the need for study of...

Development of Th1 and Th2 specific co-stimulatory molecules

T lymphocytes have an important role in most immunological responses. To exert their effector function, relatively quiescent T cells are activated during a complex interaction with antigen receptor (TcR) complex. Interaction of TcR with the major histocompatibility complex (MHC) or antibodies to TcR results in the initiation of signal transduction events and cellular activation. However, stimulation of TcR alone is insufficient to activate most of the T cells. The current dogma is that at least two signals are required. The first signal is provided by the occupancy of TcR, which is MHC restricted and second non-MHC restricted signal (co-stimulatory signal) is delivered by certain molecules present on the surface of antigen presenting cells (APC). The co-recognition of the molecules on T cell and APC is, therefore, of crucial importance as the latter event results in the transduction to specific signals that eventually determines the outcome of an immune response.

Murine T helper cells can be divided into two distinct types, Th1 and Th2, based on the patterns of cytokine secreted in response to antigenic or mitogenic stimulation. These different cytokine endow different functions to the type of cell secreting them. Th1 cells mediate several reactions suitable for combating intercellular parasites, including delayed type hypersensitivity, macrophage activation and also B cell help, though to a limited extent. Th2 cells provide strong help to B cells. Th1 and Th2 phenotypes are important as effector cells in generating a strong immune response, and are accountable for the reciprocal nature of antibody and delayed type hypersensitivity responses, because Th1 and Th2 phenotypes appear to be mutually inhibitory. It as been postulated that these two T helper subsets are not only functionally different but also have qualitatively and quantitatively distinct requirements for co-stimulation. However, till date, no co-stimulatory molecule(s) has been reported that preferentially generates either Th1 or Th2 types of responses. Recently, while analyzing the protein molecules from the surface of lipopolysaccharide-activated B cells, we found two phospho glycoproteins of 38 kD (B3) and 97-100 kD (B2) which selectively activate Th cells to secrete IL-4 and IL-5. On the other hand, a protein molecule of 150 kD (M150) present on the membrane of macrophages stimulate secretion of IL-2 and interferon-γ and proliferation of naive and ovalbumin specific $CD4^+$ T cells.

Relevance

The co-stimulatory molecules and the antibodies against them, their receptors on the target cells and the antibodies against their receptors can be of immense importance in understanding the immunobiology of various pathogenic infections like malaria, TB, leishmanisis, leprosy and as immuno therapeutic agent for selective regulation of immune system. Also, during organ transplantation antibody against Th1 co-stimulatory molecule can be of immense importance to increase the acceptability of the transplant. It may be mentioned here that development of Th1 and Th2 cells require specialized lymphocyte milieu and it is very well established that both the subsets interfere with the development of each other. In pathogenic conditions where hosts require to eliminate pathogens by developing one type of immune response, it

has often been reported that the parasites dictate host to develop the other type of response which interfere in the development of required type of immunity to eliminate the infection. Therefore, the present study proposes to understand the molecules in more details.

Aims

- To raise the antibodies and clone the genes of Th1 and Th2 specific co-stimulatory molecules identified by our laboratory.
- The cloned genes will be transfected in non-professional APCs and the transfected cells will be evaluated for their ability to help the Th cells, leading to their differentiation into Th1 and Th2 types.
- To further investigate the role of Th1 specific co-stimulatory molecule, M150 in immunobiology of $CD4^+$ and $CD8^+$ T cells.
- Finally, attempts will be made to find the human homologue of these genes.

The antibodies and/or the gene of aforesaid co-stimulatory molecules can be effectively used for immunotherapy of cancers and in other diseases like leishmaniasis, tuberculosis, etc.

Work accomplished

The hybridoma against the putative Th1 specific co-stimulatory molecule identified by us, the M150, has been successfully made. Lewis rats were immunized with the purified protein and the B cells were fused with PAIOP/3 to obtain the monoclonal antibody. The monoclonal antibody has been tested by Western blots, ELISA and immunoprecipitation for its specificity. The antibody has also been characterized and is found to be IgM suggestive of the epitope being a glycoprotein, which plays an important role in the receptor-ligand interactions.

Attempts have been made to establish the homology between B2, a Th2 specific co-stimlatory molecule, and GP96, a heat shock protein by metabolically labeling the cells. Immunoprecipitations with either anti-B2 antibody or anti GP96 antibodies yielded the same product. Hence, based on these results, a permanent cell line transfected with cDNA of GP96 (Chineese Hamster Ovary-SP6, hereafter referred as CHO-GP96) has been generated and was used as a source of secondary signal in T cell proliferation assays. The CHO-GP96 cells provided the desired signal and further cytokine analysis is underway in CHO-GP96 and T cell co-cultures.

With regard to the Th2 specific molecule that had been previously found to have homology to mouse M2 form of pyruvate kinase (PK; based on an internal peptide sequencing). We have employed 5' and 3' rapid amplification of cDNA ends from the cDNA of lipopolysaccharide activated B cells as a source of mRNA. A 700 bp fragment from the 3' end has been identified and the DNA sequence confirms the fragment as that PK. Cloning of the PK gene from the A20 cell line has also been done. The sequence differs from that of mouse M2 form of pyruvate kinase at the 5' end of the gene.

