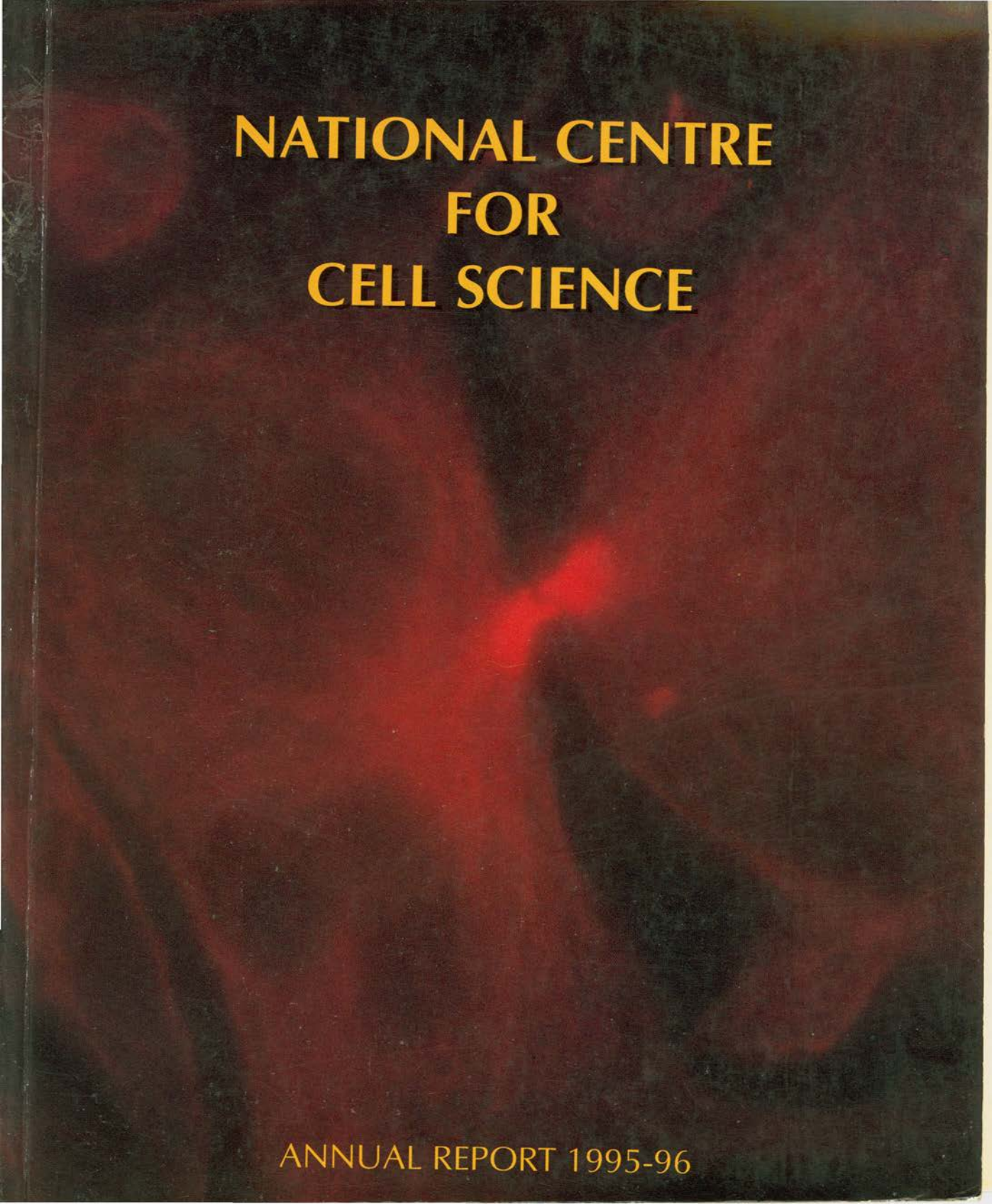
NATIONAL CENTRE FOR CELL SCIENCE



ANNUAL REPORT 1995-96

NATIONAL CENTRE FOR CELL SCIENCE

An Autonomous Institution of the Department of Biotechnology
Government of India

ANNUAL REPORT 1995-96

Pune University Campus
Ganeshkhind, Pune 411007

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 & 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 organize training programmes for technical personnel in tissue culture technology, tissue banking, cell products and related areas.

* 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 collaborate with foreign research institutions and laboratories and other international organizations in areas relevant to the objectives of the facility.

* To participate in such programmes as required in the country for the betterment of society and advancement of science and technology.

PREFACE

It is indeed my pleasure to present this Annual Report of National Centre for Cell Science (1995-96).

At the outset I would like to highlight two major changes that have taken place. First, The activities of the institute earlier carried out in two buildings, Jopasana and Jidnyasa, have now been moved into the new building complex. The construction of buildings under the first phase has been completed, which includes the main laboratory complex, animal house, infrastructure, hostel, guest house and residential quarters. Second, the institute has been renamed as the National Centre for Cell Science, to indicate its broadened scientific base, while still retaining the original framework of activities. I am confident that these changes will stimulate the growth and enthuse the staff to shoulder the added responsibilities.

An overview of the achievements during the past one year is presented below:

The NCCS cell repository now has a collection of 1127 cell cultures from 25 different species, including hybridomas. During 1995-96, 698 cultures comprising 232 different cell lines were supplied to 108 research institutes located in 59 cities. Three hundred forty individuals have registed for availing the cell supply facility. From 1st September, 1995, Rs 100/- is being charged from the user as handling charges. The cell supply shows an increasing trend in spite of this handling charges.

As part of our quest for human resource development, the staff members of NCCS continued to participate in the teaching programmes in Microbiology, Biotechnology and Zoology in the University of Pune. Similarly, training programmes in basic and specialised techniques in animal tissue culture were organized for researchers from different institutions.

Potential mitogens and differentiation agents have been detected in the spent media from fibroblsts and keratinocytes that regulate melanocyte proliferation and differentiation in vivo and were used for culturing melanocytes in vitro. These studies have allowed the development of a method for melanocyte cell transformation in vitiligo cases.

A mouse model has been developed for studying streptozotocin-induced

diabetes and its modulation. The role of free-radical scavengers in bitter gouard extract in control of diabetes is being studied.

Studies on endemic and non-endemic sera for IgG4 levels have indicated its importance in identification and detection of filaria. Efforts are in progress to establish collaboration with industries to develop this into a diagnostic kit for filaria.

Differential action of streptococcal lipoteichoic acid on oropharyngeal mucosal epithelial cells and the underlying mesenchymal cells has been identified using cultured cells.

Biological anti-oxidants, such as anti-freeze proteins, anti-freeze glycoprotens, and trehalose, have been shown to play important role in protecting bone marrow cells during cryopreservation.

In vitro studies on bone marrow cells have indicated a possible modulatory role of transforming growth factor beta in self renewal of multipotential haematopoietic stem cells.

A highly sensitive method using heteroduplex analysis and restriction fragment length polymorphism of mitochondrail DNA has been established for identification and characterization of mosquito cell lines. Partial sequence of 16S and 12S ribosomal RNA from mosquito mitochondria has been carried out.

In the end, I may add that the present strength of the scientific and technical staff is very small. Efforts are under way to induct personnel of proven scientific calibre. I understand that this would be a long-drawn process, but I hope to induct top quality researchers to join us in our endeavour to persue basic and applied research in biotechnology and allied areas of national importance.

G. C. Mishra

Director

CULTURE REPOSITORY

MEDIA REQUIREMENTS 01/04/95 TO 31/03/96

MEDIA	*QUANTITY IN LITERS*
MEM (E)	172
DMEM	150
RPMI 1640	88
IMDM	75
Ham's F 12	14
Ham's F 10	18
MM MEDIUM	22
OPTU MEM	01
BME(E)	14
McCoy's 5 A	08
Alpha MEM	05
L-15	03
Ham's F- 12 K	02
TNM-FH	01
M 199(E)	03
MEM(H)	2.5
M 199 (H)	03
H-Y	01
DMEM : Ham's F12 1 : 1 Mixture	12
BGJb	02
MCDB 153	01
TPB	01
NUTRIENT BROTH	05

MEDIA	*QUANTITY IN LITERS*
skin biopsy collection medium	02
TPVG	55
PBS (1X)	190
HBSS	10
TRYPSIN SOLUTION(2%)	2.5
SERUM	
FCS	35
NBCS	04
HS	2.5

DEVELOPMENT OF CULTURES

Molecular cross-talk between pathogenic bacteria/bacterial antigens with human mucosal and sub-mucosal mesenchymal cell system using cell culture as a model

Molecular and cellular interaction of lipopolysaccharide with human intestinal mesenchymal cells

Establishment and characterization of breast cancer cell lines from high risk ethnic groups

Growth characteristics of normal and abnormal melanocytes and their response to synthetic and natural growth factors.

Regulation of skin pigmentation (A) Regulation opf pigment transfer (B) Regulation of melanogenesis

In vitro cultivation of erythrocytic stages of Plasmodium vivax and large scale cultivation of Plasmodium falciparum

DEVELOPMENT OF CULTURES

TITLE: Molecular Cross-talk between Pathogenic Bacteria/Bacterial antigens with Human Mucosal and Submucosal Mesenchymal Cell System Using Cell Culture as a Model

Investigators: **K S Nanda Kumar**
Dipshikha Chakrvortty

General Background:

Majority of the disease caused by the bacteria originate at the mucosal site of the host.The primary step in the initiation of bacterial infections involves the adhesion,colonizationand invasion of the bacteria at the mucosal cells.Bacteria or bacterial antigens(mainly adhesins) interact with specific cell types of the mucosal region of the host leading to the cellular damage resulting in the onset of disease process.The tissue tropism involved in the bacterial interaction with cell types in the host mucosal region and the consequence of such interaction is a complex phenomenon.Non-immunocompetent cells like epithelial cells,fibroblasts, endothelial cells etc have been shown to play specific role in the out come of the mucosal infections by the bacteria.In vivo model using animals and isolated cells from susceptible site of infection of the host have limitations to study such bacteria-mucosal cell interaction.The integrity of the cell,cell physiology,architecture and ultrastructural morphology of the cells are important in studying such bacteria-host cell communication.Therefore,the present investigation was formulated to study the basic mechanism underlying the mucosal infection by the pathogenic bacteria or adhesins at themucosal cell types using cultured cells as a model.The importance of the possible molecules of the bacteria responsible for bacteria-host cell interaction provides evidence to identify a suitable molecule having therapeutic application in the prevention of bacterial colonization.

Title: Human oropharyngeal mucosal epithelial cell biology in response to Streptococcal lipoteichoic acid.

Background:

Previous studies done by the workers(KSN Kumar et al, 1991, 1992a, 1992b, 1993, 1995) and by others have shown the association between certain oral bacteria like streptococci,human oropharynx and the development of various autoimmune complications in humans and animals.The reasons for this association still remain unknown. It has also been shown that lipoteichoic acid(LTA) of streptococci exists as a possible adhesin which favours the bacteria to adhere and colonize.However the consequence following LTA binding to human oral mucosal epithelial cells has not been understood.These gave impetus to establish an in vitro model of cultured human oral epithelial cells to investigate the mechanism involved in the consequence of streptococcal LTA interaction with the oral epithelial cells.

Work Carried out:

Human pharyngeal and buccal mucosal epithelial cells(PMEC and BMEC) were cultured from 35 human foetuses obtained from MTP cases.Mucosal epithelial cells were cultured in specific medium after selective dissection of the tissue.The cell cultures were devoid of fibroblasts and other cell types.Morphological characterization of cells were studied by scanning electron microscopy.The mucosal epithelial cells showed polarity by exhibiting well formed domes.The cells were further characterized immunocytochemicallly with the help of different monoclonal antibodies.Results showed expression of specific cytokeratin molecules,desmosomal protein and extracellualr matrix molecules fibronectin,collagen IV and laminin.The PMEC and BMEC could be subcultured upto 3rd passage.Cryopreserved cells failed to grow upon revival.

