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Activation of murine thymocytes *in vivo*. Part I. Study of blastogenesis and DNA synthesis after stimulation with Concanavalin A

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Abstract

The present study establishes that a polyclonal stimulater like Concanavalin A can stimulate the murine lymphocytes *in vivo* in different lymphoid organs and peripheral blood. The process of stimulation has been investigated by studying blastogenesis and DNA synthesis of lymphocytes after injecting this substance in mice. Different doses of this substance cause differential response of blastogenesis and DNA synthesis by the lymphocytes. The implications of the findings and the necessity of the study have been discussed.

Key words: *In vivo* activation, thymocytes, Concanavalin A—blastogenesis and DNA synthesis.

1. Introduction

It has been shown earlier that some other substances than specific antigen can stimulate the T cells *in vitro* to perform cell mediated immunological functions[1–7]. The present study has been designed to determine whether a plant lectin like Concanavalin A (Con A) can stimulate the murine lymphocytes (T cells) *in vivo*. The process of stimulation has been investigated by studying blastogenesis and DNA synthesis of the lymphocytes after injecting this substance in mice.

2. Materials and methods

2.1. *Animals*

Inbred C57BL mice were obtained from Cancer Research Institute, Bombay, and maintained in our laboratory with mice-feed and water *ad-libitum*. Eight to twelve weeks old mice were used for all the experiments.

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2.2. *Blastogenesis*

C57BL mice were injected with five different doses of Con A ranging from 5 μg to 100 μg per animal intravenously (iv). The rate of blast transformation of lymphocytes in different organs like spleen, mesenteric lymph node, other lymph nodes (cervical, axilary and inguinal lymph nodes pooled together) and peripheral blood was recorded at 24 hr interval up to 96 hr. Cell suspensions from different lymphoid organs and the buffy coat of the sedimented perpheral blood in sodium citrate solution were layered on Ficoll and Hypaque solution (Sigma Co., U.S.A., Product No. F8628) and spun down at 3000 r.p.m. for 15 minutes for separation of lymphocytes from RBCs, debris, etc.

The percentage of blasts was counted according to the method described earlier[1]. Briefly, the proportion of transformed or ' blast 'cells was determined from the sum of viable medium plus large lymphocytes divided by the total viable lymphocytes, counted by hemocytometer in presence of trypan blue. The percentage count of blasts was corrected by substracting the percentage of medium and large lymphoid cells in respetive lymphoid organ of normal control mice ; the latter index usually varied from 3 to 6 per cent.

The experiments were repeated several times with different doses except with 100 μg of Con A, with which only two experiments were performed. Only the mean values are presented in the figures.

2.3. *DNA synthesis*

Synthesis of DNA was measured by the rate of incorporation of ^{3}H-thymidine into DNA as described earlier[1].

Three different doses of Con A, 10, 20 and 50 μg were used for *iv* injection and assays were made at 24 hr intervals up to 96 hr. Cells from spleen and mesenteric lymph node of experimental and control animals were collected by using tissue grinder and suspended in Minimum Essential Medium. Cell numbers were adjusted at 4×10^6 cells/ml. Minimum Essential Medium was supplemented with 10% goat serum, nystatin (50 U/ml) and penicillin-streptomycin (50 U/ml). Because of its easy availability goat serum was used instead of fetal calf serum and we observed that the viability and blastoid transformation of murine lymphocytes in the medium containing goat serum were similar as in the medium with fetal calf serum (unpublished observation). Triplicates of 250 μl cell suspension containing 10^6 cells were taken into glass culture tubes. 2μCi ^{3}H-thymidine (sp. Act. 15·8 Ci/mM, Bhabha Atomic Research Centre, Bombay) was used per tube and cells were incubated for 8 hr at 37° C in a humidified atmosphere of 7·5% CO_2 in air. The cultures were terminated by washing with cold phosphate buffered saline and precipitated with cold 10% tricholoacetic acid (TCA). The TCA precipitates were then filtered on small filter papers (Whatman filter

paper no. 3). Each residue was washed with 10 ml of 10% TCA and filter papers were dried and counted in omnifluor-toluene for total radioactivity.

