DISCUSSION

It is known for a decade that Con A and to a limited extent PHA can activate the murine lymphocytes to transform into blasts and then into cytotoxic cells *in vitro* (Stobo and Paul, 1973; Waterfield *et al.*, 1975; Heininger *et al.*, 1976; Chakravarty and Clark, 1977). Detailed study of Con A or lectin mediated activation of T cells *in vitro* has been made by several workers to understand the process of activation of the lymphocytes initiated by a stimulant like lectin or antigen. But these *in vitro* studies could not provide much physiological relevance of using lectins. This is because there was not much information about activation of lymphocytes by using the polyclonal agents in *in vivo* situation.

Objective of the present study was to find out whether the lymphocytes could be stimulated *in vivo* with Con A, PHA and FCA, and whether stimulated cells could undergo the differentiation all the way to the cytotoxic cells. Simultaneously the effects of these substances on the condition and organisation of the lymphoid cells in secondary lymphoid organs *in situ* have also been investigated by studying the histological sections of spleen and mesenteric lymph node of the animals treated with these agents.

The question, whether the lymphocytes could be stimulated *in vivo* with the polyclonal agents have been analysed from several points of view. Measure of rate of blastogenesis was the first criterion to get an idea which substance at what concentration and at what time could effectively transform the quiescent lymphocytes into blasts. The process of blast transformation is the culmination of several biochemical events and affects almost every metabolic pathway (Key, 1966; Forsdyke, 1967; Cooper, 1968; Ross and Loss, 1970; Cooper, 1972; Andersson and Melchers, 1973). Synthesis of macromolecules like DNA, RNA and protein are also accompanying events with blastogenesis and these have also been taken into account in this study.

By most of the counts defined so far, Con A seems to be the best stimulator of the lymphocytes *in vivo* and PHA is the poorest of all. Nature of blast cells and the kinetics of differentiation induced by Con A *in vivo* stimulate those of *in vitro* (Chakravarty and Clark, 1977). In both *in vivo* and *in vitro* set ups the peak of the responses was around 48 hours. This is interesting for *in vivo* set up as because the substance passes through the different systems before getting contact with the lymphoid cells in secondary lymphoid organs, whereas in *in vitro* system, the substance get direct contact with the cells from the very beginn-

ing.

It is interesting to note that pretty close to hundred percent of blasts can be obtained from different organs and peripheral blood with 100 µg Con A treatment. As 30-35% T cells are present in spleen and 65-70% of them in lymph nodes and the rest of the lymphocytes are B cells and Con A is supposed to stimulate only the T cells, it is not possible to obtain 100% blastogenesis index without participation of B cells. It is possible that the T cells stimulated with Con A *in vivo* could release certain kind of factors or lymphokines which might cause blastogenic transformation of B cells as observed by Primi *et al.*, (1979) in *in vitro* study or by being non-soluble and cross linked *in vivo* Con A is capable of stimulating B lymphocytes as it has been shown possible *in vitro* by Andersson *et al.*, (1972). There would have been another possibility of making 100% blasts by T cells only if the B cells are already dead. In favour of this possibility we observed a high percentage of dead cells, more than 50% with 100 µg Con A treatment (unpublished observation). But it was not possible to know the identity of the dead cells as B cells.

To ascertain whether there could be blastogenesis with *in vivo* treatment of Con A in absence of thymocytes, the experiment with neonatally thymectomized mice, treated with rabbit anti-mouse

thymocyte serum was carried out (anti-Thy-1 serum was not available with us). The results indicate that in vivo stimulation index with Con A is of much lower magnitude in the thymectomized and anti-serum treated mice, but not to the background level (Table-12). Thus this experiment shows that majority of the Con A-induced blasts in vivo derive from T cells in mice but the possibility of certain percentage of blasts from some cells other than T cells or T cells not susceptible to the neonatal thymectomy and anti-thymocyte serum remains.

In case of in vivo stimulation with PHA, the dose-wise effect was not well demarcated as with Con A and there was no notable peak in the response with any dose. Thus PHA was found not to be a good stimulator for murine T cells in vivo. Similar results were obtained by Stobo and his co-workers (1972) in in vitro system.

