INTRODUCTION

Specificity of immune responses as expressed in higher animals, have evolved from self non-self recognition mechanism which enable the organisms to escape from foreign invading agents. Foreignness of invading agents is usually attributed to antigenic moities on their cell surface. Acquisition of such foreignness is an usual phenomenon with neoplastic transformation and thus malignant growths are rendered susceptible to the immune response of the organism. Evidences for antigenicity of experimentally-induced tumours were demonostrated by several workers since nineteen fifties (Folly, 1953; Prēhn and Main, 1957; Prēhn, 1960; Klein et al, 1960). Their studies revealed that chemically-induced tumours transplanted to syngeneic animals are rejected, and the rejection was faster when the animals had tumour inoculum after complete excision of primary tumours. This process was considered as manifestation of immunological response induced by antigenic determinants on the tumour cell surface.

Tumour associated antigens (TAA) are broadly categorized in to three classes - oncofetal antigens, antigens of virally-induced tumours and antigens of chemically-induced tumours. Oncofetal antigens are the products of some genes in tumour cells which become silent in the course of embryonic

differention and are re-expressed due to the process of "dedifferention" (Brawn, 1970; Coggin and Anderson, 1974; Alexander, 1975). They serve as individual markers for different tumours and are antigenically poor to elicit any immune response (Baldwin et al, 1972; Fredman et al, 1974; Evans et al, 1979; Mastaka et al, 1984). Sjogren et al (1961), Sjogren (1961) and Appella and Law (1976) observed that antigenic properties of tumours induced by a particular oncovirus share some commonness even when they originate on different tissues or different strain of animals on one hand and on the other different strain of viruses induce tumours of different antigenicity even in the same tissue or in the same strain of animals. This antigenic specificity expressed on tumour cells might be determined by the antigenic constituents of viruses (Habel, 1961) after their genes being integrated with host cell genome (Temin, 1971; Gillespie et al, 1975; Rowson, 1975; Talk, 1977; Zinkernagel, 1979).

Antigenicity of chemically-induced tumours varies from tumour to tumour. The rarity of immunological cross-reactivity amongst chemically-induced tumours arising in the same inbred strain of mice was observed by many workers (Folly, 1953; Klein and Klein, 1962; Old et al, 1962; Old and Boyse, 1964; Bassambrio, 1970; Baldwin et al, 1971b). They pointed out that the variability in antigens was influenced by sex and strain of host, dose and type of carcinogen used and the route of adminis-

tration. By using a single carcinogenic agent Globerson and Feldman (1964) and Wahl et al (1974) could not find commonness in antigens on tumours developed at different tissues of the same animal. This uniqueness of antigens on chemically-induced tumour cell surface could be traced even on the cells isolated from different zones of the same murine fibrosarcoma induced by a single carcinogen (Prehn, 1970). The heterogeneity was also reflected in the differential sensitivity to different drugs which is again related to the distribution of receptors for drugs on the cell membrane (Hapner et al, 1978).

Tumour associated antigens (TAA) are likely to initiate immune responses involving several arms of host's immune system. Involvement of macrophage in tumour immunity has been shown by several workers (Nelson, 1972; Hibbs et al, 1972; Evans, 1976; Horwitz et al, 1979; Taglibaue et al, 1979; Krishnan and Orwell, 1979; Evans and Lawler, 1980; Talmadge et al, 1981). It has been shown that antibody armed or antibody-coated macrophages could destroy the tumour target cell effectively (Tosi and Weiser, 1968; den Otter et al, 1972; Krahenbuhl and Remington, 1974). But in some other reports, no correlation between macrophage content in excised tumour mass and growth of the tumour could be ascertained (Eclas and Alexander, 1974; Loveless and Happner, 1983; Ishi et al, 1984).