Streptococcal lipoteichoic acid(LTA) was dissolved in apropriate cell culture reagents not containing serum.The cultured PMEC and BMEC of passage between one and three were exposed to LTA at different concentration over a period of time.LTA at a concentration of 50-100ug/ml induced proliferation of the epithelial cells as determined by DNA

synthesis.LTA did not show cytotoxicity to the epithelial cells as studied over a period of time as revealed by MTT assay.

Indirect immunofluoresence studies using a panel of monoclonal antibodies to cytokines showed fourty percent of epithelial cells produced IL-8 in response to LTA and induction of IL-4 was marginal over the control experiments.There was no change in the cytoskeletal architecture of actin,tubulin and cytokeratins in epithelial cells in response to LTA.

In contrast to this,the direct action of LTA on cultured oral fibroblasts was different.At the above mentioned concentrations of LTA used on epithelial cultures,fibroblasts were detached from the substratum.There was a drastic decrease in the DNA synthesis and viablity of the cells. The rounding of the fibroblasts was observed as early at 2-3 hrs after the addition of LTA .The detaching effect of LTA on fibroblasts also induced the production of LDH.The action of LTA on fibroblasts could be blocked by fibronectin,serum and albumin.LTA has also induced the production of inducible nitric oxide (iNOS) in fibroblasts.

Using BMEC - fibroblasts co-cultures,it was observed that LTA(100ug/ml) could selectively remove fibroblasts from epithelial cultures.Tissue culture inserts consisted of membrane filters were set up to study whether LTA could detach fibroblasts.In this case tissue culture inserts with confluent monolayer of PMEC on the apical side were placed on a monolayer of fibroblasts cultures grown on plastic 24 well plates.However,LTA(100ug/ml)in the apical side medium failed to detach fibroblasts at the basal side studied over a period of time.However,study has not done to determine whether LTA could be transported across the membrane to the basal media in order to act on the fibroblasts.

Using different epithelial and fibroblast cell lines,it was observed that LTA was cytotoxic to fibroblast cell lines and the effect was comparatively less as compared to that observed with primary cultures of oral fibroblasts.The results derived from the above study indicate the dual action of LTA on mucosal epithelial and fibroblasts.The reasons for this remain to be elucidated.

TITLE: Molecular and Cellular interaction of lipopolysaccharide with human Intestinal Mesenchymal Cells

Background:

Gram -negative bacteria are important pathogenic organisms , responsible for development of various diseases in human . Among them, E.coli and Salmonella are associated with the developement of disease like gastroenteritis, diarrohea, urinary tract infection , food poisoning, enteric fever ect leading to morbidity and mortality. One of the important bacterial antigens of Salmonella and E.coli , which has been extensively studied is lipopolysaccharide(LPS).This toxin substance produced by E.coli has been found to exert a wide variety of biological action on mammalian cells, which are important causative agent for the initiation of the disease .Intestinal epithelial cell have been found to play significant role in the pathophysiology of diahorrea and dysentry.It is known that normal cell physiology of intestinal epithelial cell is controlled by the underlying submucosal mesenchymal cell . The invasive nature of E.coli and salmonella provides an oppurtunity for the bacteria /bacterial antigens / toxins like LPS to interact with various cell types of the submucosal region of the intestine. Among the submucosal mesenchymal cells,fibroblasts exist as a major resident cell population of all organs playing vital role in cell-cell interaction, cell growth, cell proliferation, cell adhesion by secreting many of the matrix components and growth factors.Hence,it is possible that LPS could directly act on the mesenchymal fibroblasts thereby leading to the cascade of immunobiochemical reactions resulting in the alteration in the intestinal physiology by affecting the mucosal epithelial and submucosal cells during mucosal infection by these bacteria.However , no study was done to understand the biological action of LPS of Salmonella and E.coli on fibroblast of human small intestine. Hence, the present study was aimed to reveal the molecular and cellular events involved in LPS action on this vital cell population of the small intestine.

Work carried out:

Human small intestinal lamina propria fibroblasts were cultured from 40 human foetuses of 18-20 weeks old. Lamina propia mesenchymal cells were cultured from the small intestine by careful microdissection which

removes the villi and keep the underlying fibroblast intact. The cells were cultured by both explant method and dissociation method , but successful culturing of cells was obtained by explant method. The cells thus obtained was extensively characterised for their origin by immunocytochemical staining and morphology.Presence of vimentin and absence of desmin, cytokeratin, myosin confirmed that the cells were of mesenchymal origin. The cells were studied for ECM production by indirect immunofluoresence and immunoblotting. The cells thus obtained could be subcultured upto passage 20.Cryopreservation and revival was done successfully.

LPS was purified according to the method of Westphal and Jann and further purified from the pathogenic strain of E.coli and Salmonella. Total carbohydrates, glucose, heptose, mannose galactose and octulosonic acid sugar estimation was done. Endotoxin activity was done by limulus amoebocyte lysate kit. Extracted LPS was electrophoretically separated on 8% SDS-PAGE and silver stained.

The cultured intestinal fibroblast were exposed to different concentration of LPS (10ng-10ug/ ml) in DMEM containing 1% FCS. The growth pattern was assessed by colorometric MTT assay. "S" Phase analysis was done by BrDu incorporation studies and ^{3}(H)-Thymidine uptake.LPS was found to increase cell proliferation in dose and time dependent manner.

Whether LPS could change the cytoskeletal pattern of intestinal fibroblasts,study was done over a period of time . LPS was found to induce margination of actin filaments, bundling of vimentin filaments and relocalisation of vinculin plaques. There was no effect on the b-tubulin network.

Cytokine profile of intestinal fibroblasts in response to LPS was studied by ELISA and immunoblot. It was found that IL-1, IL-6, IL-8, TNF-alpha was produced by intestinal fibroblasts in response to LPS. To study the regulation of cytokine gene expression , the total RNA from the treated and untreated cells were extracted at different time intervals. Further experiments are in progress. Hence, LPS increased cell proliferation, relocalised cytoskeletal components and induced the production of certain cytokines.Production of cytokines by fibroblast may have certain immunomodulatory action on the lymphoid cell population in lamina propria, which leads to progression of the disease.

Publications / patents / conferences:

Presented part of the research work at the Indian Association of Medical Microbiologists (IAMM) 1994, held at Armed Force Medical College,Pune.

Presented part of the research work at Society for Biological Chemistry(SBC) held at CDRI, Lucknow.

Presented part of the work at the Indian Association of Biomedical Scientist (IABMS) conference, and received Gold medal for the **best paper presentation (Dipshikha Chakravortty)**

Selected part of the research work by the Immunity to the Mucosal Infections, European Science Foundation, 1995

Selected part of the research work by the International Endotoxin Society for the 4th International Congress for Endotoxin Society to be held in Nagoya,Oct 22-25, 1996, Japan, and accepted in the *Journal of Endotoxin Research* (American society for Immuologists),USA

TITLE: Establishment and characterization of breast cancer cell lines from high risk ethnic groups

Investigators: **J. M. Chiplonkar**
Prof. A. N. Therwath, University of Paris.

Background:

Cancer of the breast is one of the commnest malignancy in women. In India, the Parsi community has a higher incidence of breast cancer. Carcinogenesis is a multistep process. It is important to understand the process of carcinogenesis. Establishing a breast epithelial cell line from tumors of high risk groups would prove to be of great value for such kind of studies.

Work carried out:

Human breast epithelial cells, both normal and malignant, are difficult to grow in vitro over extended periods of time. Experimental work was continued to set primary cultures of breast epithelial cells. Breast biopsy specimens were obtained from twenty-two Parsi patients from Breach Candy hospital, Bombay and twenty eight non-Parsi patients from Jahangir Nursing Home, Pune. Protocols standardized earlier were used for setting the primary cultures. Total thirty primary cultures were obtained. Both epithelial and fibroblast cells were present in these cultures. Of these cultures nine could be subcultured. Efforts were made to eliminate fibroblasts from these cultures by selective trypsinization, controlled EDTA treatment and use of Geneticin. The removed fibroblasts were grown separately and maintained in the culture. Standardization of optimal culture conditions for cultivation amd maintainence of the breast epithelial cells allowed two 'lines' to be maintained upto 12 passages.

Repeated attempts to immortalize the epithelial cultures failed to produce desired results. Extensive immunocytochemical characterization of these cultures was carried out. These cells expressed cytokeratin peptide 8 and cytokeratin peptide 18, which are typical of human breast epithelium. Fibroblasts from these cultures stained positive for cytokeratin peptide 19.

Publications / patents / conferences: Nil.

TITLE : Growth characteristics of normal and abnormal melanocytes and their response to synthetic and natural growth factors.

Investigators: **Dr. Manoj Mojamdar NCCS, Pune.**
Dr. Y. Tawade, K E MHospital, Pune.

Background:

Melanocytes unlike fibroblasts are not autonomous cells when grown in vitro. They need mitogens like pherbol myristute acetate (TPA) a potent tumour promotor to proliferate in vitro. The use of such tumour promotors have restricted the use of cultured meloanocytes as "cell transplants" for treatment of vitiligo cases. Therefore a search was initiated to grow melanovytes in human promotor free media using synthetic and natural growth factors.

Pigmentary disorders such as vitiligo, melanoma, melasma, nevi, prebaldism are the major disorders involving melanocytes. Proliferation and differentiation, in India the incidence of vitiligo is believed to be varying from 1-4%. All the currently available treatment modalities for the disease is not at all satisfactory. Hence a new approach 'Transplantation of cultured melanocytes' and banking these melanocytes cultured in natural mitogens has been initiated.

Work carried out:

1. Identify natural mitogens and differentiation agents from fibroblasts and keratinocytes that regulate melanocyte proliferation and differentiation in vivo and use them for culturing melanocytes in vitro.
2. Standardize methods for ' melanocyte cell transformation ' in vitiligo.

Melanocytes were grown in dermal equivalents to understand how fibroblasts requlate melanocyte proliferation and differentiation. Melanocytes co-cultured with keratinocytes were used for grafting. Assay systems for melanogenic enzymes such as tyrosinase, dopachrome tautomerase were standardized using B-16 melanoma cells.

Culture media supplemented with bFGF, cyclic AMP etc has been found to be allowing the culture and proliferation of pure melanocytes. Such

cultured melanocytes have been grafted on to dermabaded vitiligo patches in six patients. Refragmentation is monitered every month. The dermabasion followed by layering of cultured melanocytes into these area needs the immobilization of the patients for 4-8 hr. for optimum attachement of these cells to wound bed. Efforts to improve culture conditions and standardization of grafting techniques are in progress.

Publications / patents / conferences:

Pure melanocyte cultures can be marketed for treating vitiligo, burns cases etc. Clinical trials are in progress.

TITLE: Regulation of skin pigmentation (A) Regulation of pigment transfer (B) Regulation of melanogenesis

3.Investigators: (a) **Dr. Manoj Mojamdar,**
Dr. G. Raman, H L R C, Bombay.
(b) **Dr. Manoj Mojamdar**
Dr.Prof. S.B.Padhye Chemistry Dept.,
Pune University.

4. Background :

Skin pigmentation is a prominant social marker. For normal skin pigmentation to take place a co-ordinated cascade of events have to function. These include melanin synthesis inside the melanocyte and its transfer to keratinocytes.

Pigmentary disorders are cosmatically disfiguring disease. Scientific understanding of the normal biochemistry and physiology of skin pigmentation is a prerequisite to understand the process of its impairment leading to pigmentory disorders.

Work carried out:

1. Purification of melanogenic enzymes and investigation of their properties.
2. To investigate the effect of some plant compounds that are known to be useful for pigmentation.

Purification of tyrosinase, dopachrome tautomerase and protein kinase C:

10_5 B-16 melanoma cells were inoculated subcutaneously in C53BL mice. Two weaks later the mice were sacrifised and the tumoures were homogenized. A new methode for purification of tyrosinase and dopachrome tautomerase from the same homogenate has been developed. Approximately 10mg protein of pure tyrosinase and dopachrome tautomerase has been till now purified and is being used for developing monoclonal antibodies against these enzymes.