There was no sign of vacuolation in the blasts induced with PHA as it was observed in the blast cells induced with higher doses of Con A. Vacuolated nature of the cells could be the sign of hyperactivity and physiological exhaustion of the blast cells.

Usually complete Freund's adjuvant containing killed Mycobacteria is supposed to increase the immune response to a

given antigen when administered simultaneously (Bomford, 1980a). The proof that adjuvants can stimulate the reticulo endothelial system by a nonspecific mechanism is provided by experiments showing that adjuvants act as well when given separately from the antigen, as they do when mixed directly with antigen (Dresser, 1962; Pernis and Paronetto, 1962; Paraf et al., 1962; Houdayer et al., 1965). Chase (1966) proposed the explanation that after guinea pigs received 3 mg of Mycobacteria, they became very sensitive to tuberculoprotein, so that when the antigen was given, there was a rejection phenomenon, leading to cellular destruction and the elimination of a large amount of antigen.

In the present investigation, we observed that a good number of lymphocytes transform into blasts from 24 hours onward with intraperitoneal injection of the FCA. Although the peak of the blastogenesis with different doses of FCA was at 48 hours (Table 2) as in case of Con A, the increment of percentage of blasts from 24 to 48 hours was not as much as in case of Con A. Thus it seems that the FCA mediated activation of the lymphocytes for blast transformation occurs earlier than that with Con A treatment.

Besides blastogenesis, synthesis of macromolecules, like DNA, RNA and protein have also been considered for analysing the

process of activation of lymphocytes in vivo. Rate of synthesis of these substances by the lymphocytes from spleen and mesenteric lymph node have been studied here as the pattern of blastogenesis of the lymphoid cells from different sources treated with different doses of polyclonal substance were found to be comparable. Thus the experiments with spleen and lymph node cells were thought to be fairly representative ones.

With in vivo Con A treatment the rate of blastogenesis corresponds to the dose of Con A and was not correlated to the rate of DNA synthesis. The rate of synthesis of DNA was higher with lower dose of Con A (Fig. 4,5 & 12,13).

The dose of 5 µg and 10 µg Con A per animal could not elicit a reasonably higher blastogenic response; rather the doses could be considered as sub-optimal for blastogenesis. But the 10 µg dose of Con A caused maximum synthesis of DNA by the lymphocytes. Thus it seemed that the blastogenic response of the lymphocytes might not correlate with their synthesis of DNA. This may apparently seem to be contrary to the usual belief where blastogenesis and DNA synthesis are often considered synonymous for indexing blast transformation. In this context an earlier finding can be recalled where it has been shown that in in vitro condition blastogenesis can progress after suppression of DNA synthesis with hydroxyurea

(Chakravarty and Clark, 1977).

The higher dose, especially 50 µg Con A could possibly be supra-optimal for induction of DNA synthesis and thus caused low level of incorporation of ^{3}H-TdR. Similar observation was made by Steen and Lindmo (1979) in in vitro studies.

None of the doses of PHA could induce a significant level of synthesis of DNA in spleen or lymph node cells (Fig. 14 & 15). We observed that these doses could cause a feeble blastogenic response among the lymphocytes in vivo. On the basis of earlier works (Stobo et al., 1972) and our observation it can be said that PHA is not a good stimulator for murine lymphocytes whether it is in vitro or in vivo. PHA is known as a better polyclonal stimulator for human lymphocytes (Winkelstein and Craddock, 1967; Kay et al., 1970) and much detailed works regarding kinetics and site of DNA synthesis augmented with PHA stimulation in human lymphocytes have been worked out (Buckton and Pike, 1964; Ribas-Mundo, 1966; Sasaki and Norman, 1966).