3. Results

3.1. *Blastogenesis*

The dose-wise effects of Con A on blastogenesis of cell population from spleen, mesenteric lymph node, other lymph nodes and peripheral blood are shown in figs. 1a, 2a, 3 and 4 respectively. The peak of blastogenesis with the cells from all the sources irrespective of the dose of Con A, was effectively reached by 48 hr. The peak was delayed in case of cells from other lymph nodes, treated with 50 μg Con A. The responses with the dose of 5 μg and 10 μg Con A are almost at the same level in all the cases. In other three higher doses, the height of the responses correspond to the amount of Con A injected per animal. With 50 μg and 100 μg Con A per animal, percentages of blastogenesis of cells from mesenteric lymph node are higher at initial and subsequent stages.

The blast cells become more vacuolated or exhausted by 72 to 96 hr and increase in the percentage of vacuolated cells corresponds with the higher doses of Con A. The number of dead cells were found to be more in the mesenteric lymph nodes.

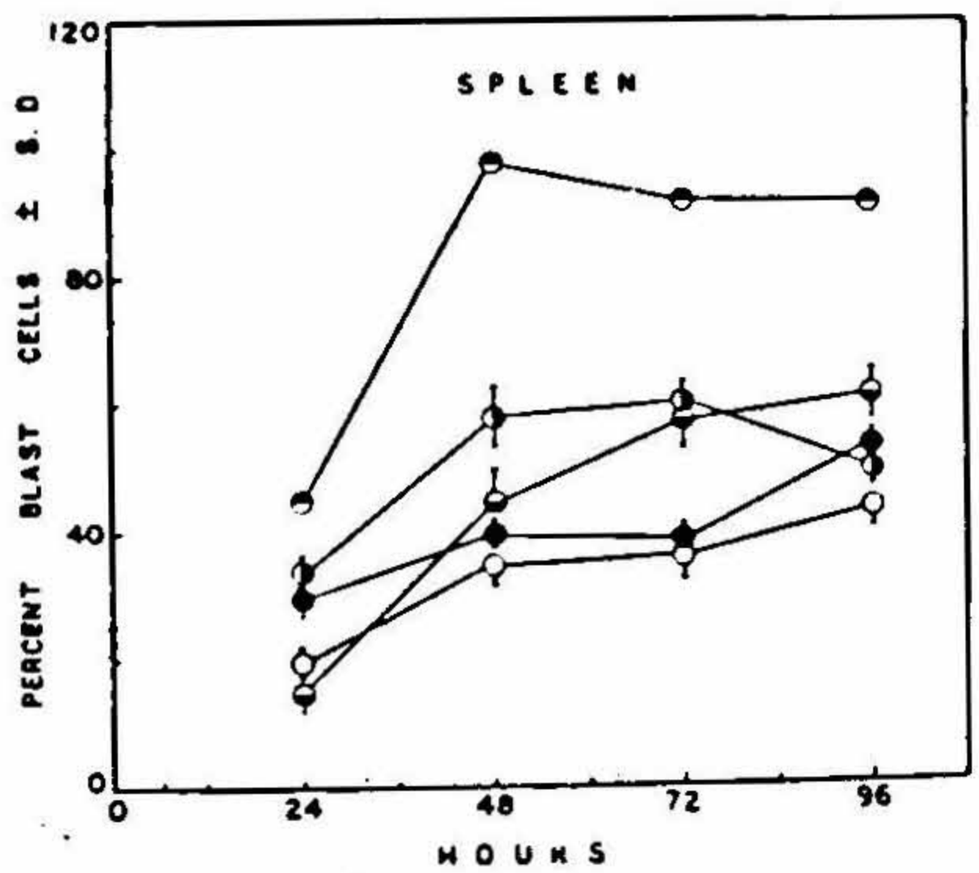

FIG. 1*a*. Kinetics of blastogenesis of lymphocytes from spleen with *in vivo* treatment of different doses of Con A. Dose of Con A per animal: O—O 5 μg; ●—● 10 μg; ⊖—⊖ 20 μg; ⊖—⊖ 50 μg and ⊖—⊖ 100 μg. (Same symbols for the different doses of Con A have been used in other figures).