Effect of Lawsone on melanogenesis:

Lawsone, a quinone isolated from Mehndi (Heena) plant has been widely used for cosmetic purposes. Sodium lawsone was tested for its action on cell free tyrosinase and for its action on B-16 melanoma cells. Na- lawsone

has been found to increase the initial binding of the substrate dopa to tyrosinase. Its effect on the other substrate tyrosine is being tested. On cells however, Na-lawsone depleted the tyrosinase activity and induced a melanogenesis. Azo-lawsone a form of lawsone dye is being developed as a specific staining agent for localizing tyrosinases in cells.

A model to study the process of melanogenesis initiation:

B-16 melanoma cells when grown in medium containing NBCS instead of FCS gradually lose their ability to make melanin. A gradual loss of tyrosinase activity without any difference in dofachrome tantomerase activity has been found in these cells adapted to NBCS. None of the known melanogenesis inducers with the exception of MSH has been found to initiate melanogenesis is these NBCS adapted cells. This system is being further developed as a mmodel system to investigate the sequential steps involved in the initiation of melanogenesis is pigment cells.

Publications / patents / conferences:

Lawsone affectts mammalian and plant tyrosinase diffrentially. Manuscript in preparation.

TITLE: In vitro cultivation of erythrocytic stages of Plasmodium vivax and large scale cultivation of Plasmodium falciparum

Investigator : Prakash Deshpande

Aims of the project :

i). Immunological studies of malaria pigment -haemozoin.

ii). Antimalarial screening

Background:

Intraerythrocytic malaria parasite uses haemoglobulin as major nutrient source. Proteolysis of haemglobilin releases haem,which when soluble is highly toxic to biological membranes.As malaria parasite lacks haem oxygenase,they are unable to cleave haem in to open -chain tetrapyrole and it is not excreated from cell, insteated haem is detoxified by convertion in to haemozoin pigment, a unique to the malaria organism.Haemozoin is released along with the merozoites when infected erythrocyes brust. Haemozoin has been implicated in drug resistence ,blood stage associated pathology and impairment of immune function.

Work carried out :

A) Monoclonal antibodies aganist haemozoin pigment:

Haemozoin pigment was partially purified from laboratory cultures of *Plasmodium falciparum* (SOHS and FAN 5HS strains) by Goldie et al. (1990) method. (Am.J.Trop.Med.Hyg.1990,43 (6)).The final pellet was resuspended in 5 ml TMK buffer (50 mM Tris-Hcl,0.025 M Kcl, 5 mM $MgCl_2$ pH 7.5).mixed by sonication and layered on 50% w /v sucrose solution in TMK buffer and spun at 100 000 x g for 45 min. The resultant pellet was washed 3 times with PBS and stored at -20 C in PBS with 1 mM EDTA Protein concentration of haemozoin has been determined by Bradford method.Blab / C mice were immunized with the pigment and positive polyclonal sera raised. Hybrides secreeting positive antibodies were produced by hybridoma technique.

Hybides were obtained 96 of 96 wells. Total 20 vials were cryopreserved (ELISA readings in OD) : Normal mouse:0.282;

Immunized mice :0.467 & 0.476.Positive hybrid supernatant

(F30/75 - 0.550, F30/50 - 0.329).Con.supernantant (0.150).

The aim for producing monoclonal antibodies is to use its supernatant for detection of haemozoin antibodies in malaria patients sera.

B) Detection of haemozoin antibodies in patients sera.

Partially purified haemozoin pigment from *P.falciparum* cultures used as antigen. Haemozoin is a pariculate matter.The soluble fraction of haemozoin in 1% SDS and 0.5% Sodium deoxycholate were also used as antigen. Blood samples (*P.falciparum*, *P.vivax*,mixed and endemic normals) were collected from Paud PHC,pune.The ELISA results were shown in table 1,2,& 3. The test has not shown antibodies to haemozoin pigment.

C) Dot ELISA : Detection of haemozoin pigment in malaria patients blood was also tried by dot -ELISA. Immunized mice polyclonanl sera was used for the detection of the pigment. About 7 *P.falciparum* blood samples,PRBCs of 2 strains / isolates (FAN 5HS & MP 14) and normal RBCs were sued in the assay. No positive results were observed.

D) Haemozoin toxicity to hepatoma cell line.

It has been reported the discoloration of the liver, spleen and brain in patients sufferig from malaria.whether the malaria pigment induces any effect on the liver.To assertain this, the effect of the pigment was studied on the Gep G2 cell line. The assessment of toxicity was dtermined by colony formation assay (Bassi et al., 1993 ; ALTA , 21 , 65 - 72. Apart from haemozoin,FAN 5 HS and NFTC 2 PRBCs extract (2% parasites), PRBCs supernantat and 0+ N RBCs extracts were tested (table 4). The haemozoin test was contaminated so its toxicity was indirectly performed using PRBC extract.There is no effect either of P RBC extract or parasite culture supernantat on the cell line.

E) Nitric Oxide production :

Nitric oxide has been implicated in the pathology of cerebral malaria.Human macrophages were islated and grown on coverslips. The macrophages were stimulated with haemozoin pigment, PRBCs extract and NRBCs extract. Nitric oxide producton was measured by Ding et al. method using Griess reagent (1% sulfanilamide, 0.1% napthyletylene

diamine dihydrocholoride, 2.5 % H_3PO_4.

No significant production of NO was observed with any stimulant (table 5).

F) There is a difference of opinion regarding the formation of malaria pigment and its associated proteins. Haemozoin derived from different strains and fractions (pellet and supernatant) were tested by ELISA for their silmilarity using normal and immunized mouse sera (The mouse was immunized with haemozoin pigment (separated from SO HS and FAN 5 HS isolate cultures).Results were depected in table 6. Significant O.D.was fond with FAN 5HS + SO HS (P), FAN 5HS +SO HS (SDS),NFTC 2 (P), FAN 5HS (P),FAN 5HS + MP 14(S) and FAN 5HS + MP 14 (P). However, The parasite extract of new isolate NFTC 2 has given very high O.D. (supernatant = s, pellet =p).

G) The antimalarial efficacy of 5 synthetic compounds were determined by (^{3}H) ethanol amine incorporation against FAN 5HS and MP 14 isolates. Five synthetic cmpounds synthesied by Dr.Padhy of Chemistry Department were tested for antimalarial activity.Compounds A5 and A6 were found effective. Results were depected in table 6.

H) Establishment of gametocyte forming P.falciparum culture:

Twelve blood samples were collected from malaria patients durung the out break of malaria in Dauaze village,Paud thesil,Pune.Six blood samples infected with P.falciparum were used for culturing. The culture of NFTC/ 2 isolate culture was successfuly established. The isolate showed very high gametocyte formation (>10% of parasitemia) at P- 10. However,the isolate lost its gametocyte forming character during 14 passage. One vial was cryopreserved.

I) Antimalarial activity of purified immunoglobulins,sera and exoantigen: Six samples (3 purified immunoglobulins,2sera and one exoantigen of P.falciparum) were tested for antimalarial activity using H3 ehanolamine incorporation. The samples were prepared by Dr.B.Ravindran, RMC (ICMR), Bhubaneshwer.

The results were depected in table 7.

Publications / patents / conferences:

Global meet on parasitic diseases, 18-22 March,1996, New Delhi. An abstract entitled "Malaria epedimic in Paud thesil,Pune,Maharashtra" was accepted for poster persentation.

Table 1. Detection of hemozoin antibodies in malarial patients sera by particulate antigen :

	sample	*no.of samples*	*O.D.range*
1.	normal control	(3)	0.195 - 0.232
2.	endemic normal	(2)	0.216 - 0.231
3.	*P.falciparum*	(4)	0.179 - 0.310
4.	*P.vivax*	(11)	0.216 - 0.232
5.	mixed	(1)	0.436

antigen : 5 ug /ml, conjugate :Ig (GAN) I:750 ,sustrate :ABTS sera : 1:100, blocking 1% BSA.

Table 2. Detection of haemozoin antibodies in malaria patients sera by soluble fraction of haemozoin in SDS and Deoxycholate

	sample	*no.samples*	*O.D.range*	
			SDS (1 %)	*Na Deoxycholate*
1.	normal control	(4)	0.075 - 0.116	0.087 - 0.111
2.	endemic normal	(2)	0.091 - 0.102	0.065 - 0.082
3.	*P.falciparum*	(5)	0.089 - 0.129	0.082 - 0.113
4.	*P.vivax*	(4)	0.073 - 0.151	0.062 - 0.127
5.	mix	(1)	0.145	0.126

antigen : 5 ug/ml, conjugate : 1.750, substrate : ABTS, serum : 1:100, blocking 1 % BSA.

Table 3. Immunogenicity of haemozoin fractions / strains to polyclonal mouse sera (haemozoin)

	strain	*Fraction*	*O.D. range*	
			normal sera	*immune sera*
1.	FAN 5HS +OS HS	(P)	0.612 - 0.114	1.220
2.	FAN 5HS +OS HS	(sds)	0.096 - 0.100	0.758 - 0.666
3.	FAN 5 HS+SO HS (sup,sds)		0.040 - 0.490	0.776 - 0.038
4.	NFTC 2	(P)	0.408 - 0.540	0.961 - 0.836
5.	NFTC 2	(S)	0.024 - 0.528	0.726 - 0.785
6.	FAN 5HS	(P)	0.401 - 0.545	0.867 - 0.769
7.	FAN 5HS	(S)	0.410 - 0.558	0.835 - 0.678
8.	FAN 5HS+MP14	(S)	0.390 - 0.393	0.818 - 0.8I8
9.	FAN 5HS+MP14	(P)	0.337 - 0.445	0.879 - 0.835
10.	NFTC 2 PRBC	extract	0.465 - 0.489	1.387 - 0.945

antigen :5 ug / ml, sera:1:100, conjugate : ant mouse 1;750 substrate :ABTS, blocking 1 % BSA.

Table 4. Effect of PRBC extract/supernatant to Hep G2 Cell line

	sample	*no.colonies*
		X = SD
1.	control	290.33 = 8.862
2.	NRBC extract	293.16 = 14.67
3.	haemozoin 50 ul /ml	- contaminated-
4.	haemozoin 10 ul /ml	
5.	FAN 5HS PRBC extract 50 ul/ml	273.77 = 13.50
6.	NFTC 2 PRBC extract 50 ul/ml	254.33 = 12.50
7.	PRBC supernantant 50 ul/ml	287.00

Table 5. NO production in human nacrophages stimulated with haemozoin / PRBC extract and supernantant.

sample	*O.D. range*
1. negative control	0.083 - 0.134
2. std.control (1mM NH_4Cl)	0.133 - 0.228
3. NFTC 2 RBC extract 25 ul/ml	0.130 - 0.173
4. NFTC 2 RBC extract 50 ul/ml	0.098 - 0.189
5. NFTC 2 antigen 50 ul/ml	0.191 - 0.229
6. haemozoin 50 ul/ml	0.114 - 0.164
7. FAN 5HS extract 20 ul/ml	0.126 - 0.131
8. FAN 5HS supernatant 50 ul/ml	0.116 - 0.147
9. NRBC extract 10 ul/ml	0.193 - 0.236

Table 6. Antimalarial effect of new synthetic compounds aganist P.Falciparum (FAN 5 HS isolate)

concentration (in nM)	*compounds*					
	A6	*A1*	*A2*	*A3*	*A5*	*CHO*
	per cent inhibition					
1. 500	78.66	41.90	47.93	57.42	72.27	67.14
2. 250	37.82	00.00	00.89	00.00	41.26	38.78
3. 125	22.07	00.00	00.00	00.00	28.09	10.39
4. 62.5	15.49	00.00	00.00	00.00	16.28	00.93
5. 31.25	07.15	00.00	00.00	00.00	16.50	00.00

Table 7. Gametocyte formation in NFTC /2 isolate

day	*ring*	*trophozoiite*	*schizonts*	*gametocytes*	*total*
0	0.53	1.20	0.13	0.40	2.26
	(23.45)	(53.09)	(5.75)	(17.69)	
1	0.65	1.99	0.20	0.46	2.50
	(26.00)	(47.60)	(8.00)	(18.40)	
2	0.52	2.46	0.17	0.36	3.51
	(14.81)	(70.08)	(4.84)	(12.25)	
3	0.42	2.52	0.27	0.36	3.51
	(11.76)	(70.58)	(7.56)	(10.08)	
4	0.57	2.63	0.17	0.49	3.86
	(14.76)	(68.13)	(4.40)	(12.69)	
5	0.49	2.51	0.32	0.52	3.84
	(12.76)	(65.36)	(8.33)	(13.54)	
6	0.62	2.42	0.27	0.61	3.92
	(15.81)	(61.73)	(6.88)	(15.56)	

Figures in parenthesis are percentage values.