All the data obtained on the sequence of the 5'- and 3'-rapid amplification of cDNA ends supports the idea that the gene shares a significant homology to mouse M2 form of pyruvate kinase, but has major differences that allow it to function in a glycosylated, membrane associated form. Cell proliferation assays with purified pyruvate kinase also support this finding that membrane purified B3 was able to proliferate $CD4^+$ cells, whereas the PK enzyme was unable to do so. A 450 bp (10 kD) fragment from the C-terminal region of the gene was cloned, and expressed in bacterial expression. Further, western blots were done using anti-PK, anti B3 and anti-19 kD antibodies, all the three purified antigens cross reacted with the above mentioned antibodies, indicating a high degree of homology between B3 and PK. The data on the 5' portion of the gene (from lipopolysaccharide activated B cells) is awaited.

Gene Therapy

Samith Chatopadhya

Ruchika Kaul
Aniruddha Deshpande

... Proper interaction of proteins with DNA is vital for a healthy state of cell. Discovery of every new DNA binding protein and its function will help us to understand the intricacies of biological process...

SMAR1, a novel T cell specific MAR binding protein: possible role in V(D)J recombination and chromatin structure modulation during cellular transformation

We have identified a novel DNA binding protein SMAR1, the expression of which is upregulated in murine lymphoid cells, specifically in double positive T cells. SMAR1 binds to a *cis* regulatory element HS1, a MAR (Matrix Associated Region) site, present next to the T cell receptor beta (TCRβ) gene enhancer Eβ. The factors binding to MAR make the region accessible for transcription as well as recombination by pulling the DNA near to the nuclear matrix where other accessory proteins of the recombination or transcription machinery associate and perform their respective functions. Similarly, in the formation of either TCR or BCR, during V(D)J recombination, the recombination activating proteins (RAG1 and RAG2) need to be associated to the nuclear matrix where all other gene segments (V, D and J) including the enhancer and additional *cis* elements are present. Since, HS1, present 400 bp away from the Eb enhancer, has conserved AT rich MAR motifs, and is conserved in both humans and mice, our lab is interested in characterizing a novel HS1 binding protein SMAR1. For the last one year, we have made a number of fusion constructs of SMAR1 that will be useful for localizing the protein within the cell and also to understand the association of SMAR1 with either Cux or SATB1(well-known MAR-binding proteins) which have been shown to bind to HS1.

Aims

- To confirm that SMAR1 is a MAR binding protein by analyzing for its binding with HS1 and other well-known MARs and to identify DNA binding motifs of SMAR1 by deleting 5' and 3' segments of HS1.
- To study the interaction of SMAR1 with well-known MAR-binding proteins like — Cux and SATB1 and perform co-immunoprecipitation studies of SMAR1 with Ku auto antigen and PARP (poly-ADP-ribose polymerase).
- To have a model system where splicing events can be studied in detail in transformed cancer cells and in various primary tissues as well.
- Construction of SMAR1 knock-out mice and isolation of SMAR1 promoter (which would subsequently be placed upstream of GFP followed by its expression in transgenic mice) to study its effect on V(D)J recombination and thereby on T-cell development.

Work accomplished

We have isolated an alternatively spliced form of SMAR1. One more alternatively spliced form has been found by another laboratory. Since the form which we have has a deletion of 117 bp, equivalent to 39 amino acids, we looked into the details of the splicing event in various primary cells and cell lines. Since SMAR1 was originally isolated from the mouse thymus, so the obvious curiosity was, is the protein present in the humans as well? The results of PCR amplification show that it is present in human T cells and various other human cancerous cell lines but not in primary cells such as NIH 3T3 (Fibroblasts), HT-1080 (Fibrosarcoma), IMR-32 (Neuroblastoma), SK-Hep1 (Liver), SK-Nep1 (Kidney), MCF-7 (Breast cancer), human neutrophils and PBMCs (poly-morpho blood mononuclear cells) isolated from human blood.

The next important question was, are the spliced forms of the SMAR1 present in both the mice and the humans? From the RT-PCR we have observed that both spliced (~300 bp) and unspliced (~400 bp) forms of SMAR1 were present in mouse thymus and spleen.

The PCR results show that the fibrosarcoma and liver have the unspliced form but not the spliced form. If the spliced form is thought to be the functional form of the SMAR1, then the protein seems to be non functional in such cells. Kidney cell line did not have any of the forms but the embryonic kidney cell line (293T cells) have the unspliced and very less of the spliced form. In T cells majority of the PCR product corresponds to the spliced form indicating that the spliced form of SMAR1 is abundant in early and mature T cells.

Since the spliced form of SMAR1 varied differently in cancerous cells i.e. it is either upregulated in some or down regulated in others but not at all present in primary cells. Hence, we are currently interested in finding out the role of this protein in cellular transformation. In this respect, we would like to have a mouse model to find out the role of novel protein SMAR1.

SMAR1 knock out mice

To make a knock-out construct of SMAR1, we are in the process of isolating 5' and 3' flanking sequences of the gene which are at least 3 to 5 kb long. For this, a mouse genome cDNA library made in Lambda FIX II vector was probed with 2.1 kb SMAR1 gene. After 4 successive screenings seven plaques were obtained. These were plated on LB agarose plates with *E. coli* strain LE392 as the host bacterium. From the plaques the DNA was isolated. The phage DNA was digested with restriction enzymes in such that they had a single site within the SMAR1 and would generate fragments which are more than 3 kb in order to get the flanking sequences of SMAR1.