Hellstrom and co-workers have shown that tumour bearing host often possesses lymphocytes which are cytotoxic to its

tumour cells in vitro (Hellstrom and Hellstrom, 1969; Hellstrom et al, 1971; Rusell et al, 1976; Vose et al, 1977; Vose, 1980). The number of such lymphocytes declined in hosts having progressive tumours. The cells isolated from solid tumours by Herberman (1974), Betz and Simar (1974); Russell et al (1976) and Lala and Kaizer (1977), were found to be of mixed type mostly Thy-1$^+$ lymphocytes, some IgM$^+$ lymphocytes, null cells and macrophages. However, it has now been accepted that, of all the cell types Thy-1$^+$ or θ^+ cells have the most crucial role in responding against malignancies, since the cytotoxic cells generated in vitro or in vivo against experimental tumours were always found to be Thy-1$^+$ or T lymphocytes (Fredman et al, 1972; Cerottini and Bruner, 1974; Wybran et al, 1974; Ting, 1976; McClusky and Bhan, 1977; Weinstein and Okan, 1980; Herberman et al, 1980; Vose, 1980; Green, 1981; Keder and Weiss, 1981). So employment of T lymphocytes, the chief mediator of tumour immunity, against malignant tumours appeared to be practicable.

Activation of immunity to malignancies by immunization with tumour cells as such is not practical for obvious reasons, like heterogeneity of tumour cell surface antigens, chances of generating some blocking factor(s) in the serum (Alexander, 1974; Sjogren et al, 1971; Hellstrom and Hellstrom, 1969; Hellstrom et al, 1970; Hellstrom and Hellstrom, 1974; Ray and Saha, 1982) and risks of inducing malignancy. Thus other means of

stimulating the host's immune system became imperative.

Mathé and others stimulated the reticuloendothelial system of tumour bearing animals with some non-specific agents like Bacillus Callamette-Guérin (BCG) to control malignant growth and obtained limited success (Mathé et al, 1970; Mathé 1971; Baldwin and Primi, 1971; Zbar et al, 1972; Bartlet et al, 1972; Hawrylko, 1977). Use of Corynebacterium parvum (Woodruff and Boak, 1966; Scot, 1974; Woodruff and Dunber, 1975; Purnell et al, 1976; Prim and Baldwin, 1977; Woodruff and Warner, 1977; Gupta et al, 1978; Meyata et al, 1983) and some other killed micro-rganisms, (Parker et al, 1974; Blast et al, 1975; Pruvell, 1975; Ray et al, 1979; Ray et al, 1980) produced some hopeful results. It had been postulated that these microrganisms mostly stimulate the macrophage arm of immune system. However, T cells are considered to be more effective to combat tumour growth and therefore stimulation of T cells rather than macrophages is likely to inhibit the tumour growth in a much effective way. Again, in view of heterogenous nature of tumour antigens, stimulation of several clones of T cells rather than a specific clone, would be more fruitful. In such a situation, stimulation of T cells in a polyspecific way is possibly more desirable.

Concanavalin A (Con A), a plant lectin and a general mitogenic agent has been shown to activate multiple clones of T lymphocytes in vitro (Stobo and Paul, 1973; Waterfield et al,

1975; Heininger et al, 1976; Chakravarty and Clark, 1977; Green et al, 1978; Chakravarty, 1980). Several of these workers and others (Clark, 1975; Bevan and Cohn, 1975; Bevan et al, 1976; Heininger et al, 1976; Bonavida and Bradly, 1976; Green et al, 1978) have observed that in vitro stimulation with Con A drives the T lymphocytes all the way to differentiate into cytotoxic cells against a variety of targets bearing non-self antigens including tumour cell lines. Moreover, some of the stimulated cells ultimately become memory cells and secondary stimulation with Con A can regenerate the cytotoxic function among these cells as in case of MLC primed cells (Chakravarty and Clark, 1977; Clark et al, 1977; Bonavida, 1977; Chakravarty, 1978).

Anaclerio et al (1974) and Waterfield et al (1976) indicated the possibility of using Con A to activate lymphocytes in the in vivo situations to generate cytotoxic cells. Subsequently, Choudhuri and Chakravarty worked out in detail the process of Con A mediated activation of murine T cells in vivo in terms of blastogenesis, DNA and other macromolecule synthesis (Choudhuri and Chakravarty, 1981; Chakravarty and Choudhuri, 1983) and differentiation of activated cells into cytotoxic cells (Choudhuri, 1983). Thus multiple clones of T cells stimulated simultaneously with Con A in vivo might be effective to mount immune response against chemically-induced malignant cells having diverse type of antigens on their surface. Investigation of such possibility has been the main

objective of the present study.

