CHAPTER – IV

EXPERIMENTAL LEUKAEMIA &
EFFECTS OF BIOLOGICAL
RESPONSE MODIFIERS
Experimental Leukaemia and Effects of Biological Response Modifiers:

It has become evident that disorders of growth control, including all cancers, arise from disruption of the signaling pathways, which normally control cell growth and differentiation. Many of these pathways are regulated by cytokines, hence, attempts have been made to use cytokines to modify behavior of cancer cells. Furthermore, since immune surveillance is important in the normal suppression of transformed cell growth, stimulation of surveillance by exogenous cytokines might give clinical benefits on top of the direct anti-tumor effects. Recently, numerous clinical trials of cytokine treatment of cancer, with varying rates of success have been recorded.

Effects of Interferons (IFN):

Taylor-Papadimitrion (1985) (252), Healy (1988) (257) has reported that several cytokines, in particular the IFNs, are involved in normal host defence. They should be applicable to combat serious infections, which do not respond to other therapies. However, the many unpleasant side-effects of systemic cytokine therapy preclude their use for mass prophylaxis against common infections like cold or influenza which are serious hazards to immunocompromised patients. Indeed many of the symptoms of these diseases are probably caused by IFNs and other cytokines produced in response to the infections. It has been found that IFN may also be able to protect normal tissues against the toxic effects of some compounds allowing higher doses to be tolerated by the patients. Such effects, albeit mediated by different mechanisms, would be analogous to the protective effects of Colony Stimulating Factors (CSF) for bone marrow during chemotherapy. On the other hand, the reports of Grander et al (258) suggest that the ability of IFN gamma (IFN-γ) to induce the expression of adhesion molecules such as LFA 1 and ICAM 1 on peripheral blood leukaemic cell surface. It has been
suggested that the existence of a large number of peripheral blood leukaemic cells during the development of CML may be related to a fault in cellular adhesion molecules. The ability of IFN-γ to induce the expression of these molecules may, therefore, prove useful in the treatment of CML patients. Kurzrock et al (1987) (259) observed a complete hematological response rate of 38% and a complete cytogenetic response rate of 19%, in a group of pH positive CML patients treated with IFN-γ. Lauta (1995) (260) reported that IFN used as a single agent is effective as fast induction therapy of pH positive CML patients, it also seems that the efficacy of IFN is superior to that of chemotherapy regarding complete cytogenetic response. The efficacy of combined IFN/chemotherapy is superior to chemotherapy alone. There are only limited data from clinical trials on the efficacy of IFN as second line treatment or as maintenance therapy.

The most successful use of IFNs against malignancy has been seen with the rare hairy cell leukaemia (Thompson & Fefer – 1987) (261). Upto 80% of patients show partial or complete remissions after IFN therapy, with clearance of the leukaemic B-cells from the bone marrow in the latter group. It has been suggested that IFN act against hairy cells by stimulating them to differentiate (Michaleviez and Revel –1978) (262). There is also some risk that rhIFN alpha may cause autoimmune disease. However, quite low doses of IFN, with minimal side effects, are often effective against hairy cell leukaemia, and the consequences of an immune response to one form of IFN may be overcome by changing to a mixture of naturally produced IFN species.

**Effects of Interleukin 2(IL2)**

Hancock et al (1990) (263) reported that activation of LAK (Lymphocyte Activated Killer) cells by IL2 has considerable clinical potential for treatment of tumors such as non-Hodgkin's lymphoma, melanoma and renal carcinoma. IL2 also has other immune enhance properties. It has been used alone or in
combination with other cytokines (IFNs, TNF) or other forms of therapy. However, its use in vivo has been limited by serious side effects including fever, nausea, tissue oedema and hypotension. Many of these probably results from vascular leakage caused by adherence of activated lymphocytes to the vascular endothelium. There is also temporary linear dysfunction and marked myelo suppression. To avoid these problems combined IL-2 treatment is beneficial for the regression of tumors such as carcinomas of the colon or kidney or malignant melanoma, that do not respond to other treatment (Semenzato-1990) (264).

