SUMMARY

As because the tumour cells possess diverse types of antigens (TAAs) on their surface, the possibility of immune response of polyclonally stimulated lymphocytes towards the tumour target cells was studied in the present investigation. First, the efficacy of polyclonally stimulated cells was tested by co-grafting them with a tumour piece explant in the anterior eye chamber of a syngeneic animal where a tumour piece transplanted alone can induce neovascular reactions or angiogenesis. Then, the stimulated cells were injected at the tumour site to note their ability in curbing the growth of the tumour. In another experimental series, the lymphocytes of the tumour bearing hosts were directly stimulated. Cytotoxic ability of the polyclonally stimulated lymphocytes towards tumour target cells was observed in ^{51}Cr release assay. As, a polyclonal T cell stimulator is supposed to activate the suppressor subset of T cells also, in one set of experiments activation of T lymphocytes was done *in vivo* after removal of suppressor T cells with cyclophosphamide treatment and their response towards tumour cells were tested in *in vitro* cytotoxic assays and in *in vivo* adoptive transfer experiments.

Following the earlier studies, we found that the dose of 50 µg of the plant lectin Concanavalin A (Con A) injected in a mouse is optimal to stimulate the T cells in terms of blastogenesis and DNA synthesis. The tumours were induced by

using 2 mg of 3'-Methylcholanthrene per animal subcutaneously. After several trials with different doses and different makes of MCA, the dose of 2 mg of MCA (Sigma Chem. Co., St. Louis, USA) per animal was found most effective in inducing fibrosarcimas in terms of early appearance and faster rate of growth of tumours.

A tumour piece transplanted in the anterior eye chamber of mouse can induce neo-vascular reactions like vasodilatation and appearance of new vessels and their branches in two phases over the cornea. A piece of lymph node containing Con A activated lymphocytes, grafted along with a tumour piece can dramatically inhibit these reactions and growth of tumour observed upto 25 days. Non-stimulated lymphocytes in control experiments could not inhibit these reactions induced by a tumour explant.(Plate 5).

The possibility of incapaciation of tumour cells by the Con A activated cells was tested by incubating the tumour explants with these effector cells in vitro for 24 and 48 hr. Incubation with Con A activated lymphocytes reduced significantly the ability of tumour pieces to induce angiogenic reactions over the cornea and to grow in the anterior chamber of eye. Their ability to incorporate radioactivity in vitro was also curbed to a great extent after incubation with stimulated lymphocytes.

The efficacy of these polyclonally stimulated cells to curb tumour growth was also studied after transferring them at

the tumour site and then measuring the size of tumours and the life span of the hosts. Three different concentrations of cells (5×10^5, 10^6 and 2×10^6/animal), was tried, of which the dose of 2×10^6 cells/animal appeared to be most effective in retarding the tumour growth; this is obvious from the slopes of the lines drawn for tumour growth. The slower growth rate of tumour enabled 15% hosts to survive beyond 150 days whereas all the control animals died within 98 days.

These observations led to an approach of stimulating hosts own lymphocytes to combat the malignant growth. Two i.v. injections of Con A, one 5 days prior to and another after 5 days of tumour induction with MCA was made, which could not influence the tumour growth very significantly. Whereas, repeated Con A injections at 10 days intervals, for 12 times starting from the day of palpable appearance of tumour inhibited the growth rate of tumour to a great extent and thereby increased the life span of the hosts.

The possibility of clonal exhaustion of responsive lymphocytes due to such repeated stimulation with a polyclonal stimulator was analysed by using normal mice and noting the changes in cellular organization of their lymphoid organs. Percentages of blasts with repeated stimulations were comparable to that of normal mice stimulated once. At the peak hour of blastogenesis the lymphocytes in spleen and lymph nodes increased in number and after that, some of them were found in the

lacunae or spaces of the lymphoid organs in histological preparations as if they were flowing out of the lymphoid organs. However, the total lymphocyte content in these organs and in per ml of peripheral blood after the wane of each stimulation, usually did not fall below the values of normal non-injected animals. Body weight of the experimental animals during the course of the injections was same as in control normal animals indicating that there was possibly not much adverse effect of repeated injections on physiology of the animals. Very low titre of antibody to Con A was detectable after three times injections of Con A and discussion has been made whether this could be one of the reasons to make repeated injections of Con A *in vivo* to stimulate host's lymphocytes comparatively less effective than adoptive transfer of effector cells directly at the tumour site.

Cytotoxic ability of these activated lymphocytes to kill radiolabelled tumour target cells in *in vitro* assay suggests the possible mechanism of immune response of Con A stimulated lymphocytes to 3'-Methylcholanthrene-induced tumour cells. Normal non-activated lymphocytes could only mount a very limited cytotoxic reaction to the tumour target cells. The possible involvement of natural killer cells in background level response has been discussed.

Removal of T cells prior to activation always abrogated the response of activated lymphocytes both in cytotoxic assays and in anterior eye chamber transplantation experiments.

Elimination of T cells was done by neonatal thymectomy and subsequent treatment of these animals with anti-mouse thymocyte serum raised in rabbit. Indeed, poor generation of blasts like 16 to 18 percent was noticeable after 48 hr of Con A injection in the animals depleted of T cells. The T cell nature of the Con A responsive cells was further confirmed by their susceptibility to the anti-thymocyte serum *in vitro*.

Although, far less the amount of Con A than the doses usually responsible for suppressor cell generation was used for lymphocytic stimulation in the present investigation, it was observed that depletion of suppressor cells with cyclophosphamide pretreatment augmented considerably the immune response of the residual T cells in cytotoxic assays and in curbing the tumour growth *in situ* on adoptive transfer. Out of four different doses, the dose of 25 mg of cyclophosphamide per kg body weight of an animal was found to be optimal for the purpose on the basis of its effectiveness to enhance the Con A response in terms of *in vivo* and *in vitro* blastogenesis. Increase in *in vivo* Con A induced blastogenesis was reflected in the increase in size and weight of the secondary lymphoid organs of the animals pretreated with cyclophosphamide and accompanied increase in ^{3}H-TdR uptake by the lymphocytes.

Feasibility of extending this model of polyclonal stimulation of T cells in other animal systems including man to combat malignancies has been suggested here.