The Xho I digested blot was probed with a 0.4 kb and a 0.6 kb probe obtained from 5' and 3' end of SMAR1 respectively. The Xho I - Sal I blot was probed with the 0.4 kb and 1.6 kb probes from the 5' and 3' ends of SMAR1 respectively. The Afl III blot was probed with a 0.9 kb and a 1.3 kb probe obtained from Sal-Afl III and Not I-Afl III digestion of SMAR1. The EcoN I blot was probed with 0.6 kb EcoN I-Not I probe.

After hybridization with the respective probes, we isolated 7-8 kb fragments from 3' regions flanking SMAR I of clone 7 and clone 2. Another fragment of about 2.2 kb obtained from clone 4 (Xho I-Sal I) digestion which was seen to hybridize to a 0.4 kb probe from the 5' end of SMAR I will be isolated for further work.

Support Units

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 standardized high quality laboratory animals viz. inbred mice, rats, rabbits etc., for the ongoing research projects of the institute. The following are the various laboratory animals maintained at the facility. We maintain several stains of mice viz. BALB/CJ, C57BL/6J, DBA/2J, SWISS, NOD/LtJ, BALB/c, Nude; among rats we have WISTAR and LEWIS. We also rabbits NEWZEALAND WHITE and mastomys MASTOMYS COUCHA.

Defined barrier practices and ethics are followed scrupulously without any exception, allowing access to identified personnel, to minimize the risk of microbial infection to the animals housed in the facility.

The breeding program for the propagation of the inbred lines is structured in a two-tier format, i.e. the Foundation colonies and the Production colonies. The animals in the foundation colonies are propagated by strict full-sib pairing only. 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 establish the mode of inheritance and being made to separate and propagate the line as a true breeding mutant strain. The mutant colony is currently at F11 level of inbreeding.

Efforts are on to develop the necessary infrastructure for the maintenance and breeding of transgenic/knock-out mice in line with the emerging demand for these animals from the scientists of the institute.

The complete technical support, advice and ethics have been extended regularly to scientists/ research scholars in various aspects of animal experimentation viz., handling of laboratory animals, collection of samples, immunizations, surgical procedures etc., including the procurement of animals.

The breeding of laboratory animals has been planned to meet the needs of scientists/research scholars for various animal experiments. The details of the animals bred in the facility, procured from various sources, and supplied for various research and development activities are given below.

SR. NO.	STRAINS/ SPECIES	ANIMALS PROCURED	ANIMALS BRED	ANIMALS SUPPLIED
1	**RATS**			
	Wistar	—	86	59
	Lewis	—	79	6
2.	**MICE**			
	BALB/CJ	—	4584	3521
	C57bl/6J	—	2014	1649
	SWISS	—	1358	468
	DBA/2J	—	299	28
	NOD/LtJ	—	90	3
	Nude (nu/nu)	34	—	34
	BALB/c*	—	191	—
3.	**MASTOMYS** (Praomis)	—	127	—
4.	**HAMSTER** (Syrian)	10	3	7
5.	**RABBIT** (NZW)	—	5	6

* BALB/c with cataract mutation.

Library and documentation

The main activities of the library and documentation have been focused on collection and dissemination of science and technology information.

The Library and documentation has added to its stocks 665 documents including books and bound volumes of periodicals during the period of 1999-2000. It subscribes 74 scientific journals and receives 16 other periodicals out of which some are subscribed by us and some are received on gratis basis.

Additional documentation facilities include local area net work confined to library activities, a number of CD-ROM databases including bibliographic, full text and factual databases, on-line access to Knight rider (DIALOG). The library is a continuing member of the Pune library network.

Publications
1999-2000

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STATEMENT OF ACCOUNTS

OF

NATIONAL CENTRE FOR CELL SCIENCE, PUNE

FOR THE YEAR ENDING 31ST MARCH, 2000.

Joshi Apte & Co.,
Chartered Accountants,

301,Trimurti Apartments,
Opp. Sahitya Parishad, Tilak Road,
1542, Sadashiv Peth, Pune - 411 030.

REPORT OF AN AUDITOR RELATING TO ACCOUNTS AUDITED UNDER SUB-SECTION (2) OF SECTION 33 & 34 & RULE OF 19 OF THE BOMBAY PUBLIC TRUST ACT 1950

Registration No. F-5282/Pune

Name of the Public Trust : **NATIONAL CENTRE FOR CELL SCIENCE (NCCS),**
PUNE. (Formerly National Facility for Animal Tissue & Cell Culture (NFATCC)