First, growth and other associated phenomena of tumour explants in the anterior eye chamber of syngeneic animals was studied in the presence of normal and Con A-activated lymphocytes. Anterior eye chamber has long been known as a privileged site for transplantation as, any allograft or heterograft (Gregoir and Symbiose, 1935) and tumour grafts transplanted therein enjoys prolonged survival. Auerbach (1961) used this method for culturing different embryonic tissues of mice in the anterior eye chamber. This technique has been adopted here to co-culture tumour explants with different combinations of activated and non-activated lymphocytes. Moreover, Folkman (1974), Gimbrone et al (1974) and Folkman and Cotran (1976) showed that a piece of malignant tumour when placed in the rabbits' corneal pocket, it induced vaso-proliferations from limbal vessels of cornea towards the tumour grafts. This vaso-proliferation was accompanied by vasodilatations and primary sprouting of capillaries and secondary development of vessels from the tip of the primary vessels. These reactions have nicely been shown to occur with a similar graft in mouse corneal pocket (Muthukkaruppan and Auerbach, 1979). This neo-vascular reaction, known as tumour-induced angiogenesis (TIA) has been shown to be induced by tumour angiogenesis factor (TAF) secreted by the tumour cells (Greenblast and Shubik, 1968; Folkman and Cotran, 1976; Weis et al, 1979; Folkman, 1982). These neovascular reactions were found to precede the growth of almost all the tumour transplants

tested at different sites (Gimbrone and Gullino, 1976; Brem et al (1978; Ziche and Gillino, 1981; Ziche and Gullino, 1982). In this study, the angiogenic reactions and growth of tumour explants grafted in the anterior eye chamber of syngeneic mouse constituted the parameters to measure the effect of activated lymphocytes on tumour explants. In another set of experiments, the tumour explants were incubated with Con A-activated or non-activated lymphocytes in vitro for 24 or 48 hr prior to implantation in the anterior eye chamber.

The efficacy of Con A activated T cells in controlling the growth of tumours was also tested by adoptively transferring at the site of the tumours in hosts.

Next, the lymphocytes in tumour bearing mice were repeatedly stimulated with Con A and the growth rate of tumour and survivality of hosts were studied. Necessary control experiment to study the effects of repeated in vivo stimulation with Con A on lymphoid system was also performed.

Efficacy of Con A generated cytotoxic cells on the tumour target cells was measured in ^{51}Cr release assay. To ascertain that Con A activated T cells are effective in anti-tumour immune response, T cells were depleted by neonatal thymectomy and subsequent treatment of the animals with anti-thymocyte serum prior to stimulation of its residual lymphocytes with Con A.

T cell population is virtually a mixture of functionally different subclasses like effector cells, helper cells and suppressor cells (Gershon et al, 1972; Cantor and Boyse, 1975a, b; Hodes and Hatchecock, 1976; Cantor and Boyse, 1978). Con A being a polyclonal stimulator for T cells, might stimulate all the clones of T lymphocytes including suppressor subset, both in normal and tumour bearing animals (Shou et al, 1976; Raff et al, 1978; Ekstedt, 1979; Eibl et al, 1980; Catalona et al, 1980). It is likely then, that the effector functions of T cells could possibly be augmented if the suppressor subset of T cells can be eliminated from the lymphocyte population.

There are several ways to deplete suppressor cells; the presence of Ly 2,3 markers and the determinants encoded by the I-J subregion of H-2 complex on their surface render them susceptible to alloantiserum against these antigens (Fujimoto et al, 1976; Perry et al, 1974; Perry et al, 1978; Green et al, 1979). They are also sensitive to low doses of radiation (Rotter and Trainer, 1975; Hellstrom and Hellstrom, 1978; Daynes et al, 1979) and the drug cyclophosphamide (Rollinghoff et al, 1977; Tagart, 1977; Green et al, 1979; Ray and Raychoudhuri, 1981; Berd et al, 1982; Berd et al, 1984). The cyclophosphamide sensitivity of this subset of T lymphocytes haa sometimes been employed to enhance the antitumour immune response (Hellstrom, and Hellstrom, 1978; Broder et al, 1978; Yu and McKhann, 1978; Ferguson and Semmons, 1978; Yu et al, 1980; Turk and Parker, 1982; Berd et al, 1982).

In this investigation an attempt has been made to enhance the response of Con A activated lymphocytes by removing the suppressor subset of T cells with cyclophosphamide treatment. The efficacy of suppressor cell depleted, Con A activated lymphocyte population was tesfed by cytotoxicity assay and by adoptively transferring to tumour bearing mice.