Fortunately, even the well tolerated doses of IL-2 produced a high level of immune modulation, characterized by increased numbers of CD56+, CD16+ or CD8+, AK cells (Gottlibe et al-1989) (183). More importantly perhaps, there is a substantial increase in the number and activity of cells able to inhibit the clonogenic growth of leukaemic blasts (Gottlibe et al-1989) (198). Leukaemic clone and cluster formation is inhibited by upto 95%. Although the clinical efficacy of IL-2 or other immuno-modulators is not yet established, it is still worthwhile to attempt to improve the immunologic efficacies of these agents while minimising their toxicity. One way to achieve this aim may be to use long term low dose IL2 infusion [Soiffer et al - 1992 (207), Higuchi et al - 1991 (212)]. Another route is to manipulate those mechanisms responsible for the down regulation of lymphocyte activation.

Moreover, a large number of non-specific Biological Response Modifiers (BRMs) have been reported to be of significant therapeutic value in different malignant conditions. Such as, sheep erythrocyte is able to stimulate the IL2 IFN secretion through the T-cell activation (Chaudhuri et al – 1990, 1991, 1992, 1993, 1998) (265-279).

For the present thesis work we chose balanced combined therapy of IL2, IFN-γ and SRBC to achieve the greater efficacy and minimise the toxicity.
Its effect on leukaemic subject has never been explored beforehand in such combinations.

**Materials:**

To serve the purpose, following materials are required:

1. Swiss Albino inbred mice.
2. 1ml syringe.
3. Ethyl Alcohol.
5. SRBC (Sheep Red Blood Corpuscles; 7% PCV saline, is prepared for the injection.)
6. Interleukin 2 (Sigma, USA, rmu).
7. Interferon γ (Sigma, USA, rmu).

**Methods:**

a) **Duration:** 6 months following injection of ENU (N-N' Ethyl nitroso urea) into new born mice (5-10 days after birth), the aforesaid BRMs are injected either singly or in combination of two or three (Group followed). Animals were sacrificed on 7th day of BRMs administration.

b) **Dose:**

i. Sheep Erythrocytes (SRBC) – 0.25ml of 7% SRBC (PCV/saline vol.) is injected.
ii. Interleukin 2 (IL2) – 5μg/kg body weight of mouse injected with 0.9% NaCl.
iii. Interferon γ (IFNγ) – 5μg/kg body wt. Injected, with 0.9% NaCl.
c) **Mode of administration of BRMs:** The above biomodulators are applied intraperitoneally either alone or in combinations as follows.

1. IL2 : (Gr. E2).
2. IFNγ : (Gr. Eγ).
3. SRBC : (Gr. ES).
4. SRBC +IL2 : (Gr. E2S).
5. SRBC +IFNγ : (GR. Eys)
6. IL2 + IFNγ : (Gr.E2γ).
7. SRBC + IL2 + IFNγ : (Gr. E2γs)

**Results:**

A) **Survival effects:** From the fig. 1, it has been found that due to toxicity of the carcinogenic agent ethylnitrosourea (ENU), 15% animals died within 10 days total lifespan (acute death), 25% of the total died due to secondary infections within 50 days, 60% of animals developed leukaemia (mixed type) and died within 150 days.

Application of Biological Response modifiers like IL-2, SRBC or IFN-γ shows increased value of Therapeutic Index (TI); SRBC was found to be more beneficial than that of IL-2 and IFN-γ, but combination of IL-2, IFN-γ and SRBC gives significant survival benefit leadings to normal.

B) **Immune Effector Mechanisms:**

1) Sheep erythrocyte ligand Binding capacity with CD2 Molecules Interaction on lymphocyte: Spontaneos E Rosetting.
2) Cytotoxicity assay by flurochrome release phenomenon (H-33342).
3) PMN mediated phagocytosis by NBT reduction assay.
4) Leucocyte Adherence Index (LAI).
5) Antigen presenting capacity of macrophages and lymphocytes : PLN assay.
Section – I

Sheep erythrocyte ligand binding capacity with CD2 molecules interactions: spontaneous E-rosetting capacity
In normal animals, the majority of the circulating lymphocytes spend their time apparently doing nothing; they are resting as Go Cells. As seen later, these dormant cells are specifically activated after contact with antigen. Antigen contact occurs as a specific surface event at the plasma membrane and generates a transduction signal which causes nuclear de-repression.