DEVELOPMENT OF TISSUE BANK TECHNOLOGY

Culture of human skin keratinocytes and their 3-D epitheliation for transplantation to burns, non-healing ulcers and vitiligo cases.

Studies on cryopreservation and revival of haematopoietiv cells

DEVELOPMENT OF TISSUE BANK TECHNOLOGY

TITLE : CULTURE OF HUMAN SKIN KERATINOCYTES AND THEIR 3-D EPITHELIATION FOR TRANSPLANTATION TO BURNS, NONHEALING ULCERS AND VITILIGO CASES.

Investigator: Manoj Mojamdar.

BACKGROUND :

Current modalities of treatment of burns are not satisfactory.The skin performs two mejor functions,

(i) to prevent water loss and

(ii) to prevent infections. Presently the methods used to cover burn wounds can carry out either one of the functions and hence there is a need to develop system that can do both the functions at least temperorily, till the wound heals.

The incidence of burns, either chemical, electrical or fire is increasing. While, upto 20% burns are handled well by burns wards. Persons with more than 20% burns die either due to water loss or due to septicimia. Bioartifical skin is therefore the most viable alternative that bneeds to be developed.

TARGETS DEFINED AND WORK CARRIED OUT:

1. Culture of normal human keratinocytes and investigating special growth supplements needed for this fastidious cell.
2. Induction of in vitro epitheliation.
3. Development of actual grafting and transport techniques.

Human skin biopsy obtained from patients have been successfully cultured, 3-D epithelia has been prepared and have been grafted with good results in burns, nevi and vitiligo cases. Several cell lines have been tested for their capacities to function as " feeder layers" for growth of human skin keratinocytes. During the case of these studies we have observed that lot of work remains to be done on immobilization of the cultured skin so as to enable us to transport the eipthelia and graft it on to burns cases.

In order to immobilize human skin keratinocytes and the preparation of skin equivalents lattice matrix made of collagen - chitosan, fellose -

chitosan, fellose - agarose etc were prepared. Fibroblasts was found to grow well as these matrix. Attempts to grow human skin keratinocytes are currently being made.

1. Extensive clinical trials of cultured eipthelia for treatment of burns in collaboration with Sion Hospital. Bombay.
2. Preparation of bio-matrix for immobilization of human keratinocytes.

Publications / patents / conferences :

(a) Intrmediate split thickness foetal skin organ cultures for treatment of vitiligo. Manuscript in preparation.

(b) Two products are bio-artificial matrix and cultured epithelia for burns treatment can be marketed.

Title : Studies on cryopreservation and revival of Haematopoietic cells.

Investigators : Mrs. Lalita Limaye
Dr.S.G.A. Rao, Stem cell biology Division, C.R.I,Bombay.
Dr.R.L.Marathe,Head of Haematology div., Jahangir Hospital Pune.

Background:

Freezing is now of common practice to store cells ,embryos and human tissues to use them at appropriate time for transplantation. Cryoprotectants such as DMSO and Glycerol together with serum are used to reduce deleterious crystal formation from free water.The use of various freezing devices allows a better control of the cooling rate and protects the cells from critical freezing stages. Bone marrow contains various cell types, at different stages of maturation and some of them are especially important during transplantation. One major problem of preservation is the damage to the cells particularly due to phase transitions that take place during freezing and thawing. This results in a considerable cell loss. It is therefore important to make attempts to get optimum recovery of cells and to study which cell population is more affected during cryopreservation.

High dose marrow ablative radio-chemotherapy regimens are a major strategy for the cure of chemotherapy-sensitive cancer for patients who cannot be cured with lower more conventional doses. Intensive chemotherapy supported by autologous marrow haematopoietic stem cells (HSC) that were previously collected and cryopreserved can salvage a significant number of patients with relapsed acute leukemia, non Hodgkin's lymphoma, Hodgkin's disease and many solid tumors. Perhaps the most significant advance in supporting patients receiving intensive chemotherapy has been the consistent ability to collect and store haematopoietic stem cells. The development of cryobiological methods for the safe preservation and storage of bone marrow provides a technological foundation for the wide spread clinical application of autologous HSC transplantation. Long term cryopreservation allows prophylactic storage for patients to be transplanted months to years later and for high risk population like people working in nuclear reactor plants and people with family history of leukemia.

The present project is undertaken for standardization and transfer of technology of bone marrow preservation. The outcome of autologous transplantation depends on many variables, especially on the recovery of adequate haematopoietic stem cells for haematopoietic reconstitution. Besides technology standardization the research aspect dealt with in the project include improvement of recovery in terms of viability and functionality of cells post cryopreservation by using additives in the freezing mixture. Attempts to replace sera have also been initiated.

Work carried out:

Initial time was utilised for standerdisation of various protocols like:collection and processing of samples, separation of haematopoietic cells, CFU assays, cryopreservation and revival. Later on these studies were carried out with a larger number of samples so as to get reproducible results for every parameter.Due to limitation of human sample size preliminary experiments are done on Mouse bone marrow.Those experiments which have given encouraging results are then performed on human bone marrow.

So far we have successfully cryopreserved and revived a total of 58 human bone marrow(F1-F58) and 45 mousebone marrow (MBM1-MBM45) and 46 fetal liver haematopoietic cells (FLI1-FLI46). The parameters used for testing the efficacy of preservation include : 1 Viability by dye exclusion and MTT 2 Nucleated cell recovery 3 Progenitor cell assays such as CFUGM,GEMM,BFU(E)CFU(E), CFU(Mix) etc. 4 %CD34cells pre and post cryopreservation 5Leishman Giemsa staining and morphological identification of cells .Certain cell lines like HL60, KG-1a and KG-1 cell line have been taken as a model of haematopoietic cells and some experimental studies are performed on the same.

1. Additives to DMSO in the freezing solution :

   a) Antioxidants: One proposed mechanism of injury during chilling is the formation of oxygen free radicals .We examined the role of antioxidants in reduction of cell damage during low temperature storage of bone marrow .Hydrophilic free radical scavengers (Catalase, Ascorbic acid, reduced Glutathione and Superoxide dismutase) and hydrophobic free radical scavenger (a Tocopheryl acetate)were used

for cryopreservation of bone marrow in combination with 10% DMSO in the freezing solution .It was found that catalase, ascorbic acid and atocopheryl acetate improved the post thaw recovery particularly in terms of early and late (CFU-GEMM and GM) progenitors. Experiments are being undertaken to study whether reduced growth factor responsiveness of cryopreserved cells is restored in presence of antioxidants.

b) Membrane stabilizers:Lipids ,sugars and amino acids exert their cryoprotective effect atleast partly by stabilizing the plasma membrane. Trehalose is a natural sugarknown to perform this function. We have used this compound and got improved recovery of HSC. (Fig. 1)

c) Antifreeze proteins and antifreeze glycoproteins are synthesized in the body fluids of certain polar fishes. They prevent recrystallization of ice during thawing and exert their protective effect. They adsorb to specific faces of small ice crystals and prevent their further growth, thus preventing recrystallization.

2. Immunophenotyping : In addition to CD 34+ cells, analysis of CD 15+, CD 11C,CD 16+, CD 45+ and CD 33+ cells, pre and post cryopreservation was done on a few samples using FACS. (Fig 2) This analysis was done in PGIMER in Dr. Ganguley's laboratory. The immunophenotyping on FACS requires further standardization.

3. Morphological identification of cell types: In addition to Leishman Giemsa staining, Wright's staining method has been standardized and is being used routinely for staining and identification of colonies (Fig.3)

4. Pre and post stimulation of cells by cytokines: Some important receptors may be shed or internalized during cryopreservation. Literature, shows that engraftment is faster in invitro lymphokine treated revived cells by culturing in presence of lymphokines like IL-3, GM-CSF or a mixture of both. These experiments have been initiated and preliminary results are encouraging.

5. Replacement of sera in the freezing mixture by low viscosity methyl cellulose: Here we have tried to replace serum by low viscosity Methyl cellulose (15, 25, 400 and 1500 centipoises). These experiments are recently initiated and are under progress.

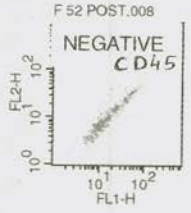
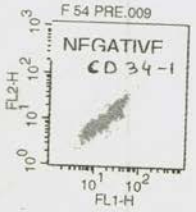
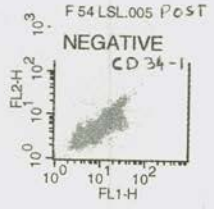
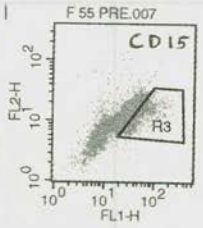
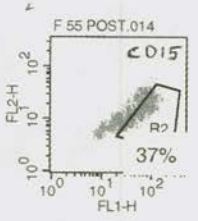
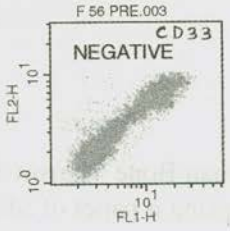
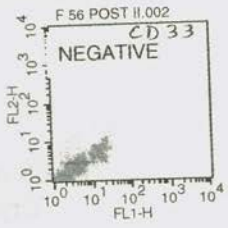
7. Cryopreservation of Haematopoietic cells: HL-60, KG1 and KG-1a cell lines are used as a model of haematopoietic cells. the cell recovery and viability (by MTT dye exclusion) of thawed cells that were cryopreserved with or without additives to DMSO in the freezing mixture was studied. Results of one experiment are shown in fig. 4.

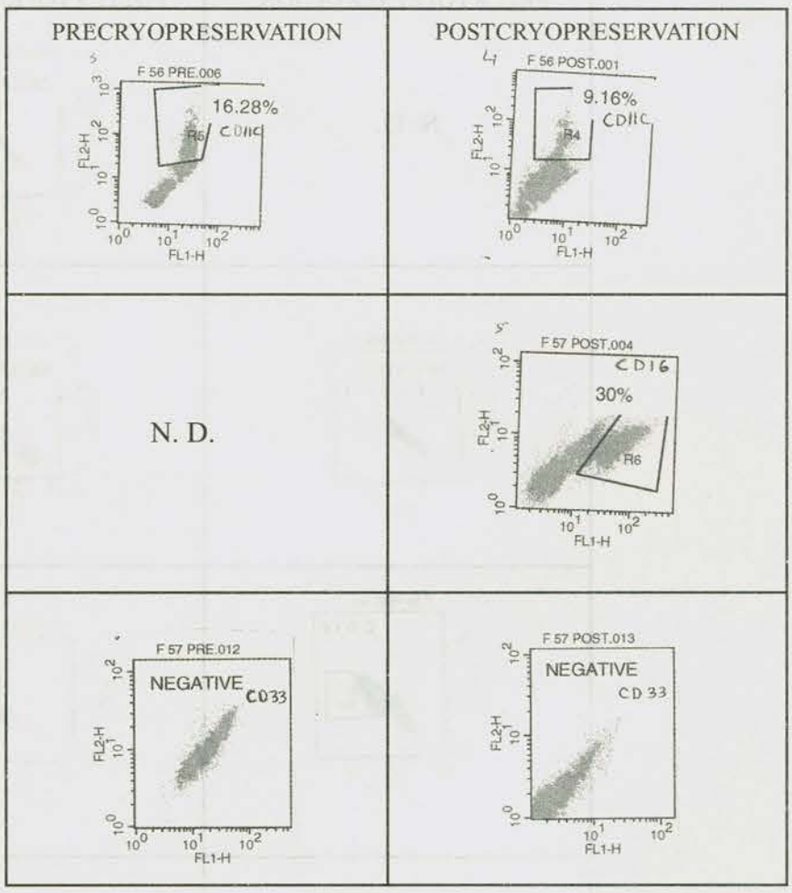
Publications / patents / conferences:

(a) Bone marrow cryopreservation: improved recovery due to bio-anti-oxidant additives in the freezing solution. Communicated to Cryobiology.