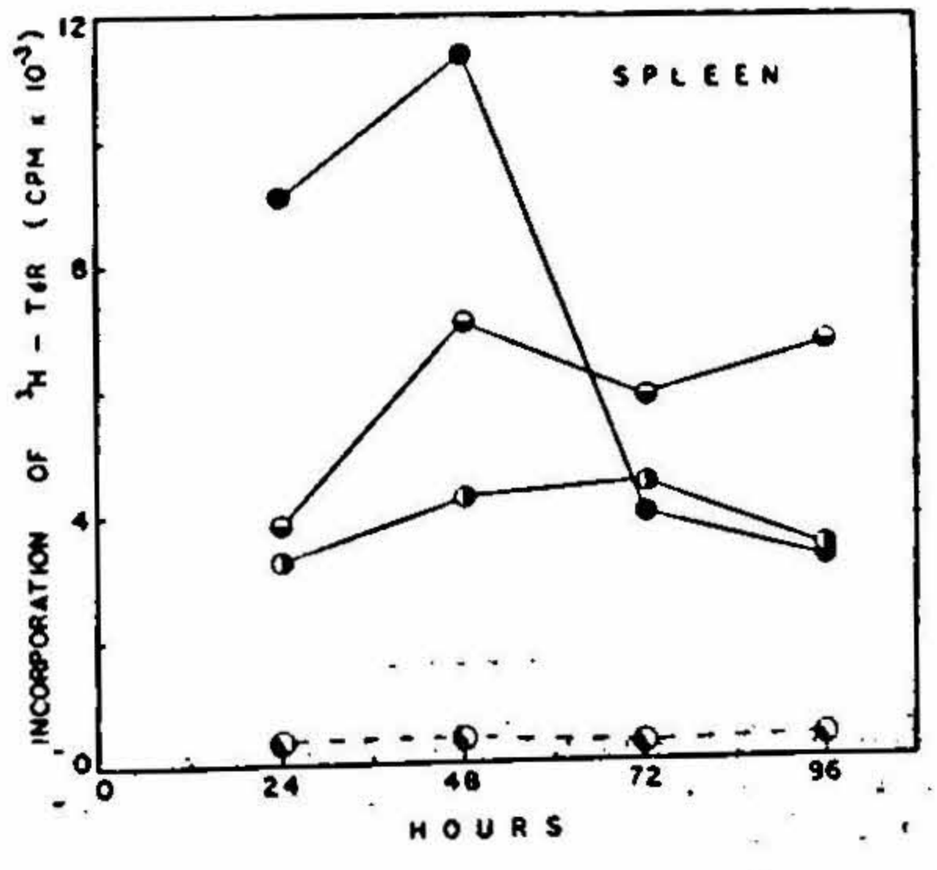

FIG. 1*b*. Pattern of incorporation of ^{3}H-TdR by splenic lymphocytes at different hours after *in vivo* stimulation with different doses of Con A (O—O control, without Con A treatment. Same symbols have been used in other figures.).