For the year ending : 31.3.2000

(a) Whether accounts are maintained regularly and in accordance with the provision of the Act and the Rules	:	YES
(b) Whether receipts and disbursements are properly and correctly shown in the accounts	:	YES
(c) Whether the Cash balance and vouchers in the custody of the manager or trustee on the date of audit were in agreement with the accounts	:	YES
(d) Whether all books deeds accounts,vouchers or other documents or records required by the auditor were produced before him	:	YES
(e) Whether a register of movable and immovable properties is properly maintained, the changes therein are communicated from time to time to the regional office and the defects and inaccuracies mentioned in the previous audit report have been duly complied with	:	Registers of Movable and Immovable properties are maintained.
(f) Whether the manager or trustee or any other person required by the auditor to appear before him did so and furnished the necessary information required by him.	:	YES
(g) Whether any property or funds of the Trust were applied for any object or purpose other than the object of purpose of the trust	:	NO
(h) The amounts of outstandings for more than one year and the amounts written off, if any.	:	NO
(i) Whether tenders were invited for repairs or construction involving expenditure exceeding Rs. 5000/-	:	YES

(j)	Whether any money of the public trust has been invested contrary to the provisions of Section 35.	:	NO
(k)	Alienation's, if any, of the immovable property contrary to the provisions of section 36 which have come to the notice of the auditor	:	NIL
(l)	All cases of irregular, illegal or improper expenditure or failure or omission to recover monies or other property belonging to the public trust or of loss or waste of money or other property thereof and whether such expenditure, failure, omission, loss or waste was caused in consequence of breach of trust or misapplication or any other misconduct on the part of the trustees or any other person while in the management of the trust.	:	NIL
(m)	Whether the budget has been filed in the form provided by the rule 16 A.	:	YES
(n)	Whether the maximum and minimum number of the trustees is maintained.	:	YES
(o)	Whether the meetings are held regularly as provided in such instrument.	:	YES
(p)	Whether the minute book of the proceedings of the meeting is maintained.	:	YES
(q)	Whether any of the trustees has any interest in the investment of the trust.	:	NO
(r)	Whether any of the trustees is a debtor or creditor of the trust.	:	NO
(s)	Whether the irregularities pointed out by the auditors in the accounts of the previous year have been duly complied with by the trustees during the period of audit.	:	Please see our General Remarks as per Annexure - I.
(t)	Any Special matter which the auditor may think fit or necessary to bring to the notice of the Deputy/ Assistant Charity Commissioner.	:	Please see our General Remarks as per Annexure.

PUNE

DATE: 14 JUNE, 2000

For JOSHI APTE & CO.

Sd/-
Partner
Chartered Accountants
Auditors

Joshi Apte & Co.,
Chartered Accountants,
301,Trimurti Apartments,
Opp.Sahitya Parishad, Tilak Road,
1542, Sadashiv Peth, Pune - 411 030.

AUDIT REPORT

We have examined the Balance Sheet of National Centre For Cell Science (Formerly National Facility For Animal Tissue & Cell Culture), Pune as at 31.03.2000 and the Income and Expenditure Account for the year ended on that date which are in agreement with the books of account maintained by the said Trust.

We have obtained all the information and explanations which to the best of our knowledge and belief were necessary for the purpose of the Audit. In our opinion, proper books of account have been kept by the Trust so far as appears from our examination of books, subject to the comments given below :
See our General Remarks as per Annexure I and Significant Accounting Policies followed as per Annexure II.

In our opinion and to the best of our information and according to explanations given to us, the said accounts give a true and fair view,

I. In the case of Balance Sheet, of the state of affairs of the above named Trust as at 31.03.2000 AND

II. In the case of Income and Expenditure Account, of the deficit for its accounting year 31.03.2000.

PUNE

DATED: 14 JUNE, 2000

For **Joshi Apte & Co.,**
Chartered Accountants,

Sd/-
(P.J. Apte)
Partner

Joshi Apte & Co.,
Chartered Accountants,
301,Trimurti Apartments,
Opp.Sahitya Parishad, Tilak Road,
1542, Sadashiv Peth, Pune - 411 030.
Tel.: 4333188, 4332991, TeleFax: 020-4335673
Email: joap@vsnl.com

ANNEXURE I

NATIONAL CENTRE FOR CELL SCIENCE

GENERAL REMARKS FOR THE YEAR ENDED 31.03.2000

1. We have conducted the audit of NCCS as per our appointment vide Ref.No.509/CAV/FRM/CENTRAL GOVT. COMP/ANIMAL, March 7th, 2000 and our acceptance dated 22.03.2000.

2. Out of the grants received during the year under audit from Govt. of India, Ministry of Science and Technology and Department of Bio-Technology grants of Rs. 2,69,05,000/- received for incurring capital expenditure are added directly to Capital Grants from Department of Bio-Technology under the heading 'Other Earmarked Funds'.

3. It is informed to us that as per Memorandum of Understanding between NCCS & University of Poona, the latter has allotted land measuring about 10 acres for construction of NCCS complex including laboratories and residential accommodation for NCCS's staff members, students, fellows & visitors. Title of the land allotted by the University of Poona for constructing these buildings shall continue to be vested in the name of University of Poona (Govt. of Maharashtra).

   Construction and service group of Department of Atomic Energy, Govt. of India is constructing the NCCS complex with the help of amounts deposited by the trust with them. The aggregate amount so deposited with the C & S group, Dept. of Atomic Energy along with other payments made is carried forward as 'Capital Work in Progress'.