(b) Trehalose as a useful additive in the freezing mixture for cryopreservation of bone marrow cells. Manuscript in preparation.

(c) Anti-freeze proteins and anti-freeze glycoproteins for cryopreservation of bone marrow cells. Manuscript in preparation.

PRECRYOPRESERVATION	POSTCRYOPRESERVATION
N. D.	
	
	
	



Figure

FACS Analysis of Human Bone Marrow Samples Before and After Cryopreservation using a panel of Monoclonal Antibodies.

INDIGENOUS TECHNOLOGY AND RENDERING EXPERT SERVICES

Identification and characterization of protective antigens in lymphatic filariasis

INDIGENOUS TECHNOLOGY AND RENDERING EXPERT SERVICES

TITLE: Identification and characterization of protective antigens in lymphatic filariasis

Investigator: **P. B. Parab**

Aims:

1) To identify and characterize protective and diagnostic antigens in lymphatic filariasis.

Background:

Lymphatic filariasis is most important vector borne disease prevalent in tropical and sub tropical regions of the world affecting nearly 100 million people. In India, Wuchereria bacrofti and Brugia malayi nematodal infections are causative agents of this disease. Owing to difficulties in obtaining human filarial parasites and lack of animal model we have tried to analyse Setaria digitata (Bovine filaria) antigens which could be useful for diagnosis and prophylaxis in lymphatic filariasis.

Work carried out:

i) Analysis of Setaria digitata adult worm antigens cross-reactive to human filarial serum.

Our earlier studies showed definite cross reactivity between Setaria digitata antigens and infected human sera. However, there was no distinction between various groups, such as endemic normals, microfilaraemics and clinical filarial sera was obtained. Recently it was reported that measurement of IgG4 antibodies to filarial antigen resulted in improved diagnostic specification in lymphatic filariasis due to W. bacrofti infection. Thus now a differential diagnosis based on the presence of specific IgG4 subclass in filarial sera is possible. We have therefore analyzed total Ig and IgG4 subclass antibodies in sera of lymphatic filariasis reactive to soluble adult Setaria digitata (Bovine filarial worm) antigen using enzyme linked immunoassay. Setaria digitata reactive IgG4 antibody levels were higher in themicrofilaraemics as compared to the endemic normals and elephantiasis sera. Frequency of a positive reaction in themicrofilarawemics was 93.3 % and was much higher than the endemic normals and elephantiasis sera. Fifty sera from endemic area and ten from non-endemic area were tested in ELISA. IgG4 antibodies in microfilaraemics recognized

5 distinct antigens of setaria digitata amongst them 40 KDa and 28 KDa antigens found to be very specific as evident by immunoblot studies. Endemic normal sera and sera from chronic filarial disease did not show any rectivity to these antigens. Thus determination of filaria specific IgG4 levels would facilitate the detection of active infection (microfilaraemia) in endemic area of filariasis.

ii.. Immunoscreening of B. malayi L3, cDNA expression library with rabbit antibodies to w. bacrofti infective larvae.

B. malayi L3, cDNA library was immunoscreened with rabbit antibodies to w. bancrofti L3 to clone antigens that are expressed by infective filarial larvae - Ten clones were selected and plaque purified. PCR analysis was done to determine insert sizes (ranges 1.3 to 3.5 kb) and five clones were selected for subcloning and partial sequence analysis. Preliminary results showed that 2 of the clones were full length Brugia paramyosin and one clone each of myosin and tropomyosin. One clone found to have similarity to C. elegans Calmodulin like gene. Further studies are in progress. These studies were carried out at the Infectious Diseases Division of the Jewish Hospital at Washington University Medical Centre, St. Louis, MO, USA.

2. Development of monoclonal antibodies to co-stimulatory molecules present on the surface of activated B cells and on macrophages.

Monoclonal antibodies against costimulatory molecules on antigen presenting cells wpould facilitate the studies on regulation of immune responses. Attempts are being made to immunize hamsters with purified preparations of M150, B1, B2 and B3 antigens. Heterohybridoma were produced against B3 and M150 antigens. Against B3 antigen 93 hybrids were produced 5 hybrids were cloned. Further 3 clones were tested by ELISA and Immunoblot. One clone 34.51.69 showed specific activity agaist 40 kDa B3 antigen. Against M KKDa antigen total 17 hybrids were produced, 2 hybrids were further cloned and 3 clones were tested by ELISA assay. Further characterization of these clones are underway.

Publications / patents / conferences:

(a) Detection of Setaria digitata specific IgG4 subclass antibodies in lymphatic filarial sera. Global meet on Parasitic Diseases, sponsored by the Indian Society of Parasitologists, New Delhi, 18-22 March, 1996.

(b) Efforts are in progress to collaborate with industries for the development of a diganostic kit for filaria.

BIOMEDICAL AND BIOTECHNOLOGICAL APPLICATIONS

Stromal cell biology: Definition of factors triggering cell-cell interaction in the haematopoietic cells

Identification of melanoma oncogene(s) and characterization of the oncogene protein product.

Use of mitochondrial DNA restriction fragment length polymorphism for characterization of mosquito cell lines.

Identification of factors in supernatants from stimulated lymphocytes on growth and diffrentiation of Neuroblastoma cells

Studies on the interaction of differentiated neuroblastoma cells with extracellular matrix components

Investigation on beta cell protective mechanisms as means of reducing incidence of diabetes.

Foetal liver infusion: Its mode of action, efficacy of cryopreserved cells and its potential application in the management of cancer.

Regeneration of pancreatic beta cells

BIOMEDICAL AND BIOTECHNOLOGICAL APPLICATIONS

TITLE: Stromal cell Biology : Definition of factors triggering cell-cell interactions in the hematopoietic cells

Investigators: **Dr.(Mrs) V.P.Kale**
Dr.L.C.Padhy, TIFR, Bombay

Background :

Blood cell formation or haemopoiesis is a complex process where the multipotent stem cells present in few numbers, ordinarily resident in the bonemarrow micro environment, orchestrate a complex sequence of events in co-operation with a large number of soluble cytokines/growth factors. 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.

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

Several experiments have documented the importance of hemopoietic environment consisting mainly of bone marrow stroma. The bonemarrow micro environment is defined by the stromal cells and the ECM component surrounding them. In long term bone marrow culture experiments, it has clearly been shown that bone marrow derived stromal cells play a crucial role in colony formation. Several experiments have underscored the need for cell-cell interactions that allow effective interaction or cross-talk between the differentiating hemopoietic cells and the supportive stromal cells. In addition, several cytokines,interleukins, growth factors, such as c-kit ligand (SCF), TGF, TNF etc. are required to convey specific signals to the differentiating progenitor cells at various stages of differentiation process. The role of various cytokines, interleukins etc. in this process is reasonably understood to allow designing of more precise experiments.

A line of our work is related to the still poorly understood problem : "What determines or triggers cell-cell interactions in the hemopoietic cells?" This undoubtedly is a central issue, because if the deterministic factors modulating specific cell to cell contact could be identified, it may pave the way to modulate haemopoiesis in a pre-determined manner, at least, in

cell cultures. Secondly, this understanding may aid in the identification of defective steps in a human disease process.

Work Carried out:

We reported that bone-marrow mononuclear cells released soluble factors (such as TGF/FGF) in the medium in response to crythropoietin. These factors and some membrane bound receptors (such as fibronectin receptors a5b1 integrin) were shown to be involved in a specific type of cell-cell interaction involving tthe bone-marrow stromal cells. We also found that the treatment of stromal cell with active conditioned medium lead to enchanced colony formation.

Marrow stromal cells are known to be heterogenous in nature consisting of cells of at least seven differentiation types viz. fibroblasts, smooth-muscle, adipocytes, endothelial adventitial reticular, macrophage and osteoblasts. We attempted to determine whether the cytoadhesive effect was uniform across all these cell types or was restricted to some subset. As a first step to resolve this issue, we used a panel of antibodies to make phenotypic characterization of these cells, both before and after exposing the cells to conditioned medium as well as to TGFB.

The stromal cells did not show positive staining with oil red O, Macl as well as pancytokeratin which are cell specific markers for adipocytic, macrophage, and epithelial cell differentian respectively, both before or after treatment. Staining of cells with a monoclonal antibody against chondrolin sulphate showed a diffuse pattern all over the cells. The staining pattern did not change after treatment with CM or B1. A characteristic pattern was observed when the cells were stained for FSP1, a fibroblast specific marker (sigma). The stromal cells were intensely positive for FSP1 indicating their fibroblastoid nature. The staining pattern, however, showed a drastic change after CM/B, treatment. The fluorescence initially became restricted to cellular margins and after longer incubations most of the cells become negative (Fig).

Antibodies to VIII and VWF were used as markers for endothelial cells. The cells before treatment did not show positively for these markers but after the treatment with CM/B1, however, the cells became positive for both markers within 6 hrs.

We used BMA1, which is an antibody specific for human endothelial differentiation marker, to check whether the cells are indeed undergoing phenotypic change from fibroblastoid to endothelial type. The cells showed a very weak reactivity towards this antibody before CM/B1, treatment. After the treatment however, the cells became intensely positive for this marker.

The results indicate that the treatment of stromal cells with CM as well as TGF B induce an endothelial phenotype on them as evidenced by positive results with VWF, VIII and BNAI markers while losing the fibroblastoid nature as evidenced by loss of reactivity to FSP1. This observation supports our earlier finding of capillary morphogenesis induced on stromal cells by CM as well as B

Publications / patents / conferences:

(a) A possible role of erythropoietin as a modulator of haematopoietic microenvironment leading to improved cell-cell interaction and colony formation. Manuscript in preparation.

(b) A possible modulatory role of transforming growth factor beta in multipotential haematopoietic stem cell self renewal. Manuscript in preparation.

TITLE: Identification of Melanoma oncogene/s and characterization of the Oncogene protein product.

Investigators : Dr Anjali Shiras
Dr L C Padhy
Molecular Biology Division
TIFR : Bombay

Objectives of the project :

1. Preparatiion of an expression cDNA laboratory from clone M3 and M3 transfected cells using the automatic directiional cloning approach.
2. Characterization of the expression cDNA library generated by tranfection into NIH 3T3 for various markers like G418 resistance , transfection ability, and various morphological phenotypes.
3. Subcloning of the clones of interest into the plasmid CEV27 and transfection of the plasmid DNA into NIH 3T3 for scoring of the desired phenotype.
4. identificaton of the gene/s responsible for transformatin and gene sequence.

Background information:

Melanoma, one of the most metastatic cancer is on the rise all over the world. The status about involvement of oncogenes in melanoma is not certain, though there are reports about involvement of genes like c-KIt, the receptor for mast cell growth factor, c-met protooncogene and ski oncogene. There has been a strong association also reported for N-Ras involvrdment in melanoma.

The present project is aimed at studying the genes involved in transformation in the mouse melanoma cell line clone M3 as the model system using the approach of directional cloning which has not been used earlier for these studied in melanoma.

Work carried out:

An expression cDNA library was prepared from mRNA of transfectants and clone M3 using the automated directional cloning appraoch. the vector

TITLE: Use of mitochondrial DNA restriction fragment length polymorphism for characterization of mosquito cell lines.