Rosette formation represents a basic technique by which antigen-binding lymphocytes can be visualised. The applicability of this approach is limited principally by the experimental design to ensure that non-specific binding is avoided. It is, however, difficult in this type of experiment to include a satisfactory specificity control. The approach adopted here is to show that the number of antigen-binding lymphocytes increases after immunisation.

The mature T lymphocytes of humans also carry receptors which, fortuitously, bind to determinants on the surface of sheep red blood cells. When mixed with SRBC each T cell binds a cluster of SRBC to its surface forming a rosette. Such E (erythrocyte) rosettes not only provided a convenient way to identify and count human T cell but also a way to separate T-cells from B cells. After mixing peripheral blood lymphocytes with SRBC, the rosetted T cells can be separated from B cells by their increased density. So here we have chosen this method to identify the healthy and active T-lymphocytes in the leukaemic and BRM treated groups.

Materials:-

1. Sheep Erythrocyte (1%PCV/Saline volume).
2. Spleen derived lymphocytes (Percoll separated at 1.077).
3. RPMI-1640 media without FBS.
Methods: 3-4x10^6 lymphocytes are taken in a test tube with 0.25 ml of 1% SRBC and incubated into 37°C for 15 min. After incubation, it is briefly, centrifuged and incubated at 4°C for 18 hrs. Then 2.5% glutaraldehyde is added to the cell pellet with gentle mixing and count is taken into Neubauer Chamber under microscope. The method followed was adopted from Kinchington. D and Ng, T, 1997 (270) with modification.

Results: The functional assessment of the lymphocytes sensitive to sheep erythrocytes (SRBC) showed the decreased rosette forming capacity in mice with induced leukaemia. (P=<0.001) Administration of IL-2 in leukaemic mice is found to elevate the rosette forming capacity moderately (P=<0.001). But in interferon treated subjects the rosetting capacity is significantly increased almost reaching normal value (P=<0.001). SRBC on the otherhand is found to be by far in best stimulator of rosette formation in leukaemia induced mice. When used in combinations with SRBC, IFN-γ or SRBC, IL-2 & IFN-γ such results continue to remain the same, IL-2, however, maintains a lower value in combination with SRBC or IFN-γ. (P=<0.001) (Fig. 2).

Comments: Formation of Erythrocyte rosette or ‘E-rosette’ between T-cell and sheep RBC (SRBC) not only separate T-lymphocyte from the B-lymphocyte, but also indicate the presence if active lymphocytes through their receptor ligand interaction. From the above data we have found
LEGENDS TO FIGURE:

Fig. 2:

Represents spontaneous E-Rosetting capacity of spleenic lymphocyte (SP60) of different groups of animals including normal control: Significant decrease of rosetting capacity of SP60 has been noted in ENU induced leukaemic mice which following BRMs treatment showed significant label of improvement in E-Rosetting capacity, SRBC(s) has got a notable effect (Chapter – IV, Sec. – I).
that during leukaemic condition maturation of lymphocytes are arrested but after the applications of biological response-modifiers (BRMs) there is increasing in receptor functions which could be represented in 'E-rosette' formation. Above data conclude that specific modifier like interferon-γ (IFN-γ) or nonspecific modifiers such as SRBC help in maturation of T lymphocytes in a regulated manner. Another important aspect of BRM administration in leukaemic mice concerned the generation of more active T lymphocytes with a probable immuno-potentiation.
Section – II