4. Following project grants are carried forward in the Balance Sheet as Liabilities as these grants are received for specific projects: —

	Particulars	Unspent Balance
1.	Fetal Liver	200,404.05 Cr.
2.	Biotech Associates	15,888.10 Cr.
3.	H.L.R.C. Project	23,739.00 Cr.
4.	U.G.C. Project	22,400.00 Dr.
5.	CSIR Fellowship (JRF)	24,609.50 Cr.
6.	CSIR (Emeritus Scientist)	134,647.00 Cr.
7.	Indo French	700,000.00 Cr.
8.	Horse Shoe Serum	45,292.00 Cr.
9.	HIV Infection	531,555.00 Cr.
10.	Targetted Modulations	287,008.00 Cr.
11.	Bone Marrow	846,000.00 Cr.
12.	Immuno Genetic Engg.	581,156.00 Cr.

5. During the year 1997-1998, the Trust had signed a Memorandum of Understanding with CSIR for leasing out two properties 'JOPASANA' and 'JIDNYASA' at an annual lease rent of Re.1/-. A clause should be incorporated in the MOU to share the earning of Joint Venture to compensate the loss of Revenue of NCCS on account of lease rent.

For Joshi Apte & Co.
Chartered Accountants

Sd/-
Partner

DATED: 14 JUNE, 2000

ANNEXURE '2'

NATIONAL CENTRE FOR CELL SCIENCE, PUNE

SIGNIFICANT ACCOUNTING POLICIES FOLLOWED

1. The financial statements are prepared on cash basis.
2. Fixed Assets are carried at cost. No depreciation is provided on Fixed Assets upto 31.03.2000
3. As per the system consistently followed, entire expenditure on consumables is charged to Income & Expenditure Account, Opening and Closing Stocks of consumables are not accounted for.
4. Contributions to Provident Fund are charged to Income & Expenditure Account.
5. Foreign Exchange transactions are recorded at the rates prevailing on the dates of payment.
6. Revenue is recognized only when service is rendered and payment is received.
7. The trust does not hold any Investment in its own name.
8. Retirement benefit will be accounted on cash basis as and when due.
9. Government grants are shown in the Income and Expenditure account as and when received.
10. Expenditure on Research & Development activities relating to Government recognized in-house Research and Development laboratories comprising of salaries and wages, stores, consumed and miscellaneous expenses during the year, amounted to Rs. 8,27,19,599.82 including on capital account Rs. 2,68,87,865.25 (In 1998-99 Rs. 8,81,12,920.55 including on capital accounts Rs. 4,27,41,628.50).

PUNE

DATED: 14 JUNE, 2000

For **Joshi Apte & Co.,**
Chartered Accountants,

Sd/-
Partner

NATIONAL CENTRE FOR CELL SCIENCE, PUNE

BALANCE SHEET AS ON 31.03.2000

As on 31.03.99 Amount in Rs.	FUNDS AND LIABILITIES	As on 31.03.2000 Amount in Rs.	As on 31.03.99 Amount in Rs.	PROPERTY AND ASSETS	As on 31.03.2000 Amount in Rs.
	Trust Funds Or Corpus			**Immovable Properties: (at cost)**	
	Balance as per last Balance Sheet		168,708,998.01	As per Schedule 4.	178,586,935.01
NIL	Adjustments During The Year (Give Details)	NIL		Balance as per last Balance Sheet Additions During the year Less: Sales During the year Depreciation upto date	
	Other Earmarked Funds: (Created under the provisions of the Trust Deed or Schedule or out of the Income)		NIL	**Investments** Note: The market value of the above investment is Rs.	NIL
338,997,581.71	As per Schedule 2	365,902,581.71			
	Loans (Secured Or Unsecured)			**Furniture, Fixtures & Other Assets**	
	From Trustees		166,550,560.45	As per Schedule 5.	183,560,488.70
NIL	From Others	NIL		Balance as per last Balance Sheet Additions During the year Less: Sales During the year Depreciation upto date	
	LIABILITIES				
	For Expenses				
	For Advances				
	For Rent & Other Deposits		NIL	**Loans (Secured or Unsecured) Good/doubtful**	NIL
	For Sundry Credit Balances			Loan Scholarships Other Loans	
298,122.15	As per Schedule 3	3,445,798.65			

As on 31.03.99 Amount in Rs.	FUNDS AND LIABILITIES	As on 31.03.2000 Amount in Rs.	As on 31.03.99 Amount in Rs.	PROPERTY AND ASSETS	As on 31.03.2000 Amount in Rs.
	Income & Expenditure Accounts:			**Advances**	
	Balance as per last Balance Sheet		834,968.50	As per Schedule 6.	1,911,844.00
				To Trustees	
	Less: Appropriation if any			To Employees	
				To Contractors	
NIL	Add: Surplus as per Income &	NIL		To Lawyers	
	Less: Deficit Expenditure A/c.			To Others	
			NIL	**Income Outstanding**	NIL
				Rent	
				Interest	
				Other Income	
				Cash & Bank Balances	
			1,967,578.93	As per Schedule 7	3,478,928.11
				a) In Current Account With	
				b) With The Trustee	
				c) With the Manager	
				Income & Expenditure Accounts:	
				Balance as per last Balance Sheet 1233597.97	
			1,233,597.97	Add: Deficit 576586.57	1,810,184.54
				As per Income & Expenditure A/c.	
339,295,703.86	**Total Rs.**	**369,348,380.36**	**339,295,703.86**	**Total Rs.**	**369,348,380.36**

As per our report of even date

Income Outstanding (If Accounts are kept on cash basis) = NIL

The above Balance Sheet to the best of my/our belief contains a true Account of the Funds and Liabilities and of the Property and the Assets of the Trust.