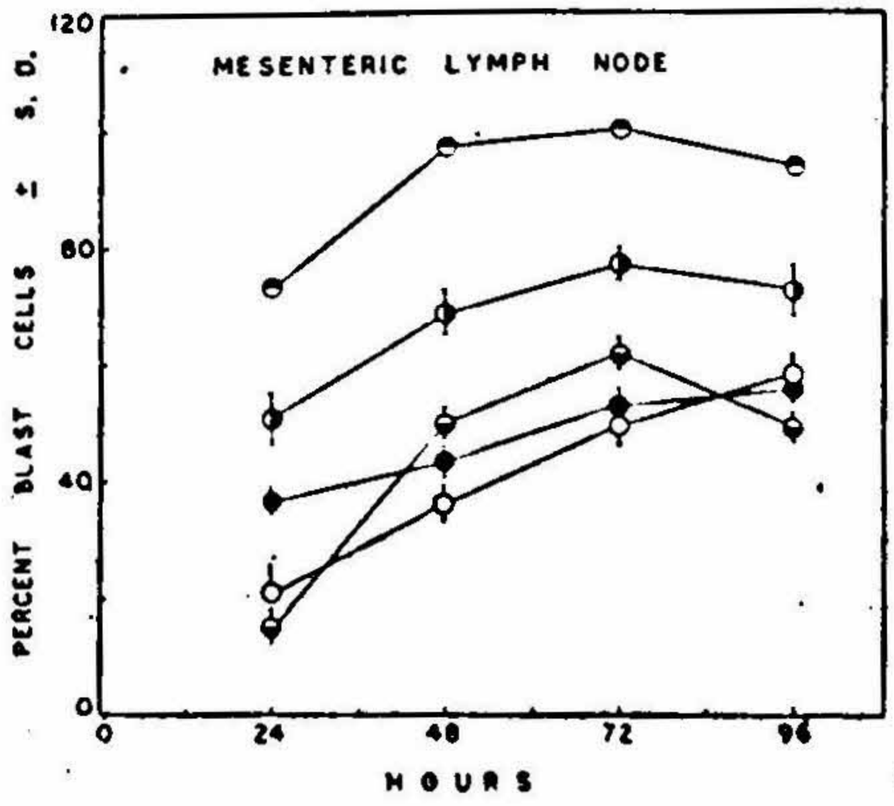

FIG 2*a*. Kinetics of blastogenesis of lymphocytes of mesenteric lymph node with *in vivo* treatment of different doses of Con A.

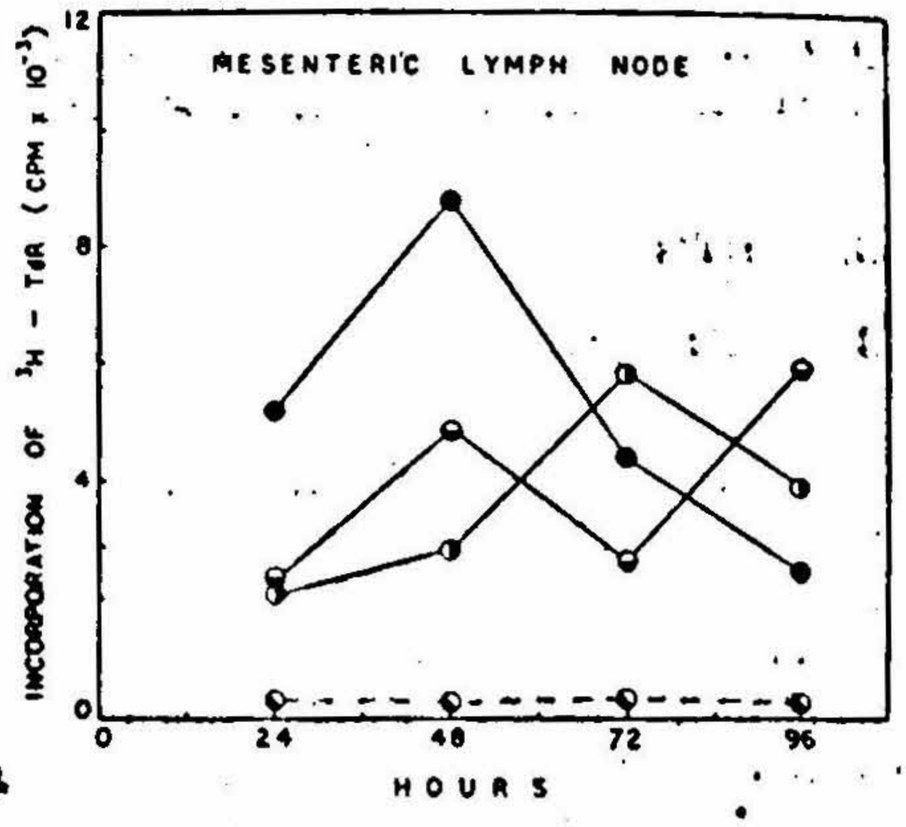

FIG. 2*b*. Pattern of incorporation of 3_H–TdR by lymphocyte of mesenteric lymph node at different hours after *In vivo* stimulation with different doses of Con A.