Cytotoxicity assay by fluorochrome release phenomenon (H33342)
Lymphocytes from immunized donors can kill target cells \textit{in vitro} in the absence of complements, and this form of cytotoxic reaction can readily be made quantitative by counting viable cell loss or measuring release of radioactive chromium ($^{51}$Cr). In allogenic system showing one-hit kinetics of target cell destruction $\frac{1}{2}$ per cent of lymphocytes appear to be active (Wilson 1965) (271), but in tumour systems the fraction is smaller. The cells active in this reaction appear to be T-lymphocytes, for educated thymus cells (Spleen cells taken from mice which have been lethally irradiated, and repopulated with allogenic thymus cells) are active in the mouse, and activity is lost after treatment with anti-antiserum (Cerattiai, Nordin & Brunner 1970 a, b) (272 a,b). Cytotoxic activity is found predominantly in a subpopulation of lymphocytes, which is larger, more pyroninophilic, and more radio-resistant than the average (Ginsburg 1968) (273). Such cells designated as cytotoxic T cells has cluster character CD8.

**MATERIALS:**

1. Lymphocytes
2. Dalton's lymphoma (Target)
3. Hoechst 33342 (Fluorochrome)
4. RPMI Media.

**METHODS:** Cytotoxic Efficacy:

1. **Preparation of effector-cells:** (E) From the single cell suspension of spleen, lymphocytes were separated from the neutrophils by density gradient method through percoll layer ($1.077$). Then lymphocytes were collected centrifuge and washed in 0.95 Saline twice and then harvested in non FBS RPMI media.
Preparation of Target cells: For target cell preparation Dalton’s lymphoma were collected from the peritonium of mice and then washed in normal saline and finally harvested in FBS-media.

Labelling of Target: Tumor cells were taken into centrifuge with Hoechest 33342 fluorochrome (6kg/10⁶ cells) and incubated for 15 min. at 37°C. After incubation it washed by PBS and ready for experiments.

Lysis of Cells: For the lytic experiment, effector and target were taken at a ratio of 10:1 and incubated for 18 hrs at 37°C. Cells were centrifuged and the supernatant was taken to read in a flurometer (400 & 490 nm. Exciting emission wavelength). Then reading were taken at 400 and 590 nm (Chaudhuri et al, 1998).

RESULTS: It has been found that the functions of the cytotoxic lymphocytes (CD-8) also suffered a significant decrease in cytolytic properties against the target in leukaemic condition (P=<0.001).

As far as the cytotoxic efficacy of the lymphocytes are concerned the BRM group of cells revealed significant increase in cytotoxic efficacy; Treatment of IL-2 either alone or in combinations to the leukaemic mice showed significant trigger in both the cases.(P=<0.001). Administration of IFN-γ do not, however, modify the cytotoxic efficacy in leukaemic mice when treated as above. In combination with SRBC, interleukin or both, the cytotoxic efficacy supercede the effects of single therapy of SRBC or interleukin. Nevertheless, these results are beneficial compared to those found in untreated leukaemic mice. The data have been presented in fig.3.
LEGENDS TO FIGURE:

**Fig. 3:**

Cytotoxic efficacy of spleenic lymphocytes (SP60) of different groups of animals including normal (N), ENU induced leukaemia (E) and those treated with BRMs either in single or in combinations doses (E2rs). (Chapter – IV, Sec. – II).
SP-60

CTL ASSAY
COMMENTS: Cytotoxic efficacy of lymphocytes in different groups including the leukaemic and BRM treated leukemic mice revealed significant outcome to ensure the beneficial effects of BRMs on leukemic animals. The data showed the individual capacity of IL-2 IFN-γ and SRBC in modulations the Cytotoxic immune-efficacy of lymphocytes mostly of CD8 category although some natural killer cells (NK) might have also been included. Analysis of data also showed that IFN-γ administrated in vivo could not satisfactorily modify the function when compared with IL-2 effects. SRBC was nevertheless, effective almost equally. The most important outcome was the overall significant increase of CTL activity in animals receiving IL-2, IFN-γ and SRBC in combinations. It seems reasonable that IL-2 and SRBC could overcome the inhibitory effects of both leukaemia and IFN-γ under the event and reorganise the cytokine network for stimulated activity.
Section – III
PMN mediated phagocytosis by NBT reduction assay
PMN MEDIATED PHAGOCYTOSIS BY NBT REDUCTION ASSAY:

Polymorphonuclear Neutrophils constitute more than half the circulating white cell population in most species and are characterized by multilobed nucleus and characteristic cytoplasmic granules. These include primary (azurophilic) and secondary (specific) granules, first isolated by Cohn and Hirsch (1960) (274). More recently, a third granule type has been identified (Baggiolini 1980) (275). The granules contain an armoury of enzymes, proteins and glycosaminoglycans believed to participate in many of the functions of the cell. Neutrophils are essential for effective host defence, specifically the phagocytosis and killing of pathogens in tissues. Their critical role in this process is exemplified by a group of individuals whose neutrophils are deficient in the CD 11/CD18 groups of adhesive glycoproteins. The rapid localization of these circulating cells to sites of local insult and their ability to penetrate through the vessel wall and migrate into the tissues represent key elements of neutrophil function. Such an accumulation of inflammatory leucocytes in response to local injury was first described by Dutrochet (1824) (276), who observed the emigration of blood cells from vessels in the tails of tadpoles. Additionally, these cells are highly phagocytic and, upon stimulation, mount an impressive oxidative burst and generate a variety of highly reactive metabolites are also considered to be important contributors to the paradoxical injurious potential of these cells in inflammatory lesions (Fantone and Ward, 1982) (277). The present section involves the assessment of PMN mediated phagocytosis in different groups of animals as described before.

MATERIALS:
(1) Spleenic Neutrophil. (Percoll density at 1.084).
(2) Dalton's Lymphoma (Target).
(3) Nitroblue tetrazolium chloride (NBT, Loba).
(4) RPMI 1640 Media. (Gibco, BRL, USA).
(5) Percoll (Pharmacia, Sweden).
(6) Pyridine (Sigma USA)
(7) 0.1N HCL (Chilled).
(8) Lipopoly saccharids (LPS, Sigma, USA).

METHODS:

(1) Preparation of Effector Cells: (E):

From the Single cell suspension of spleen, Neutrophil were separated from the lymphocytes by density gradient centrifugation method through percoll at 1.089 layer. The cells were collected at the interface and taken into centrifuge tube and washed in 0.9% saline twice and then harvested at RPMI.

(2) Preparation of Target cells: (T):

Tumour cells or Dalton's lymphoma were taken as a target for the NBT reduction assay. Lymphoma cells were aspirated from the mice and it was maintained at non-FBS-RPMI 1640 media after washing with 0.9% saline.

(3) Preparation of NBT (Nitroblue tetrazolium chloride):

1% NBT solution was prepared from the stock in a saline and centrifuged to set clear solution.
NBT Reduction assay:

To demonstrate the experiment, effectors and targets were taken at 10:1 ratio with 100 µl of 1% NBT solution and 15 µl LPS and then it was kept into 37°C incubator for 18 hrs. After incubation experiment was stopped by 0.1% chilled HCL solution, and then was centrifuged for 10 min. After centrifugation 3 ml pyridine was added to each tube containing the pellet. The samples were cooled and centrifuged to get clear bluish supernatant. The color was read at 530 nm in a spectrocolorimeter.

Results:

Results reveal that the neutrophil mediated immune function in the leukaemic animal suffer a simultaneous diminution in their phagocytic properties against the target. The phagocytic as well as secretory capacity of the neutrophil are found to be affected under the conditions (P=<0.001), but responded to variable degree of stimulations following applications of cytokines and non-specific BRM like SRBC (P=<0.001). As evident from the results interleukin-2 (IL-2) has minimum effect (P=<0.001) whereas IFN-γ shows a significant increase (P=<0.001) in phagocytic property as evident from NBT reduction assay. When treated with SRBC, it shows significant benefit but the most significant results were obtained after the treatment of SRBC + IL-2 + IFNγ in combination (P=<0.001). The data have been presented in fig 4.