Different parameters for stimulation of RES like increment in the weight of spleen and liver (Halpern et al., 1964), phagocytic activity of the RE cells (Halpern et al., 1966; Berman et al., 1967), rate of antibody synthesis (Havas

and Andre, 1955), resistance to viral infection (Gorhe, 1967) with introduction of Corynebacterium parvaum in mice have been studied earlier. In the present investigation we observed that complete adjuvant containing killed Mycobacterium can stimulate the lymphoid system of mice in vivo as measured by blastogenesis and the synthesis of macromolecules. Regarding DNA synthesis we observed that a treatment with 0.3 ml adjuvant caused the maximum incorporation of radioactive precursors into DNA. It seems that there could be two cycles of DNA synthesis in course of 96 hours, the first peak might be around 24 hours. This was more evident with the dose 0.3 ml FCA per animal. Similar peaks of DNA synthesis at 48 hours and 96 hours were observed with the treatment of 20 µg Con A.

The two peaks in the incorporation of ^{3}H-TdR might represent two cell cycles and thus indicating that the first cell division augmented with FCA treatment occurs comparatively earlier than this in case of Con A treatment. Of course the observation of Zank et al., (1971) should be taken into account in this regard where they showed that the variation in the peak of incorporation was not always related with cell division, a temporary decrease in the rate might even be seen in mid-S phase of the cell cycle.

The replication of DNA and mitosis are very late events in the course of lymphocyte activation. Before lymphocytes enter the S phase they express function and growth. The central role in the acquisition of function or in cellular growth is played by the induction of increased protein synthesis. Whether the early increase in protein synthesis requires the formation of new mRNA is unclear. New mRNA species have not been unequivocally detected by hybridization studies in mitogen activated lymphocytes (Neiman and McDonnell, 1971). Using cell free system it has been suggested that protein synthesis of activated lymphocytes is regulated by translational processes (Ahern and Kay, 1973; Ahern et al., 1974).

In the present investigation the rate of synthesis of RNA and protein in the lymphocytes activated with Con A, PHA and FCA have been studied at 24 hours interval upto 96 hours. The main purpose of this study was to measure the rate of synthesis of these substances with the treatment of different doses of the polyclonal substances and to find whether there was any correlation between the pattern of synthesis of RNA and protein with that of DNA and the process of blastogenesis as such.

In general the dose or the substance which could not elicit a good blastogenic response also failed to stimulate the synthesis of RNA and protein. For example, PHA could not induce

a reasonable level of incorporation of radioactive precursors into newly synthesized RNA and protein (Fig. 20,21 & 28,29) and was also a poor inducer for blastogenesis (Fig. 8 to 11). Similarly the dose of 50 µg Con A per animal caused better incorporation of radioactive uracil and leucine at 48 hours both in spleen and lymph node cells than the other doses (Fig. 18,19 & 26,27). Only notable exception was the dose of 0.5 ml FCA which was not as poor for inducing blast transformation as it was for induction of RNA and protein synthesis.

In view of our earlier discussion it seems that the process of blast transformation might be more closely related with the synthesis of RNA and protein than that of DNA. The former relation is possibly an expected one as because large blast cells are supposed to synthesize more of building blocks for differentiation.

So far it seems that Con A and FCA can activate the murine lymphocytes to a reasonable degree in terms of blastogenesis and synthesis of different types of macromolecules. Now the question regarding the mechanism of activation of the lymphocytes in vivo may arise. Possibly molecular mechanisms for triggering small lymphocytes in the path of differentiation

of the effector cells remain same for *in vitro* and *in vivo* situation, and difference may occur in terms of certain circumstantial factors which will be discussed later.

A good number of papers have been published during the last decade to explain certain molecular mechanisms of activation of lymphocytes *in vitro*, and some of them will be discussed here briefly.

Lymphocytes without being exposed to external stimuli are arrested within the G-1 phase of the cell cycle; they do not express demonstrable function. When antigens or mitogens bind to the surface of the cells, a sequence of metabolic events is triggered, leading to proliferation and or expression of function. According to several authors, reaction of mitogens or antigens with the plasma membrane alone is sufficient to activate resting lymphocytes (Greaves and Bauminger, 1972; Betel and Van der Berg, 1972; Andersson *et al.*, 1972). The activation, therefore, must be initiated by a process in which the plasma membrane undergoes interaction with a ligand (stimulant).