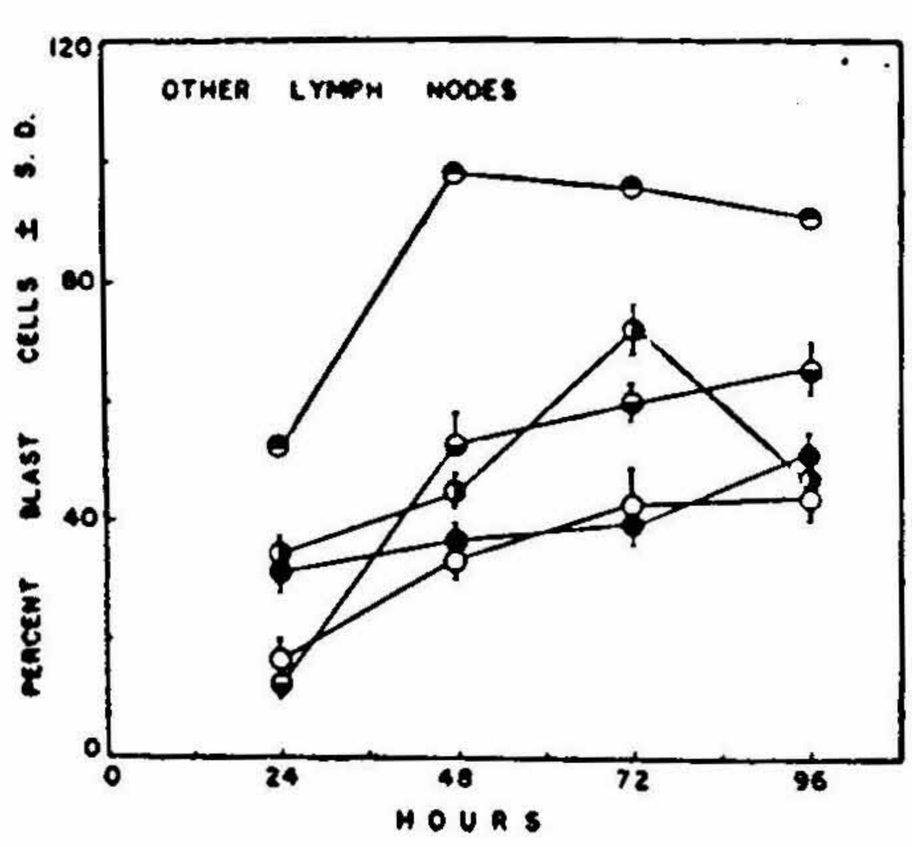

FIG. 3. Percentages of blast obtained at different hours in other lymph nodes with treatment of different doses of Con A.

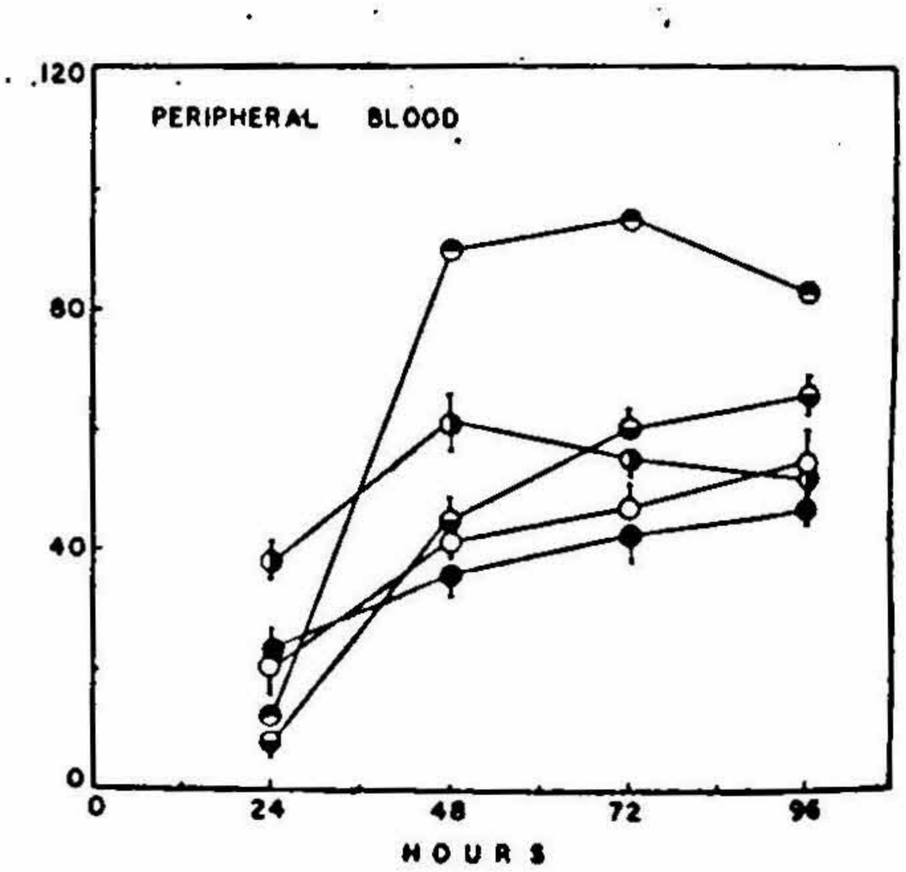

FIG. 4. Percentages of blast obtained at different hours in peripheral blood with treatment of different doses of Con A.