Sd/-
Chartered Accountants
Auditors

Dated at: 14 JUNE, 2000

Dated at: 14 JUNE, 2000

Sd/-
TRUSTEES

THE BOMBAY PUBLIC TRUSTS ACT, 1950

SCHEDULE IX [VIDE RULE 17(1)]

NATIONAL CENTRE FOR CELL SCIENCE, PUNE (R. NO. F-5282)

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDING 31.03.2000

FOR THE YEAR ENDED 31.03.99 AMOUNT IN RS.	EXPENDITURE	FOR THE YEAR ENDED 31.03.2000 AMOUNT IN RS.	FOR THE YEAR ENDED 31.03.99 AMOUNT IN RS.	INCOME	FOR THE YEAR ENDED 31.03.2000 AMOUNT IN RS.
NIL	To **Expenditure in respect of Properties** Rates, Taxes, Cesses Repairs and maintainance Salaries Insurance Depreciation (by way of provision or adjustments) Other expenses	NIL	NIL	By **Rent** (accrued) (realised)	NIL
			NIL	By **Interest** (accrued) (realised)	NIL
				On Securities On Loans On Bank Accounts	
NIL	To **Establishment Expenses**	NIL			
341,780.00	To **Remuneration to Trustees**	337,816.00			
NIL	To **Remuneration** (in the case of math) to the head of the math, including his household expenditures, if any.	NIL	NIL	By **Dividend**	NIL
NIL	To **Legel Expenses**	117,000.00	NIL	By **Donations in cash or kind**	NIL
3,000.00	To **Audit Fees**	3,150.00	43,110,154.00	By **Grants**	54,947,961.00
5,898.00	To **Contribution & Fees**	7,283.00		By **Income From other sources (in details as far as possible)**	

NATIONAL CENTRE FOR CELL SCIENCE, PUNE

SCHEDULE 1

EXPENDITURE ON THE OBJECTS OF TRUST

As on 31.3.99 Amount in Rs.	Particulars		As on 31.3.2000 Amount in Rs.
14,372,887.00	Salaries and Allowances		13,858,304.00
—	Fellowships		1,811,139.00
2,518,269.00	Works on Contract		2,589,917.00
17,330,160.05	Consumables		18,182,562.57
1,178,212.00	Travel		1,103,800.00
131,535.00	Consultancy		140,195.00
	Contingencies		
166,333.75	Printing & Stationery		119,581.30
536,070.00	Staff Recruitment Exps.		405,570.00
7,857.00	Newspapers & Periodicals		9,301.50
807,397.40	Telephone Telex		637,927.80
97,292.05	Postage		119,619.00
147,909.80	Meeting, Hon. & Misc. Expenses		156,820.65
105,872.00	Vehicle Expenses —	Petrol	162,138.00
37,058.10		Repairs	67,619.60

As on 31.3.99 Amount in Rs.	Particulars	As on 31.3.2000 Amount in Rs.
6,890,938.00	Water & Electricity	**15,307,714.00**
41,697.00	Labour Charges	**385.00**
268,474.90	Misc. Purchases	**347,235.65**
8,502.00	Conveyance	**6,390.50**
13,849.00	Repairs & Maintenance	**15,122.00**
36,850.00	Inspection & Other Fees	**15,500.00**
138,077.00	Maintenance Contract	**246,695.00**
—	Insurance	**16,136.00**
8,468.00	Seminar	**23,486.00**
—	Training Expenses	**23,326.00**
176,905.00	Technology Transfer	—
45,020,614.05	**Total**	**55,366,485.57**

NATIONAL CENTRE FOR CELL SCIENCE, PUNE

SCHEDULE 2

OTHER EARMARKED FUNDS

			Amount in Rs.
a)	**Capital Grants from DBT New Delhi:**		
	Balance As per last Balance Sheet		**338,154,180.71**
	Grants for Capital Expenditure		**26,905,000.00**
		Total A	**365,059,180.71**
b)	**Vehicle Fund:**		
	Balance as per last Balance Sheet		**400,000.00**
		Total B	**400,000.00**
c)	**Liquid Nitrogen Fund:**		
	Balance as per last Balance Sheet		**443,401.00**
		Total C	**443,401.00**
	Total A + B + C		**365,902,581.71**