One of the early effects of mitogen stimulation is aggregation of stimulant-binding receptors into "patches" which may subsequently merge to form a "cap". The observation that all

stimulatory agents acting through receptors are at least divalent, whereas the corresponding monovalent forms have no mitogenic effect, has been taken to indicate that receptor cross-linking is crucial for the triggering of lymphocyte activation (Woodruff et al., 1967; Fanger et al., 1970). Some of the mitogen enters the cell by endocytosis, and seems to be concentrated in cytoplasmic vacuoles which may contain lysosomal enzymes (Biberfeld, 1971; Barat and Avrameas, 1973).

Several hypothesis have been put forward as to how such a "signal" of the binding of the ligand to the surface receptor can be transmitted across the plasma membrane. It has been suggested that some of the surface receptors are connected with the cytoplasmic system of microtubuli, possibly via actin and myosin containing filaments and that the initiation of mitogen response is caused by alterations in this system which are dependent upon redistribution of mitogen receptors. So receptor mobility may be considered to be of crucial importance for the initiation of blastogenic response. As an essential part of the basis for this hypothesis, Steen and Lindmo (1979) have observed that hyperoptimal concentrations of Con A inhibit both patching and capping of receptors, and at the same time the blastogenic response, as measured by the incorporation of ^{3}H-TdR, is considerably suppre-

ssed. Furthermore, similar concentrations of the divalent succinyl-Con A, which does not inhibit patching and capping significantly, do not suppress blastogenic response either (Heininger et al., 1976). Certain criticism for this hypothesis has also been put forward by Yahara and Edelman (1975).

The plasmamembrane of activated lymphocytes exhibits gross changes measured by an increase in membrane fluidity (Yahara and Edelman, 1973; Barnett et al., 1974; Ferber et al., 1974) or an accelerated turnover of membrane phospholipids (Fisher and Mueller, 1968; Resch et al., 1972; Resch and Ferber, 1975). So the permeability for nucleosides (Peters and Hausen, 1971a), metabolites like sugars (Peters and Hausen, 1971b), amino acids (Mendelsohn et al., 1971; Van der Berg and Betal, 1971) or ions such as K^+ (Quastel and Kaplan, 1971) and Ca^{++} (Freedman et al., 1975) increase.

Membrane bound enzymes such as ATPases (Quastel and Kaplan, 1971) or lipid metabilizing enzymes (Fisher and Mueller, 1968; Resch et al., 1972; Ferber et al., 1974) are also activated. The rapidly induced disbalance of intracellular cyclic nucleotides i.e. increase in cGMP and concomitant decrease in cAMP (Hadden et al., 1972; Hadden, 1974) may also be regulated by the cyclases located within the plasma membrane (Smith et al.,

1971).

So far, generalized mechanisms which might be responsible for activation of lymphocytes *in vivo* as *in vitro* have been discussed. Now discussion is needed for the factors which are different in *in vivo* system and might influence the process of activation.

The dose of mitogen especially Con A used here for *in vivo* stimulation can be considered low. Waterfield *et al* (1975) used between 60 and 480 µg Con A per mouse to generate cytotoxic cells but they did not take into account any other parameters for activation of the cells. In an earlier publication, Waterfield and his co-workers (Anaclerio *et al*., 1974) used the dose of 120 µg Con A to generate blast cells and cytotoxic cells in CBA mice. We observed that the dose of 50 µg Con A is quite effective in generating good number of blasts, causing synthesis of macromolecules to a reasonable level and ultimately cytotoxic differentiation of the lymphocytes. Some cells other than lymphocytes possibly do have binding sites for Con A and the substance goes through the different systems and a series of enzymes before getting contact with the lymphocytes; even then in our study it is notable that a small amount of the substance can elicit the responses. Several

reasons can be put forward to explain the situation, such as binding sites for mitogens on the cell types other than lymphocytes might remain bound to some sugar compounds similar to α-D-Methylmannoside, competitor for Con A binding sites and too many mitogen molecules per lymphocyte might not be needed for the activation of the cell. Further controlled in vitro experiments would only help to clarify these points.