Comments:

The nitroblue tetrazolium reduction assay (NBT assay) is a satisfactory method to measure the activity of neutrophil which can kill the tumour
Fig. 4:

Phagocytic efficacy of polymorpho nuclear neutrophil from spleen (SP75) showing suppression in leukaemic mice (E) and significant improvement in phagocytic efficacy following combination (E2rs) BRMs therapy (Chapter – IV, Sec. – III).
Fig. 4

SP-75 ASSAY

NBT REDUCTION

COUNTS
cells through phagocytosis. So the stimulation of neutrophil phagocytic
capacity can reduce the tumour load during treatment of leukaemia.
Our findings also reveal that stimulation of phagocytosis by neutrophils
can be achieved after the treatment of specific or non-specific
biomodulator like IFN-γ/IL-2 or SRBC respectively. The combination of
BRMs including SRBC, thus, was much effective even in the face of
tumorigenic inhibition of PMNs. Such effects were found unique
phagocytosis in PMNs against tumour.
Section – IV

Leucocyte Adherence Index (LAI)
LEUCOCYTE ADHERENCE INHIBITION ASSAY

Leucocyte when allowed to rest in glass or plastic petry dishes in presence of a suitable nutrient media, exhibit sticky adherence with some of the population. These cell types mostly comprising monocyte and macrophages constitute a potential group of phagocytic killer against invasive targets (Metnikoff, 1884) (278). The degree of adherence were found to correlate the phagocytic defence in a linear fashion (Lachman, 1993) (279). Thus Leucocytes isolated from different groups of animals when allowed to incubate at 37°C in a nutrient media, are expected to exhibit the adherence and adherence inhibition (AI) at the surface of the plastic dishes reflecting the relative immunocompetence of the cells concerned. The present section embodies such adherence efficacy of that particular type of cells under the event of Leukaemia development and following administration of some BRMS in leukaemia animals.

Materials and Methods:

(i) Haemocytometer chamber in a sterile moist petridish.
(ii) Normal Saline.
(iii) Syringe (2ml sterile with needle).

METHODS:

(i) Cells in media were counted and sized to approximately $5 \times 10^6$/ml and were charged in the hemocytometer chamber and the distribution of the cells were recorded as the initial count.

(ii) The cells on to the chamber were then incubated at 37°C for one hour at the end of which they were gently washed off with prewarmed (37°C) 0.9% saline.
After washing (thrice, gently) the final count of these cells were recorded.

The adherent index has been calculated as: $\text{LAI(\%)} = \frac{\text{Initial count} - \text{Final count}}{\text{Initial Count}} \times 100$.

**Results:**

From the above (Fig. 5) it has been found that leucocyte adherence was reduced ($P<=0.001$) during ENU induced leukaemic conditions. It was increased significantly ($P<=0.001$) when treated with IL-2. But IFN-γ or in combination of IFN-γ + IL-2 can not be beneficial ($P<=0.001$) than that of SRBC ($P<=0.001$) alone (Fig. 5)

**Comments:** Macrophages are very important immunocytes in tumour immunology, that was found to be activated by SRBC, interleukin-2 and interferon-γ as well, but the latter showing the usual relative inhibitory effects on the above score. The adherence capacity of macrophages was found to be significantly elevated following SRBC and IL-2 treatment. Application of IFN-γ minimise the sum total effect which is supposedly the masking inhibitory effect of IFN-γ. The adherence effect under the conditions represent a high macrophage activity with respect to phagocytosis or cytotoxic killing.
LEGENDS TO FIGURE:

Fig. 5:

Adherence index of splenic lymphocytes before & following applications of BRMs in leukaemic mice (E). Notably single BRMs were found to be more effective whereas combinations (E2rs) failed to improve adherence capacity (Chapter – IV, Sec. – IV).
Section – V

Antigen presenting capacity of macrophages and lymphocytes:
PLN assay
POPLITEAL LYMPH NODE ASSAY: (PLN Assay).