3.2. *DNA synthesis*

Patterns of DNA synthesis by the spleen cells with treatment of three different doses of Con A have been presented in fig. 1b. With all three different doses maximum level of incorporation of radioactive thymidine occurred at 48 hr. However, at different

hours, the rate of DNA synthesis with the dose of 50 μg Con A is not appreciably different. Furthermore, the rate of synthesis of DNA is higher with lower dose of Con A.

DNA synthesis by cells of mesenteric lymph node follow the pattern as in the case of spleen cells with a few variations (Fig. 2b). Similar to the spleen cells, the rate of synthesis of DNA with 10 μg Con A is higher than that with other doses. But there is not much difference in the height of incorporation of ^{3}H-thymidine (^{3}H-TdR) with the treatment of 20 μg and 50 μg Con A. With the latter dose, the peak of synthesis was at 72 hr. Possibly there are two peaks at 48 hr and at 96 hr with the treatment of 20 μg Con A. Similar tendenoy has also been observed with the spleen cells (Fig. 1b).

4. Discussion

Our findings indicate that substances like a plant lectin, Con A can stimulate the lymphoid system of mice *in vivo* and cause blastoid differentiation of the lymphocytes. The nature of blast cells and kinetics of differentiation *in vivo* simulate these of *in vitro* treatment of lymphocytes with Con A[6]. In both *in vivo* and *in vitro* set-ups the peak of the responses was around 48 hr. This is interesting for *in vivo* set-up as the substance passes through different systems before coming into contact with the lymphoid cells in secondary lymphoid organs whereas in *in vitro* system the substance gets direct contact with the cells from the very beginning. The *in vivo* blastogenesis with Con A indicates that this substance does not get totally neutralized, digested or excreted before exerting its stimulatory effects to the lymphoid cells. This fact will help one to use this substance *in vivo* for certain specific purposes, especially to stimulate the lymphoid system against some specific antigenic moieties.

Although this substance is generally considered as polyclonal, we and others[1–7] have shown that the lymphoid cells, stimulated with Con A can differentiate into cytotoxic killer cells and can cause lysis of specific target cells. This lysis has deen shown not to depend on the bridging of effector and target cells by tetravalent Con A molecules as the cytotoxic reaction was made in the absence of Con A and in the presence of its competitor α-D-methyl mannoside[1, 8].

It is interesting to note that pretty close to hundred per cent of blats 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[9]. 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 (unpublished observation) high percentage of dead cells, more than 50% with 100 μg Con A treatment

(percentage of blasts was counted in reference to viable lymphocytes only as outlined in Materials and methods). We are pursuing certain experiments with neonatally thymectomized mice to resolve these possibilities.

The rate of blastogenesis corresponds to the dose of Con A and was not correlated to the rate of DNA synthesis. Rate of synthesis of DNA was higher with lower dose of Con A (Fig. 1b). As the kinetics of blastogenesis with lymphocytes from different sources after treatment of 5 μg and 10 μg Con A are almost at the same plane and at lower level, it seems that these doses are sub-optimal for blastogenesis. But the 10 μg dose of Con A cause maximum synthesis of DNA by the lymphocytes. Thus it seems that the dose-wise blastogenic response of the lymphocytes might not correlate with their synthesis of DNA.

The higher dose, especially 50 μg Con A, caused low level of ^{3}H-TdR incorporation in our study. This could be as this dose is being supra-optimal for induction of DNA synthesis as similar observation was made by Steen and Lindmo[10] in *in vitro* studies.

Further investigation about cytolytic property of blast cells generated *in vivo* with a polyclonal stimulator like Con A might help to select an effective dose of the substance for generating effector cells for *in vivo* immune responses against different types of antigen.

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