Investigators: **M. S. Patole**
Y. S. Shouche
S. G. Kshirsagar

STANDARDIZATION OF HETERODUPLEX ANALYSIS (HDA) FOR CHARACTERIZATION OF INSECT CELL LINES.

HDA is a simple technique used for detection of mutations in genes. It involves heat denaturation and fast denaturation of mixture of DNA which contains normal wild type gene and the gene to be tested. This DNA mixture is separated on polyacrylamide gel by electrophoresis and presence of multiple bands arising due to heteroduplexes is indicative of mutation in the gene. In the present study we have used HDA for identification of lineage of cell line which is one of the most important aspect of cell line characterization. 12 and 16S Ribosomal RNA (rRNA) sequences located on mitochondrial DNA are utilized in this study. rRNA genes are unique with respect to having stretches of conserved and variable sequences amongst related organisms.

Using oligonucleotides specific for insects, mitochond-rial 16 and 12S rRNA genes were amplified by polymerase chain reaction from cell lines and larvae. To confirm the lineage and to eliminate the possibility of cross contamination by already established cell lines, HDA was utilized for new moth cell lines developed from larva and pupa of *Spodoptera litimura*. HDA analysis performed with mixture of rRNA gene segments from cell lines and *S. litimura* larvae showed only single homoduplex band, indicating the cell lines are not contaminated and are indeed derived from *S. litimura*. But when rRNA gene segment was taken from *S. frugiperda* (from which popularly used cell line Sf-9 and Sf-21 are developed), HDA analysis showed 4 DNA bands indicating formation of heteroduplex due to variation in the gene sequence of new cell lines and *S. frugiperda*. This showed that the cell lines are not derived from *S. frugiperda*. The variation in the gene sequences were confirmed ny DNA sequencing of rRNA genes from new cell lines and larvae of *S. frugiperda* and *S. litimura*. HDA analysis was extended for characterization of other insect cell lines viz. mosquito cell lines. It was found that HDA analysis can be successfully utilized for

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Table 2 Transition and transversion differences in the sequenced reagion of the mosquitoes

	A.st.	*A.quad.*	*A.albo.*	*A.walb*	*Cu.tri.*	*Cu.bi.*
A.st.	-	11	9	9	14	14
A.quad.	19	-	15	11	11	13
A.albo.	30	25	-	7	12	12
A.walb.	25	20	11	-	8	10
Cu.tri.	27	24	19	18	-	1
Cu.bi.	27	22	17	16	4	-