In vivo system would possibly differ significantly from in vitro system on account of cell migration or recruitment which is almost nil in vitro. There remains the possibility of migration of different quantum of different types of cells at the site of the activation of lymphocytes and then modulating the process of activation. It may be worthwhile to persue the matter in future; at present it may be concluded that the factor of cell migration might not have a stronger influence in the process of activation in vivo as the kinetics of the response in vivo stimulate the in vitro pattern. However, except suppressor cells no other cell type has yet been implicated in influencing the process of blastogenesis. Possible role of the suppressor cell population will be discussed later in the context of applicability of the method of mitogen mediated in vivo activation of the lymphocytes.

Limitation of nutrients and removal of metabolic waste products in in vitro set up are other significant points of diffe-

rence with in vivo set up. Usually non stimulated cells in in vitro culture die off soon; this could be due to high rate of utilization of the nutrients by the metabolically more active stimulated cells and good amount of waste products from these cells. Such things are not expected in vivo and thus might not cause death to most of the non stimulated cells. Although we observed a good number of dead cells in the cell suspension prepared from the spleen and lymph nodes of the animals injected with higher doses of Con A, the reason for this cell death could be other than the limiting factors of in vitro culture condition as discussed later.

why should FCA be considered as polyclonal stimulator?

Although Con A, PHA and FCA are generally considered as polyclonal stimulators, it has been shown earlier that the lymphoid cells, stimulated in vitro with Con A, can differentiate into cytotoxic killer cells and can cause lysis of specific target cells differing in MHC (Waterfield et al., 1975; Bevan et al., 1976; Heininger et al., 1976; Chakravarty and Clark, 1977; Falkhoff and Dutton, 1977; Chakravarty, 1978; Thomson and Jensen, 1980; Waterfield et al., 1981). This lysis has been shown not to depend on the bridging of effector and target cells by tetravalent Con A molecules as the cytotoxic reaction was made in absence of Con A

and in presence of its competitor ∝-D-methyl mannoside (Clark, 1975; Chakravarty and Clark, 1977).

It has originally been proposed by Wilson (1965) and Rosenau (1968) that T-cell mediated cytolysis requires intimate contact between the effector cell and its homologous target. Berke et al., (1981) postulated that the lectin dependent cell mediated cytolysis occurs as a consequence of an interaction between the effector cell surface receptor and a target cell-major histocompatibility directed antigen. It has been shown that when killer and target cells are separated either by a semipermeable membrane or by suspension in a viscous medium such as agarose or dextran, cytolysis does not occur (Cerottini and Brunner, 1974; Henney, 1974a,b). Cytolysis results from single collision between effector and target cell and is dependent on a viable killer cell (Berke et al., 1969; Henney, 1971). One cytolytic T lymphocyte can kill more than six target cells and can do so sequentially in time (Brunner et al., 1970; Berke et al., 1972). Malter (1979) suggested that target cell lysis does not start with a surface phenomenon similar to complement lysis, but a process involving practically the whole cell.

Intensive works have been done by many workers regarding the cytotoxic ability of the effector cells generated in vitro with

mitogens. But *in vivo* differentiation of cytotoxic T cells by using these substances have not yet been studied in detail. One of the objectives of this study was to measure the cytotoxic ability of the T cells activated *in vivo* with the treatment of Con A, PHA and FCA.

So far we referred to activation of lymphocytes by these polyclonal agents in general, not in reference to a specific cell type as it was known from *in vitro* experiment that except Con A, the other two substances are capable of stimulating to some extent both B and T cells (Freeman *et al.*, 1966; Stobo, 1972; Greaves and Janossy, 1972; Janossy *et al.*, 1973) Cytotoxic immune response of these activated lymphocytes has been considered here as T cell function as this function is known to be mediated mainly by the T cells.