NATIONAL CENTRE FOR CELL SCIENCE, PUNE

SCHEDULE 3

LIABILITIES

Sr. No.	Particulars	Opening Balance Amount in Rs.	Additions Amount in Rs.	Expences Amount in Rs.	Closing Balance Amount in Rs.
1	Fetal Liver	200,404.05	-	-	**200,404.05**
2	Biotech Associates	15,888.10	-	-	**15,888.10**
3	H.L.R.C. Project	23,739.00	-	-	**23,739.00**
4	U.G.C. Project	-	-	-	**-**
5	Canteen Deposit	5,000.00	-	-	**5,000.00**
6	Security Deposit	500.00	-	-	**500.00**
7	Deposit Maintainance Contract	50,000.00	-	-	**50,000.00**
8	CSIR Fellowship (JRF)	(162,839.50)	962,445.00	774,996.00	**24,609.50**
9	CSIR (Emeritus Scientist)	-	391,340.00	256,693.00	**134,647.00**
10	Indo French	-	700,000.00	-	**700,000.00**
11	Horse Shoe Serum	-	126,000.00	80,708.00	**45,292.00**
12	HIV Infection	-	760,000.00	228,445.00	**531,555.00**
13	Targetted Modulations	-	770,000.00	482,992.00	**287,008.00**
14	Bone Marrow	-	846,000.00	-	**846,000.00**
15	Immuno Genetic Engg.	-	581,156.00	-	**581,156.00**
	TOTAL	132,691.65	5,136,941.00	1,823,834.00	**3,445,798.65**

NATIONAL CENTRE FOR CELL SCIENCE, PUNE

SCHEDULE 4

IMMOVABLE PROPERTIES AS ON 31.03.2000 (AT COST)

Particulars	As on 31.3.99 Amount in Rs.	Additions during the year Amount in Rs.	**Total as on 31.3.2000 Amount in Rs.**
A] BUILDING:			
1) Jopasana	6,026,554.30	—	**6,026,554.30**
2) Jidnyasa	6,914,265.25	—	**6,914,265.25**
TOTAL	12,940,819.55	—	**12,940,819.55**
B] CAPITAL WORK IN PROGRESS			
NCCS Complex at Pune University campus			
1) Amt. deposited with C & S Group Dept.of Atomic Energy, Govt. of India	130,900,000.00	8,000,000.00	**138,900,000.00**
2) Other payments	24,868,178.46	1,877,937.00	**26,746,115.46**
TOTAL	155,768,178.46	9,877,937.00	**165,646,115.46**
GRAND TOTAL Rs.	168,708,998.01	9,877,937.00	**178,586,935.01**

NATIONAL CENTRE FOR CELL SCIENCE, PUNE

SCHEDULE 5

FURNITURE, FIXTURES AND OTHER ASSETS AS ON 31.03.2000

SR. NO.	PARTICULARS	AS ON 31.03.99 AMOUNT IN Rs.	ADDITIONS DURING THE YEAR AMOUNT IN Rs.	DELETION DURING THE YEAR AMOUNT IN Rs.	AS ON **31.03.2000 AMOUNT IN Rs.**
A	Furniture & Fixture	17,159,553.73	377,668.00	—	**17,537,221.73**
B	Library Books	12,624,975.35	2,557,726.25	—	**15,182,701.60**
C	Equipment	135,824,679.37	14,074,534.00	—	**149,899,213.37**
D	Vehicles	741,352.00	—	—	**741,352.00**
E	Equipment under Fetal Liver Project	200,000.00	—	—	**200,000.00**
	TOTAL	166,550,560.45	17,009,928.25	—	**183,560,488.70**

NATIONAL CENTRE FOR CELL SCIENCE, PUNE

SCHEDULE 6

ADVANCES

As on 31.03.1999 Amount in Rs.		As on 31.03.2000 Amount in Rs.
	A) To Employees:	
400,479.00	Motor Cycle Advance	**707,340.00**
22,500.00	Festival Advance	**33,300.00**
49,000.00	Computer Advance	**43,120.00**
—	House Building Advance	**905,534.00**
471,979.00	**TOTAL A**	**1,689,294.00**
	B) Deposits:	
173,000.00	Telephone Deposit	**173,000.00**
27,150.00	Gas Deposits	**27,150.00**
200,150.00	**TOTAL B**	**200,150.00**
	C) UGC Projects:	
162,839.50	CSIR (JRF & SRF)	—
—	UGC Projects	**22,400.00**
162,839.50	**TOTAL C**	**22,400.00**
834,968.50	**TOTAL A + B + C**	**1,911,844.00**

NATIONAL CENTRE FOR CELL SCIENCE, PUNE

SCHEDULE 7

CASH BANK BALANCES

As on 31.03.1999 Amount in Rs.		As on 31.03.2000 Amount in Rs.
	Balance with Bank of India:	
1,956,578.93	- in Current Account NCCS	**247,529.46**
—	- in Current Account Project	**3,220,398.65**
1,000.00	- in Fixed Deposit	**1,000.00**
10,000.00	**Petty Cash in Hand**	**10,000.00**
1,967,578.93	**Total Rs.**	**3,478,928.11**