ALIGNED SEQUENCES OF MOSQUITO MITOCHONDRIALL 16S rRNA

```
Ctri     NANTNANNNTCNNNNNANNNNNNNANNNANNNTNNCNTAATTTGCCCACTGATAAAAATT
Cbi      NANTNANNNTCNNNNNANNNNNNNANNNANNNTNNCNTAATTTGCCCACTGATAATAATT
Aalbo    NANTNANNNTCNNNNNANNNNNNNANNNANNNTNNCNTAACCTGCCCACTGGTAAAAATT
Awal     NANTNANNNTCNNNNNANNNNNNNANNNANNNTNNCNTAACCTGCCCACTGATTT--ATT
Aqadri   NANTNANNNTCNNNNNANNNNNNNANNNANNNTNNCNTAATTTGCCCACTGATAT----T
Asteph   NANTNANNNTCNNNNNANNNNNNNANNNANNNTNNCN---CCTGCCCACTGAATT---TT

Ctri     AAAGGGCCGCAGTATTTTGACTGTGCGAAGGTAGCATAATCACTAGTCTTTTAATTGGAG
Cbi      AAAGGGCCGCAGTATTTTGACTGTGCGAAGGTAGCATAATCAGTAGTCTTTTAATTGGAG
Aalbo    AAAGGGCCGCAGTATTTTGACTGTGCGAAGGTAGCATAATCAGTAGTCTTTTAATTGAAG
Awal     AAAGGGCCGCAGTATTTTGACTGTGCGAAGGTAGCATAATCAATAGTCTTTTAATTGAAG
Aqadri   AAAGGGCCGCAGTATTTTGACTGTGCGAAGGTAGCATAATCAATAGTCTTTTAATTGAAG
Asteph   AAAGGGCCGCGGTATTTTGACCGTGCGAAGGTAGCATAATCAATAGTCTTTTAATTGAAG

Ctri     GTTGTATGAATGGTTGAATGAGATATATACTGTCTTTTTTAAAATTATATAGAATTTTAT
Cbi      GTTGTATGAATGGTTGAATGAGATATATACTGTCTTTTTTAAAATTATATAGAATTTTAT
Aalbo    GCTGTATGAATGGTTGAATGAGATATATACTGTCTTTTTTAAATTTTTATAGAATTTTAT
Awal     GCTGTATGAATGGTTGAATGAGATATATACTGTCTTTTTTAAATTTTTATAGAATTTTAT
Aqadri   GCTGGATGAATGGTTGAATGAGATATATACTATTTTTTTTTAAAATTTTATAGAACTTTAT
Asteph   GCTGGATGAATGGTTGAATGAGATATATACTGTTTTTTTTAAAAATTTTTTAGAACTTTAT

Ctri     TTTTTAATTAAAAAGTTAAAATAAAATTAAAGGACGAGAAGACCCTATAGATCTTTATTT
Cbi      TTTTTAATTAAAAAGTTAAAATAAAATTAAAGGACGAGAAGACCCTATAGATCTTTATTT
Aalbo    TTTTTAATTAAAAAGTTAAAATATAATTAAAAGACGAGAAGACCCTATAGCTATTTATTT
Awal     TTTTTAATTAAAAAGTTAAAATGTAATTAAAAGACGAGAAGACCCTATAGATCTTTATTT
Aqadri   TTTTTAGTTAAAAAGCTAAAATTTAATTAAAGGACGAGAAGACCCTATAGATCTTTATTT
Asteph   TTTTTAATTAAAAAGTTAAAATATAATTAAAAGACGAGAAGACCCTATAGATCTTTATTT

Ctri     TTGTTAT-TTATAAATTAAAAAGAATTTTAAAATTTATAATTTAATAAAAAATTTTATTG
Cbi      TTCTTAA-TTATAAATTAAAAAGAATTTTAAAATTTATAATTTAATAAAAAATTTTATTG
Aalbo    TTTTTAA-TTATAAGTTAAAAAGAATA-TTAAATTTATAGTTTTATAAAAAATTTTACTG
Awal     TTGTTAG-TTATAAATTAAAAAGAATAATTAAATTTATAATTTAATAAAAAATTTTACTG
Aqadri   TTA-------ATAAATTATAAAGAATT-TAAAATTTATATTTTAA-ATAAAATTTTACTG
Asteph   TTATAAAATTATAATTTAT---GAATTTTAATAATTATA---TAATAAAAAATTTTACTG

Ctri     GGGTGATATTAAAATTTAAAAAACTTTTAAAATTTATTAACATAAATATATGAATAAATG
Cbi      GGGTGATATTAAAATTTAAAAAACTTTTAAAATTTATTAACATTAATATATGAATAAATG
Aalbo    GGGTGGTATTAAAATTTAATTAACTTTTATTATTTGTTAACATTAATATATGTATATTTG
Awal     GGGTGGTATTAAAATTTAATAAACTTTTATTATTTATTTACATTA-TATATGAATATTTG
Aqadri   GGGTGGTATTAAAATTTAATAAACTTTTATTTTTAATTAACATTGATTTATGAATTTAAG
Asteph   GGGTGGTATTAAAATTAAATTAACTTTTATTATTTATTAACATAAATTTATGAATAAAAG

Ctri     ATCCAGTTTTATTGATTAAAAAATTAAGTTACCTTAGGGATAACAGCGTAATTTTTTTTT
Cbi      ATC-AATTTTATTGATTAAAAAATTAAGTTACCTTAGGGATAACAGCGTAATTTTTTTTT
Aalbo    ATCCAATTTTATTGATTAAAAAATTAAGTTACCTTAGGGATAACAGCGTAATTTTTTTTT
Awal     ATCCAGTTTTATTGATTAAAAAATTAAGTTACCTTAGGGATAACAGCGTAATTTTTTTTT
Aqadri   ATCCTGTATTATGGATTAAAAAATTAAGTTACCTTAGGGATAACAGCGTAATTTTTTTAG
Asteph   ATCCTATTTTAT-GATTAAAAATTTAAGTTACCTTAGGGATAC--ACGTAATTTTTTTAG

Ctri     AGAGTTCATATCGAGAAAAAAGATTGCGACCTCGATGTTGGATTAAGAGTTATTTTTAGG
Cbi      AGAGTTCATATCGACAAAAAAGATTGCGACCTCGATGTTGGATTAAGAGTTATTTTTAGG
Aalbo    AGAGTTCTTATCGAGAAAAAAGATTGCGACCTCGATGTTGGATTAAGAATTATTTTTAGG
Awal     AGAGTTCTTATCGACAAAAAAGATTGCGACCTCGATGTTGGATTAAGAGTTATTTTTAGG
Aqadri   AGAGTTCTTATCGATAAAAAAGATTGCGACCTCGATGTTGGATTAAGAGTTATTTTTAGG
Asteph   AGAGTTCTTATCGATAAAAA-GATTGCGACCTCGATGTTGGATTAAGAGTTATTTTTAGG

Ctri     TGTACAAGTTTAAAG
Cbi      TGTAGAAGTTTAAAG
Aalbo    TGTAGAAGTTTAAAG
Awal     TCTAGAAGTTTAAAG
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TITLE: IDENTIFICATION OF FACTORS IN SUPERNATANTS FROM STIMULATED LYMPHOCYTES ON GROWTH AND DIFFERENTIATION OF NEUROBLASTOMA CELLS.

Investigators: **Padma Shastry**
Rathna

Mouse cell line Neuro-2a was used in the study for identification of factors generated from con A stimulated mouse splenocyte cultures that influence the growth and differentiation in neuroblastoma cells. The supernatant collected at 120 h. following stimulation (m-120) which demonstrated an inhibitory activity on proliferation of neuro-2a was used. At 20% conc. m-120 was nontoxic to cells by MTT assay in 24 h. assay. Separation of components in this supernatant was done using amicon filteration unit with cut off 25,000 m.w. and assessed for activity. The inhibitory activity was retained in fraction 25,000 m.w. Further characterization of this fraction revealed the active components to heat labile (90 o for 5 min.) and acid stable (pH 3.0). Anti TNF alpha and anti IFN gamma antibodies neutralized the inhgibitory activity in M-120 by 53.14 and 71.4% respectively. However, TNF alpha and IFN gamma individually did not have any effect on the growth of Neuro-2a at any of conc. in the range (0.1-500 i.u.) tested./ NGF-beta at conc. 5000pM demonstrated protective effect on the inhibition of proliferation,k when the Neuro-2a cells were pretreated with NGF beta and then treated with M-120 in 48 h assay.

Publications / patents / conferences: Nil.

TITLE: Studies on the interaction of differentiated neuroblastoma cells with extracellular matrix components.

Investigators: **Padma Shastry**
Rathna

Neuroblastoma is the most common extracranial tumor of childhood.

It is thought to arise from neural crest cells due to the arrest of differentiation. Neuroblastoma is a very good model to study neuronal differentiation and cell matrix interactions because of its ability to undergo spontaneous as well as induced differentiation into neuroganglioma, the most differentiated form of the tumor and that these neural crest cells migrate all over the embryo making contact with the surrounding extracellular matrix for guidance.

The present work is being carried out to study the changes associated with differentiated neuroblastoma cells with respect to their interaction with the extra cellular matrix components.

Two mouse neuroblastoma cell lines lines Neuro-2a and NB 41 A3 were chosen for the study. Staurosporine a potent protein kinase C inhibitor was used to induce differentiation.

The following studies have been carried out.

1. *Proliferation assay.* Proliferation assay was carried out to study dose and time dependent effcet of staurosporine on the growth of neuroblastoma cells. Cells were treated with staurosporine concentrations ranging from 1nM to 100nM for 24, 48, and 72 hours. Effect on proliferation was studied by their ability to incorporate tritiated thymidine.
2. *Measurement of viable cell number by MTT assay.* Neuroblastoma cells were treated with various concentrations of staurosporine ranging from 1nM to 100nM and incubated for 24, 48, and 72 hours. The effect on cell number was studied by their ability to reduce MTT to MTT formazan a colored product which can be quantitated by measuring the absorbance at 570nM.
3. *Cell cytotoxicity* Neuroblastoma cells were treated with 1nM to 100nM staurosporine for 12 hours. At the end of this time period the the viable cell number was counted by thier ability to exclude trypan blue dye.

4. *Morphological studies* . The cells were treated with 20 nM concentration of staurosporine and incubated for 48 hours. and phase contrast pictures were taken to show the change in the morphology.
5. *Acetyl choline esterase activity* Cells were treated with 20nM concentrations of staurosporine and incubated for 48 hours. The enzyme activity was measured by the method of Ellman . The method is based on the ability of the enzyme to hydrolyze acetyl thiocholine a substrate analogue which inturn is made to react with a reducing agent that forms a colored product having maximum absorbance at 412nM.

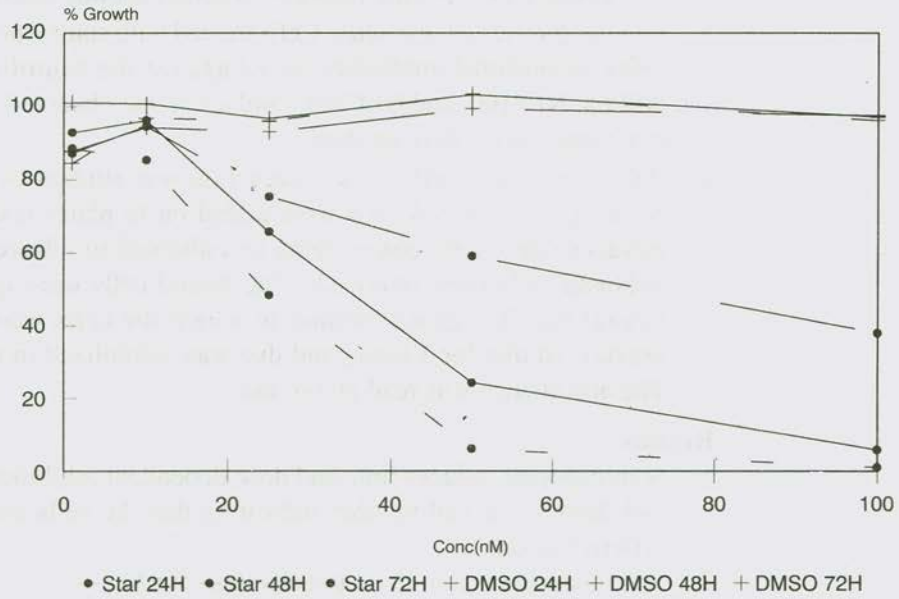
   The specificity of the reaction was tested by carrying out the reaction in the presence of a specific inhibitor of acetyl choline esterase BW 284 C51.
5. *Immunofluorescence studies.* Cells treated with staurosporine were stained using monoclonal antibodies raised against the neurofilament proteins (NFP68, NFP160, and NFP200) and a-tau and observed using fluorescent dye tagged secondary antibody.
6. *Adhesion assay.* Cell matrix interaction was studied by binding studies. Staurosporine treated cells were added on to plates coated with various extracellular matrix components and allowed to adhere for 1 hour. The unbound cells were removed . The bound cells were quantitated by the neutral red dye uptake method in which the cells were incubated with neurtal red dye for 3 hours and dye was solubilized in acidified ethanol. The absorbance was read at 540 nm.

Results

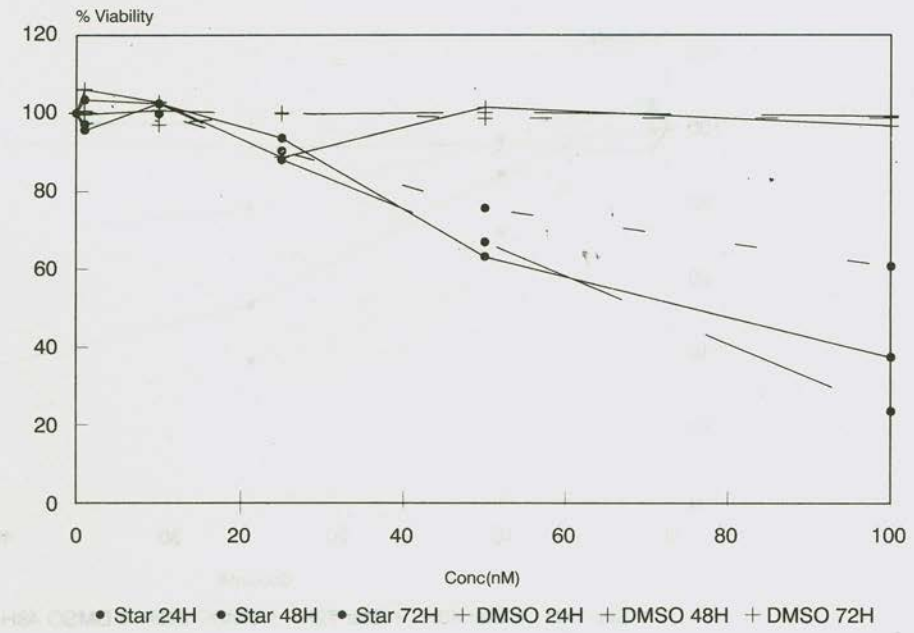
1. Staurosporine induces time and dose dependent inhibition of proliferation and decrease in cell number indicating that the cells may be undergoing differentiation.
2. Staurosporine is non toxic to cells upto 50nM.
3. Staurosporine induces the formation of long neurites indicating morphological differentiation of neuroblastoma cells.
4. Staurosporine induces functional differentiation of NB 41 A3 cells as assesed by acetyl choline esterase activity. This activity was abolished using a specific inhibitor of the enzyme.
5. Staurosporine induces increased binding sites for collagen type 1 in Neuro-2a cells.

Publications / patents / conferences:Nil.

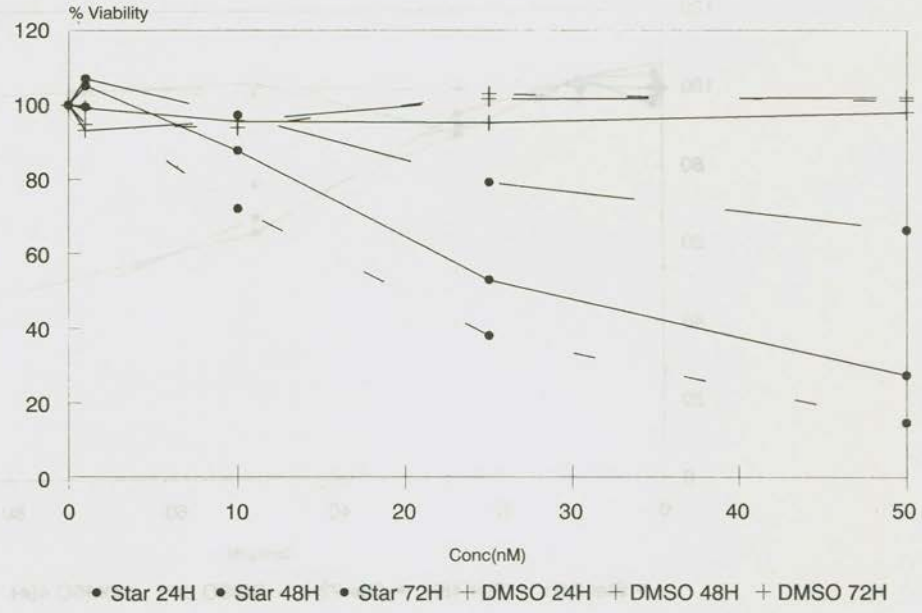
Proliferation Assay by 3H thymidine incorporation in Neuro-2a



Cell number by MTT in NB41A3



Cell number by MTT
in Neuro-2a



TITLE : Investigations on Beta cell protective mechanisms as means of reducing incidence of diabetes

Investigators : R.R. Bhonde
Sandhya Sitasawad

BACKGROUND :

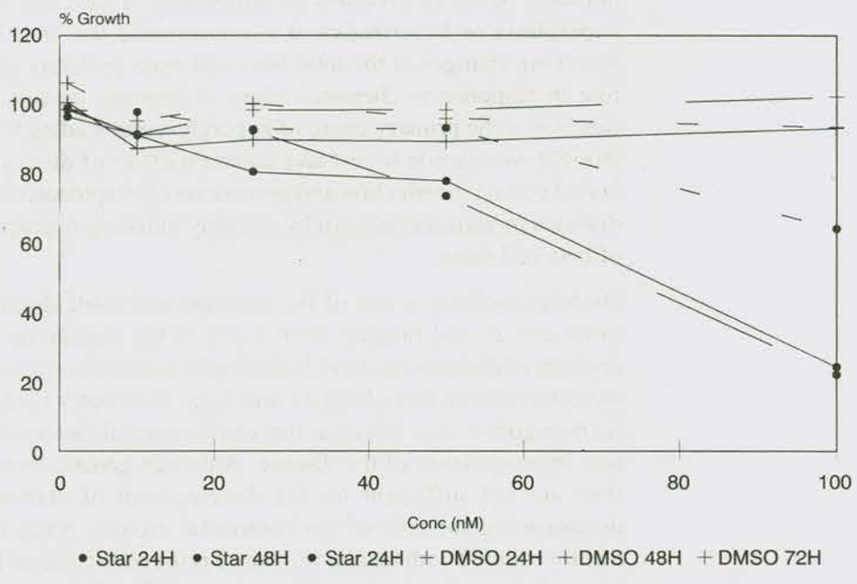
The capacity of the islet organ to respond to diabetogenic stimulus by Beta cell renewal may be one of the key factors that determines the development, course and outcome of diabetes mellitus. In a broad sense diabetogenic influences are continuously active in the organism and are normally balanced precisely by adjustment of Beta cell function through hyperplasia or hypertrophy. It is conceivable that long-term adaptation involving changes in the total Beta cell mass probably play an important role in response to chemical injury or immune assault. Since beta cell function is the primary cause of hyperglycemia leading to diabetes, it was thought worthwhile to test and ascertain effect of dietary substances and herbal extract in reduction and prevention of streptozotocin (STZ) induced diabetes in vivo and in vitro by possibly inducing regeneration and repair of beta cell mass.

Diabetes mellitus is one of the non-communicable diseases prevalent in India and abroad ranging from 1-5% of the population. Studies on the etiology of diabetes involves both genetic and environmental factors. Since the concordance rate of Type I and Type II diabetes is more than 50% in monozygotic twins, it is clear that environmental factors play an important role in progression of the disease. Although genetic factors are essential they are not sufficient for the development of diabetes thus further documenting the role of environmental factors. Since insufficiency of insulin coupled with insulin resistance is the root cause of Type II diabetes. The only hope for the recovery and reversal of diabetes is to induce Beta cell regeneration and to inhibit further Beta cell loss. With this view in mind studies were undertaken to investigate Beta cell protective mechanisms.

WORK CARRIED OUT :

(a) Standardization of techniques especially isolation and cultivation of islet of the langerhans, estimation of lipid peroxidation, testing viability and

Proliferation Assay by 3H thymidine incorporation in NB41A3.



functionality of islets.

(b) Induction of diabetes in mice by streptozotocin, estimation of glucose in blood and urine

WORK DONE TILL DATE :

1) Isolation and cultivation of islet of langerhans
2) Estimation of lipid peroxidation
3) Testing viability and functionality of islets
4) Maintenance of Rat Insulinoma Cell line (RIN 5mF)
5) Induction of diabetes in mice by streptozotocin
6) Estimation of glucose in blood and urine

Work carried out during last year (1994-1995) :

a) Recently it has been shown that streptozotocin (STZ) induced diabetes could be prevented by treatment with D-glucose and 5-Thio-D-glucose. However the mechanism of action of these sugars in protecting Beta cells and isolated islets against STZ toxicity is not known. One of the mechanism postulated for STZ induced Beta cell damage is by the generation of oxygen derived free radical species. Hence in vitro and in vivo studies were undertaken to measure STZ induced lipid peroxidation. Part of the in vitro data has been reported in the annual report of 1994-1995 and was abstracted in XIX All India Cell Biology Conference held at IICB, Calcutta (February 23-25, 1996).