Cytotoxicity of the effector T cells generated with different doses of Con A and the dose of PHA and FCA which were most effective for generating blasts have been considered in this study. PHA as a poor stimulator for blast transformation has again proved itself poor for generating cytotoxic cells. Thus results with PHA possibly indicate that a good level of blastogenesis is a prerequisite for differentiation of cytotoxic cells in a T cell population. This fact has also been indicated by others in other

contexts (Stobo et al., 1972; Stobo and Paul, 1973).

Con A as the best stimulator for blastogenesis has been able to induce a good number of T cells to differentiate into cytotoxic killer cells as revealed from the Table 6. FCA generated effector cells expressed cytotoxicity at a level in between those with PHA and Con A. The dose of 0.3 ml FCA per animal caused a significant level of blast transformation and synthesis of the macromolecules in a lymphocyte population in vivo, but the degree of cytotoxicity of these cells was not that high. This is possibly because FCA generated a good number of blast cells both in B and T cell populations and then only a fraction of the blast cell population could mount the cytotoxic reaction.

Specificity of cytotoxic reaction has been tested with non-stimulated and stimulated syngeneic effector cells (Table 6,7), and it was found that non-stimulated cells cannot mount cytotoxic reaction and this reaction cannot be generated against self-antigen. For specificity test, target cells from third party have not been used as polyclonal substances are known to produce multiple clones of effector cells with specificity for varied targets (Heininger et al., 1976).

Con A as a polyclonal stimulator is supposed to stimulate the suppressor cells too (Dutton, 1972). That aspect has not been looked into in the present investigation as it does not come into the main purview of this study, and no significant interference of suppressor cells in cytotoxic response of the effector T cells has been encountered. Of course Con A treatment may not always generate the suppressor cells. For example, it has been shown that *in vivo* administration of Con A (Ekstedt *et al.*, 1977) or *in vitro* treatment with low concentrations of Con A (Nespoli *et al.*, 1977), does not seem to lead to the formation of suppressor cells which can interfere with polyclonally stimulated B-cell mediated humoral response *in vitro*.

Before suggesting any physiological relevance of *in vivo* stimulation of T cells with polyclonal agents, exploration of the fact, how do these substances affect the cells *in situ* is possibly needed. That is why histological sections of spleen and mesenteric lymph node of the mice treated with different doses of Con A and 0.3 ml of adjuvant have been studied. As PHA was found from our study not to be much suitable for *in vivo* stimulation of murine lymphocytes, treatment with this compound

has not been included in this section.

No severe effect of three different doses of Con A or 0.3 ml of FCA was observed on the lymphoid cells or their organisation in the secondary lymphoid organs. In course of blastogenesis, gradual change in size of the cells and in consequence change in white pulps, organized area of lymphocytes, can nicely be visualized in the histological sections of spleen and mesenteric lymph node.

It is apparent with the histological studies that the lymph node has been affected more with in vivo Con A treatment than the spleen in terms of higher percentage of blast transformation at early hour, lesser density of the cells due to cell migration or cell death. This could be as because 65 to 70% T cells are present in lymph node and only 30 to 35% lymphoid cells are T cells in spleen and Con A does mostly affect the T cells. Another factor might also be coupled with this, the density of Con A receptors are comparatively more on the cells of lymph nodes than that on spleen cells; but no such evidence has yet been available. Density of the cells in secondary lymphoid organs of animals treated with higher dose of Con A has been found to be lesser than that of the animals treated with lower dose of Con A (Plate 3). The lesser density of the cells in lymphoid organs

* There is no evidence, no rationale for such an observation.

could be due to two different reasons. The cells might have transformed at a faster rate with higher dose of Con A, and migrated out of the spleen and lymph node into circulation or some of the cells died off. Although existence of blast cells in the blood vessels of the spleen and the lymphatic spaces of the lymph node have been observed and the percentage of blasts in peripheral circulation was significantly high, it can only be an indirect proof but not unequivocal evidence for migration of the blasts from the lymphoid organs to circulation.