The work of Metchnikoff with wandering mesodermal cells in starfish larvae established the existence of specialized host cells adapted to serve a protective role through ingestion and removal of invading microorganisms [Metchni-Koff, 1884 (278), Chernak and Tauber 1988 (280)]. In mammals, the phagocytic cells of Metchnikoff exist as neutrophils and macrophages. The latter are extra-ordinarily versatile cells that play a central role in specific immunity as well as in non-specific aspects of host defence. In the specific immune response macrophages present antigen to lymphocytes and serve as supportive ‘accessory’ cells to lymphocytes, which is accomplished at least partly through release of soluble factors. Their capacity to ingest and kill invading micro-organisms is fundamental to their non-specific protective function, and they release an enormous number of factors involved in host defence and inflammation. This broad array of essential functions places macrophages at the centre of the contemporary concept of immunity. The role of these cells as ‘antigen presenting cells’ (APC) has largely been established from the work of unanne and Benaceraff (281). APC has successfully been arrayed by Sorensen’s popliteal lymph node (PLN) assay. The present section involves APC of cells prepared as above.

Materials:

1. 1% starch solution.
2. 0.9% Nacl (Normal Saline).
3. Phosphate Buffer solution (pH 7.2).
4. Spleen lymphocyte.
5. Peripheral macrophages.
6. Forceps.
Methods:

(a) **Injection of Starch Solution**: 1% starch solution was injected intraperitonealy into leukaemic as well as biological response modifier treated mice at a volume of 3 ml each.

(b) **Collection of Macrophages & Lymphocytes**: 6 days after injection of starch solution, macrophages were collected aseptically from the peritoneum of the leukaemic mice into normal saline. Then lymphocytes were collected from the spleen as described before. Then both cells were sized at $1 \times 10^5$/ml and heat inactivated (50°C) lymphocytes were prepared for injection.

(c) **Injection of macrophages & Lymphocytes Into Rat**: Prepared dose of macrophages and lymphocytes ($1 \times 10^5$ cells) were injected into Rat's right and left footpad respectively.

(d) **Collection of Lymph Node**: 6 days after injection of cells in the foot pad, animals were sacrificed and then left and right lymph nodes were collected carefully and then weight of the lymph nodes were taken.

Results: The antigen presenting capacity as represented in terms of poplitateal lymph node assay showed (Fig. 6) that induction of leukaemia mildly stimulated the APC of macrophages from their normal counterpart. But administration of SRBC and IL-2 *in vivo* revealed a significant beneficial effects ($P<0.001$) on antigen presentation. But application of interferon-γ showed a decreased value ($P<0.001$) in ENU induced leukaemic mice. The ENU treated group, however did not show any benefit whereas, IL-2 + IFN-γ were used in combination, rather the
LEGENDS TO FIGURE:

Fig. 6 (a & b):

Comparative antigen presenting capacity of Mφ (macrophages) and Ly (Lymphocytes) from spleen of different groups of animals.

(a) represents the data of normal groups with BRMs and,

(b) data representing cells from ENU treated mice with different groups of mice. In all the cases macrophages were found to be more potent APC than Ly and, further, the combination BRMs therapy significantly stimulating in both normal and ENU treated mice (Chapter – IV, Sec. – V).
PERCENTILE WEIGHT OF PLN

M = Macrophage
LY = Lymphocyte

NORMA
PLN ASSAY

+2.39
+1.37
+1.32
+1.26
+1.09
+0.68
+0.37
+0.00
+2.39
+1.51
+3.9

PERCENTILE WEIGHT OF PLN
value was reduced to a insignificantly lower level (P=>0.001). Antigen presenting capacity (APC) was found to be activated and benefited after the treatment of SRBC, interleukin-2 (IL-2) and interferon-γ in combination (P=<0.001).

**Comments:**

From the above data it has been shown that the activity of macrophages which is represented as antigen presenting capacity when treated with SRBC and IL-2 either alone or in combination. But IFN-γ does not show any beneficial performance in case of activity of macrophages. Rather IFN-γ decreases the value of APC of macrophages. So it can be concluded that SRBC or IL-2 help in tumor killing through increase the activity of macrophages through APC. The overall immune responsiveness is, therefore, was found to be beneficial with the treatment of IL-2 SRBC and IFN-γ in combination under the leukaemic event.