NATIONAL CENTRE FOR CELL SCIENCE, PUNE

STATEMENT OF RECEIPTS PAYMENTS FOR THE YEAR 1999-2000

RECEIPTS		AMOUNT IN Rs.	PAYMENTS		AMOUNT IN Rs.
TO OPENING BANK BALANCE:			**BY CAPITAL EXPENDITURE:**		
As on 01.04.99					
Petty Cash		10,000.00	University Site	9,877,937.00	
Bank of India		1,956,578.93	Furniture	377,668.00	
Bank of India Fixed Deposit		1,000.00	Equipment	14,074,534.00	
			Library books	2,557,726.25	
					26,887,865.25
TO GOVERNMENT GRANT:			**BY OTHER EXPENDITURE:**		
(Dept. of Biotechnology					
Ministry of Science & Technology					
Govt. of India			1. Fellowship	1,811,139.00	
Capital	26,905,000.00		2. Salary/Allowences	13,858,304.00	
Revenue	54,565,000.00		3. Remuneration to Director	337,816.00	
		81,470,000.00	4. Professional service charges	2,589,917.00	
			5. Consumables/Overheads	18,182,562.57	
TO INTEREST:			6. Travel	1,103,800.00	
against grants		382,961.00	7. Consultancy	140,195.00	
			8. Water/Electricity Corpn. Chgs	15,307,714.00	
					53,331,447.57
TO INTEREST ON VEHICLE LOANS:		29,910.00			
			BY CONTINGENCIES:		
TO RECOVERY OF ADVANCES:					
Vehicle advance	167,789.00		1. Labour charges	385.00	
Festival advance	59,700.00		2. Postage	119,619.00	
Computer Advance	5,880.00		3. Vehicle		
House Building	38,858.00		Petrol	162,138.00	
		272,227.00	Repairs	67,619.60	

RECEIPTS		AMOUNT IN Rs.
TO GRANTS-IN-AID:		
Indo French	700,000.00	
Immnuolysis Genitic Engg.	581,156.00	
Science Day	10,000.00	
CSIR (Emeritus Scientists)	391,340.00	
CSIR (JRF/SRF/RA)	962,445.00	
U.G.C. Project	60,000.00	
Hybridoma	225,000.00	
Horse Shoe	126,000.00	
HIV Infection DNA Vaccine	760,000.00	
Targetted Modulation	770,000.00	
Bone Marrow	846,000.00	
		5,431,941.00
TO CELL LINE HANDLING CHARGES		220,160.00
To MISC. RECEIPTS:		
Guest House	40,642.00	
Registration Fees	600.00	
Facs	—	
Income Tax	15,875.00	
		57,117.00

PAYMENTS		AMOUNT IN Rs.
4. Contribution to Charity Commissioner	7,283.00	
5. Maintenance Contract	246,695.00	
6. Staff Recruitment Exps.	405,570.00	
7. Printing & Stationary	119,581.30	
8. Telephone/Telex	637,927.80	
9. Inspection fees	15,500.00	
10. Audit fees	3,150.00	
11. Misc. purchases	347,235.65	
12. Repairs & Maintenance	15,122.00	
13. Newspaper & Periodicals	9,301.50	
14. Conveyance	6,390.50	
15. Meeting & Misc. Expenses	154,720.65	
16. Legal Expenses	117,000.00	
17. Insurance	16,136.00	
18. Other Exp.	2,100.00	
19. Seminar / Symposium	23,486.00	
20. Training Expenses	23,326.00	
		2,500,287.00
BY PROJECT:		
CSIR (Dr. Iqbal)	256,693.00	
CSIR (JRF/SRF)	774,996.00	
UGC	84,991.00	
Hybridoma	225,000.00	
Production Of Horse Shoe	80,708.00	
HIV infection	228,445.00	
Targetted Modulation	482,992.00	
Science Day	10,000.00	
		2,143,825.00

RECEIPTS	AMOUNT IN Rs.	PAYMENTS	AMOUNT IN Rs.
		BY VEHICLE ADVANCE	474,650.00
		BY FESTIVAL ADVANCE	70,500.00
		House BUILDING ADVANCE	944,392.00
		BY CLOSING BALANCES:	
		a) Balance with Bank of India, Projects	3,220,398.65
		b) Balance with Bank of India, NCCS	247,529.46
		c) Fixed Deposit	1,000.00
		d) Cash in hand	10,000.00
TOTAL	**89,831,894.93**	**TOTAL**	**89,831,894.93**

As per our report of even date
For Joshi Apte & Co.
Charatered Accountants

Sd/-
Partner
Date: 14 JUNE, 2000

Sd/-
Dr. G.C. Mishra
Director, NCCS, Pune

Sd/-
S.M. Hendre
Accounts Officer
NCCS, Pune

NATIONAL CENTRE FOR CELL SCIENCE, PUNE

REPLIES TO THE GENERAL REMARKS AT ANNEXURE I OF THE AUDIT REPORT FOR THE YEAR ENDING 31.03.2000

1. The appointment of auditor's is being made as per the suggestion of Comptroller and Auditor General, Government of India, New Delhi.

2. The allocation of grants has been done as per budgeted grants received from Department of Biotechnology, Ministry of Science and Technology, Government of India.

3. The title deed of the land allotted by the University of Pune to establish NCCS complex is as per the MoU, signed between the University of Pune and NCCS. The construction of NCCS complex at Pune University Campus, is being undertaken by C&S Group, DAE as deposit work. Hence, payments made as advance on account of deposit work is being shown as capital work in progress until the construction accounts are finalised.

4. The project grants received are for a speciftic research work. The projects are continued. The grants will be utilised in due course of time for the purpose for which it was released by the funding authority.

5. As regards sharing of earnings of joint venture undertaken with CSIR, the matter is being persued with CSIR headquarter, New Delhi.