It was not easy to ascertain the percentage of dead cells in the histological preparation. But a good percentage of dead cells has been observed in the suspension of spleen and lymph nodes from the animals treated with higher dose of Con A (Fig.32). The index is slightly higher with mesenteric lymph node cells of the animals treated with 50 µg Con A. However, more cell death with higher rate of blastogenesis in spleen and mesenteric lymph node could be due to the delicate condition of the cell membrane of the blasts and their susceptibility to the stress of mechanical separation of the cells from spleen and lymph nodes. This seems more plausible when we take into account the lesser percentage of dead cells in the leucocyte cell population of the peripheral blood which was collected without any mechanical stress of tissue grinder and passage through the

Is FCA directly mitogenic to T cells or does it act via helper T. The proliferation in first 72 hrs should be of helper T cells

syringe. The percentage of dead cells in peripheral blood samples are pretty close to the background count (Fig.33).

In contrast to Con A treatment, in vivo treatment of FCA affects the spleen cells more than lymph node cells (Plate 6 & 7). Rationale for this difference is difficult to suggest at present. A possible suggestion could be that FCA might affect B cells more than T cells and it is known that the spleen harbours more of B cells than T cells. Apparently notable degree of loss in cell density or cell death has not been observed in histological sections of the lymphoid organs from the FCA treated animals.

Thus these polyclonal agents can be considered as substances without having much adverse effect on the lymphocytes. Possibly it can be proposed now that the lymphoid cells of an organism activated with polyclonal agents may generate in vivo, multiple clones of cytotoxic killer T cells against target cells bearing unknown or multiple varieties of antigen. Malignant cells can easily be equated with such type of target cells. Polyclonal stimulation would then provide an immunological tool to combat the malignant cells where cancer cells cannot be used for immunization as a prophylactic measure.

The contention is polyclonally activated cells can kill MHC unrelated targets : Are autologous malignant cells MHC unrelated ?

At the time of therapeutic use, if it is necessary to increase the efficacy of the cytotoxic cells, the suppressor T cells can possibly be removed by some already worked out techniques. It has been shown by several authors (Askenase et al., 1975; Hellström and Hellström, 1978) that certain doses of cyclophosphamide can be used to deplete selectively suppressor T cells and thereby eliminating their effect in cytotoxic differentiation of T cells (Rollinghoff et al., 1977). The differential sensitivity of suppressor T cells over other T cell subpopulations to different agents like 2,4,6 Trinitrophenyl conjugate to D-glutamic acid D-lysine, and allo-anti Ia serum can also be used to eliminate suppressor T cell selectively (Yamamato et al., 1977; Primi et al., 1979).

Present investigation has mainly been concerned with primary response of the lymphocytes activated in vivo with the polyclonal agents. Chakravarty and Clark (1977) have shown that at the end of primary stimulation Con A is capable of generating effector memory cells in mouse lymphoid cells in vitro. Thus mouse lymph node cells exposed 12 days previously to Con A behave qualitatively, as defined in their study, differently from unprimed mouse lymphocytes. Similar study would have been necessary to understand whether the polyclonal stimulation can generate memory

T cells *in vivo* or not. Thus further studies regarding time required for secondary stimulation, efficacy of the effector cells after this stimulation, organisation of the lymphoid cells *in situ* after repeated stimulation, possibility of this type of stimulation in tumour bearing host etc will be required before contemplating the use of the polyclonal substances for clinical purposes and attributing physiological relevance to the usage of Con A, PHA or FCA.

In conclusion, the present investigation has analysed the process of activation of mouse lymphocytes *in vivo* by Con A, PHA and FCA in terms of blastogenesis, synthesis of macromolecules and condition of lymphocytes *in situ* and found Con A and FCA as potent stimulators for the lymphocytes and capable of generating cytotoxic T cells which can lyse the target cells bearing non-self antigen. Thus the present investigation shows the feasibility of using the polyclonal substances *in vivo* which do not get digested or excreted before inducing the lymphocytes. The present study also suggests a possible relevance of using the polyclonal stimulators for combating malignant cells immunologically.