   In continuation of these in vitro studies in vivo studies were undertaken to test the efficacy of D-glucose and aqueous extract of bittergourd in reducing incidence of STZ induced diabetes. Malondialdehyde (MDA) levels as an index of lipid peroxidation were measured in the above experiments.

Effect of bittergourd extract :

The effect of pre-treatment of bittergourd extract was studied on STZ induced diabetic mice. The degree of damage caused by STZ was assessed by measuring lipid peroxidation in pancreatic tissue and estimation of glucose to know the degree of hyperglycemia induced. A group of 30 mice (4-6 week old, Balb/c male) was taken as a single unit and was divided

into 6 groups of 5 each. Mice were pre-treated with 10% and 50% aqueous extract of bittergourd (fruit pulp) for 48 hrs. Fifty percent mice were then injected with STZ (200 mg/kg body weight) alongwith STZ treated groups and untreated controls. The induction of diabetes was confirmed by estimating glucose levels in blood and urine. All the groups of mice were sacrificed and lipid peroxidation was estimated in pancreas.

It was observed that levels of lipid peroxidation in controls, 10% and 50% aqueous extracts was comparable. STZ alone induced high levels of lipid peroxidation which was reduced to less than 50% by 10% and 50% aqueous extracts. Similarly it was found that pre-treatment with bittergourd extract helps in reducing hyperglycemia induced by STZ (Fig.1).

B) In vitro studies:

Effect of pre-treatment of 55, 10% and 20% bittergourd aqueous extract was treated on STZ induced lipid peroxidation in RIN (Rat insulinoma) cell line and freshly isolated islets from Balb/c mice. It was observed that all the three concentrations reduced STZ induced lipid peroxidation to less than 50% in RIN cells whereas 1% aqueous extract reduced about 50% STZ induced lipid peroxidation in isolated islets (Fig. 2)

Publications / patents / conferences:

Hypoglycemic effect of bitter gourd fruit extract in STZ-induced diabetic mice is mediated through reduced lipid peroxidation. Manuscript in preparation.

TITLE : Fetal Liver Infusion : Its mode of action, efficacy of cryopreserved cells and its potential applications in the management of cancer.

Investigators : DR.(MRS) V.P.KALE
Dr.(Mrs) L.S.Limaye
Dr.(Mrs) V.Kochupillai, Chief, IRCH, New Delhi

Background :

This project has been undertaken as a collaborative study with Dr.Vinod Kochupillai, AIIMS, New Delhi, to investigate the mechanism of autologous bone-marrow recovery, observed in several patients of aplastic anemia, acute leukemia after fetal liver infusions. The results of their clinical trial as well as the results reported by several authors indicate that fetal liver cells have the capacity to stimulate pa-tients stem cells without the use of preconditioning regi-men. However, the mechanism of this autologous recovery is not clear at present.

The proposal has been designed with the following objectives

i) To study the proliferative capacity of fetal liver hematopoietic stem cells compared to adult bone- marrow.

ii) To study the mechanism of hematopoietic recovery induced by fetal liver hematopoietic cells, using co-culture studies in vitro.

iii) To standardise the method of cryopreservation of fetal liver cells.

iv) To assess the efficacy of cryopreserved fetal liver cells using committed stem cell assays.

v) To assess the possible application of fetal liver hemo-poietic stem cells in the management of cancer, if above studies succeed.

Work carried out :

We reported last year that the hematopoietic cells from livers dissected from fetuses from MTP cases were isolated and cryopreserved. The efficacy of cryopreservation was assessed by the CFU assay carried out before and after cryopreservation. We also prepared fetal liver extracts from various samples to check whether these cells secrete diffusible factor(s) which may be responsible for stimulation of adult bone-marrow cells.

We carried out several experiments to assess the ability of the fetal liver extracts to stimulate the adult bone-marrow cells. For this purpose, we used in vitro CFU-GEMM assay system. The fetal liver extracts were used in combination of usual growth factors namely 5637 cem and erythropoietin (GEMM.CM). The colonies were scored after 14 days and the percent stimulation was calculated by using following formula:

$$\% \text{ stimulation} = \frac{\text{AV.CFU GEMM with CM (GEMM.CM)} - \text{AV.CFU GEMM without CM (GEMM)}}{\text{AV.CFU No. without CM}}$$

The results of some representative experiments were given in the table.

Sr. No.	*FLI No.*	*BM No.*	*% stimulation*
1	FLI.19	T-2	60
2	FLI.19	T-6	58
3	FLI.19	T-7	227
4	FLI.23	T-10	26
5	FLI.26	T-17	42
6	FLI.26	BM 193	55
7	FLI.26	BM 196	104
8	FLI.26	BM 205	300

Publications / patents / conferences:

Foetal liver infusion: Its mode of action, efficacy of cryopreserved cells and its potential application in the management of cancer. Manuscript in preparation.

TITLE : Regeneration of pancreatic Beta cells.

PARTICIPANTS : Dr. R.R. Bhonde

BACKGROUND :

The pancreatic beta cell is highly specialized for its vitally important function-the production and release of insulin to regulate the blood glucose level. The number of functionally intact beta cells in the islet organ is of decesive importance for the development, course and outcome of diabetes mellitus. Since all forms of diabetes are characterised by an insufficient extent of beta cell replication needed to compensate for the loss or dysfunction beta cell occuring in diabetes, elucidation of regenerating potential in experimentally induced diabtic animals would be of interest as an alternate therapy for diabetes. The pathogenesis of diabetes has been viewed by some as a balance of destructive and regenerative processes. The therapeutic goal would then be to downregulate the destructive processes. As 3% of adult islets are still capable of proliferation,a specific factor may be used to enhance beta cell regeneration and tilt the balance toward regeneration in a patient with diabetes.

It is therefore important to define the growth factors responsible for proliferation of Islets and eventually to identify the Islet stem cell. Identification of Islet stem cell and the stimulus necessary to make the stem cell proliferate into progeny of islets will enhance our understanding of diabetes and perhaps offer novel approches to the cure of diabetes .

OBJECTIVES

1. To find out factors affecting growth of beta cells in vivo and in vitro.
2. To ascertain role of exocrine pancrease / liver in promoting beta cell proliferation.
3. To induce beta cell regeneration for reversal of diabetes.

WORK CARRIED OUT :

A. In vivo studies:—

In order to find out whether partial hepatectomy plays any role in improving diabetic state, male wistar rats (6) weighing 200 g were made diabetic by

intraperitoneal streptozotocin injection (200 mg/kg body). Partial hepatectomy was carried out on three diabetic rats and three control non-diabetic rats. Blood and urine glucose was tested daily in control and experimental animals for a period of 8 days. It was observed that blood glucose level of control and partial hepatectomised rats was comparable 110 mg/100ml and 90 mg/100ml respectively and no glucose was detected in their urine.However , blood glucose level of diabetic rats (without partial hepatectomy) was 440 mg/100 ml with urine glucose ++++ve, where as diabetic and partial hepatectomised rats exhibited no sugar in urine and blood glucose of 200 mg/100 ml. The data indicates that partial hepatectomy reduced hyperglycaemia and inhibited glycosuria in diabetic animals. Further studies on histopathology and insulin content of pancreas of all the control and experimental groups are in progress.

B. In vitro studies :

To find out how far glucose and nicotinamide help in promoting beta cell proliferation, the effect of 16mM glucose alone or in combination with nicotinamide (50ug/ml)was tested on proliferation of beta cells isolated from mouse islets. It was found that glucose treatment alone induces hypertrophy in beta cells as revealed by protein contents of cells whereas glucose in combination with nicotinamide induces hyperplasia as evidenced by increase in cell number as compared to untreated control cultures.Results indicate that nicotinamide therapy may help in triggering beta cell proliferation in hyperglycemic subjects.

Publications / patents / conferences :

(a) Gudrun Ulrich Merzenich and Ramesh R.Bhonde Donor site, age and health affect fibroblast growth in culture. In vitro 31:7:494-996 (1995)

(b) Bhonde R.R. Dietary management of pancreatic beta cell homeostasis and control of diabetes Medical Hypothesis 46:4:357- 361 (1996)

(c) Smita S.Kulkarni, V.S. Padbidri and R.R.Bhonde :Comparative studies on the susceptibility of certain primarycultures to coxiella burnetii. Ind. J.Microbiology 13(3) 145-149 (1995)

(d) Cynthia B.Elias and Ramesh R. Bhonde Encapsulation of islets : A new

tool for islet cryopreservation and transplantaion. XIX All India Cell Biology Conference held at IICB, Calcutta during Feb.23-25, 1996.

(e) Bhonde R.R. & Sitaswad S.L. Glucose treatment protects pancreatic beta cells from streptozotocin toxicity by reducing levels of lipid peroxidation. XIX All India Cell Biology Conference held at IICB, Calcutta during Feb.23-25, 1996.

SUPPORTING UNITS

Library and documentation

Animal house

SUPPORTING UNITS

Library and documentation

For sustained advancement of research and developement programmes, easily accesisible comprehensive information is an absolute pre-requisite. With this objective in view NCCS, Libtrary and documentaton facility over the years has developed neccessary infrastructure and facilities to meet the information requirments of the centre. The Library and documentation facility of the centre added to its stocks 500 documents including books, reports, serial monographs and bound volumes of periiodicals during the period 1995-96.

The electronic information sources available in the library which include number of CD-ROM databases such as Medline, Biotechnology abstract and few of the current protocols titles are also subscriped during the year.

Facilities for direct access to international database hosts like DIALOG is made available to users by way of renewing DIALOG ONLINE password. The library continued to provide services like electronic mail, internet access, current awareness services, SDI serviceon a regular basis on the latest R & D in any specific area chosen by the user. Library continued to extend its information consolidation srrvices such as Press vision, Bio-vision having relevance to Biotechnology on front line R & D activities.

The library is continued to be a member of Pune Library Network and is actively participating in the consortium.

PUBLICATIONS:

1. N V RAMAKRISHNA: Press vision service at National facility For Animal Tissue And Cell Culture : A case study [In press journal: ISALIC BULLRTIN].
2. N V RAMAKRISHNA : Inter library cooperation (ILC) in Pune Biiomedical libraries : utility f journal articles at National Facility For Animal Tissue And Cell Culture. [In press journal Annals of Library science and documentation].
3. N V RAMAKRISHNA

   Experiences with CD-ROM Databases at Natiional Facility For Animal Tissue & Cell Culture library (Communicated)

4. Comparative evaluatioin of ON-LINE and CD-ROM searches im Biomedical sciences - A preliminary study (Communicated)

ANIMAL HOUSE

The animal house for small laboratory animals became fully functional in the Jidnyasa building. Humidity, ventilation and temprature is regularly monitored and maintained. Health monitoring of animals is done regularly by faecal sample examination for parasitic load, blood examination for haemoprotozoans and skin and hair examination for mites and other ecctoparasites. All the animals used for various experiments were healthy and were free from the above infestations.

The details of the animals procured from National Institute of Virology, bred in the Animal house and supplied for various R & D activities is given below.

NO	*SPECIES STRAIN*	*ANIMALS PROCURED*	*ANIMALS BRED*	*ANIMALS SUPPLIED*
1	RAT			
	1.Wistar	17	59	41
	2.C. F.	13	05	
	3.Lewis	04		
2	MICE			
	1.C57BL	16	30	17
	2.Balb\c	30	244	142
	3.Swiss	30	192	157
	4.nu\nu	30		19
3	Mastomys	11	38	01
4	Gerbils	04		04
5	Hamster	09	18	02