ACTIVATION OF MURINE LYMPHOCYTES *IN VIVO*: BLASTOGENESIS AND DNA SYNTHESIS AFTER STIMULATION WITH BACTERIAL ADJUVANT

T. K. CHAUDHURI AND A. K. CHAKRAVARTY
Centre for Life Sciences, University of North Bengal, Darjeeling 734 430, India.

ABSTRACT

Freund's complete adjuvant containing killed mycobacteria has been used in this study to stimulate the lymphoid cells *in vivo*. The degree of stimulation with three different doses of this substance was measured in terms of blastogenesis and DNA synthesis by the lymphoid cells in spleen, lymph nodes and peripheral blood. The implication of the findings has been discussed.

INTRODUCTION

IT has been shown by several workers that certain substances other than specific antigen can stimulate the T cells *in vitro* to provide helper factor in antibody response[1,2] and to perform cell mediated immunological functions[3-9]. We have shown that a plant lectin like Concanavalin A (Con A) can stimulate the murine lymphocytes (T cells) *in vivo*[10]. In this investigation, complete Freund's adjuvant known as general stimulator for reticuloendothelial system, has been used for stimulation of lymphocytes *in vivo* in mice. The process of stimulation of lymphoid cells in spleen, lymph nodes and peripheral blood has been investigated by studying blastogenic transformation of the cells; DNA synthesis in the course of blastogenesis has also been measured in the case of spleen and lymph node cells.

MATERAL AND METHODS

Animals

Inbred C57BL mice, obtained from Cancer Research Institute, Bombay and reared in our Centre with mice-feed from Hindusthan Lever Limited., Bombay and water *adlibitum* were used for the experiments. Eight to twleve week old mice were used for all the experiments.

Measure for Blastogenesis

Freund's complete adjuvant (Difco, U.S.A.) containing killed mycobacteria was injected intraperitoneally with three different doses, 0.1, 0.3 and 0.5 ml per animal. The rate of blast transformation of lymphocytes in different organs like spleen, mesenteric lymph node, other lymph nodes (cervical, axillary and inquinal lymph nodes pooled together) and peripheral blood was recorded at 24 hr intervals upto 96 hr. Cell suspensions from different lymphoid organs and the buffy coat of the sedimented peripheral blood in sodium citrate solution were layered on Histopaque (Sigma Co., U.S.A. Product No. F 8628) and spun down at 3000 RPM for 15 minutes for separation of lymphocytes from RBCs, debris, etc

The percentage of blasts was counted according to the method described earlier[3]. 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 subtracting the percentage of medium and large lymphoid cells in respective lymphoid organ of normal control mice; the latter index usually varied from 3 to 6%.

Measure for DNA synthesis

DNA synthesis was measured by the rate of incorporation of ^{3}H-Thymidine into DNA as described earlier[3]. 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 μ/ml) and penicillin-streptomycin (50 μ/ml). Goat serum was used instead of fetal calf serum as it was easily available. It was observed that 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, 2 μ Ci ^{3}H-thymidine (sp. Act. 15.8 Ci/mM, Bhabha Atomic Research Centre) was used per tube and the 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% trichloroacetic acid (TCA). The TCA precipitates were then filtered on small filter paper (Whatman filter paper no. 3) Each residue was washed with 10 ml of 10% TCA and filter papers were dried and counted in omniflour-toluene for total radioactivity.

TABLE 1

Percentage of blast cells from different Lymphoid organs and Peripheral blood after injecting different doses of Freund's complete Adjuvant in Mice

Adjuvant Dose per animal	0.1 ml				0.3 ml				0.5 ml			
Hours	24	48	72	96	24	48	72	96	24	48	72	96
Spleen	37.5 ±1.4	43.8 ±2.7	50.0 ±3.1	45.4 ±3.0	46.8 ±1.6	51.2 ±1.9	54.6 ±3.0	40.6 ±2.6	38.4 ±2.3	47.3 ±1.8	50.3 ±1.7	44.9 ±0.9
Mesenteric lymph node	40.3 ±2.4	47.1 ±2.0	50.9 ±2.4	44.0 ±1.1	44.4 ±1.6	54.7 ±1.5	57.7 ±1.4	41.2 ±4.7	44.1 ±1.8	53.2 ±1.8	51.6 ±1.4	48.3 ±1.8
Other lymph nodes	35.7 ±1.8	44.9 ±2.0	51.6 ±0.6	46.2 ±2.6	40.6 ±5.7	49.2 ±8.4	52.5 ±0.9	46.2 ±1.8	46.0 ±1.0	51.8 ±1.1	47.1 ±1.8	42.5 ±2.2
Peripheral Blood	38.9 ±1.9	48.7 ±1.8	55.0 ±1.7	50.9 ±2.3	43.6 ±0.6	51.2 ±0.4	51.5 ±0.6	44.1 ±1.6	39.9 ±1.7	46.0 ±1.0	47.4 ±1.4	45.7 ±2.0

Control Experiment—Incomplete Adjuvant 0.3 ml injected per animal:
Percentages of blasts in different organs at different hours varies from 4% to 7%.

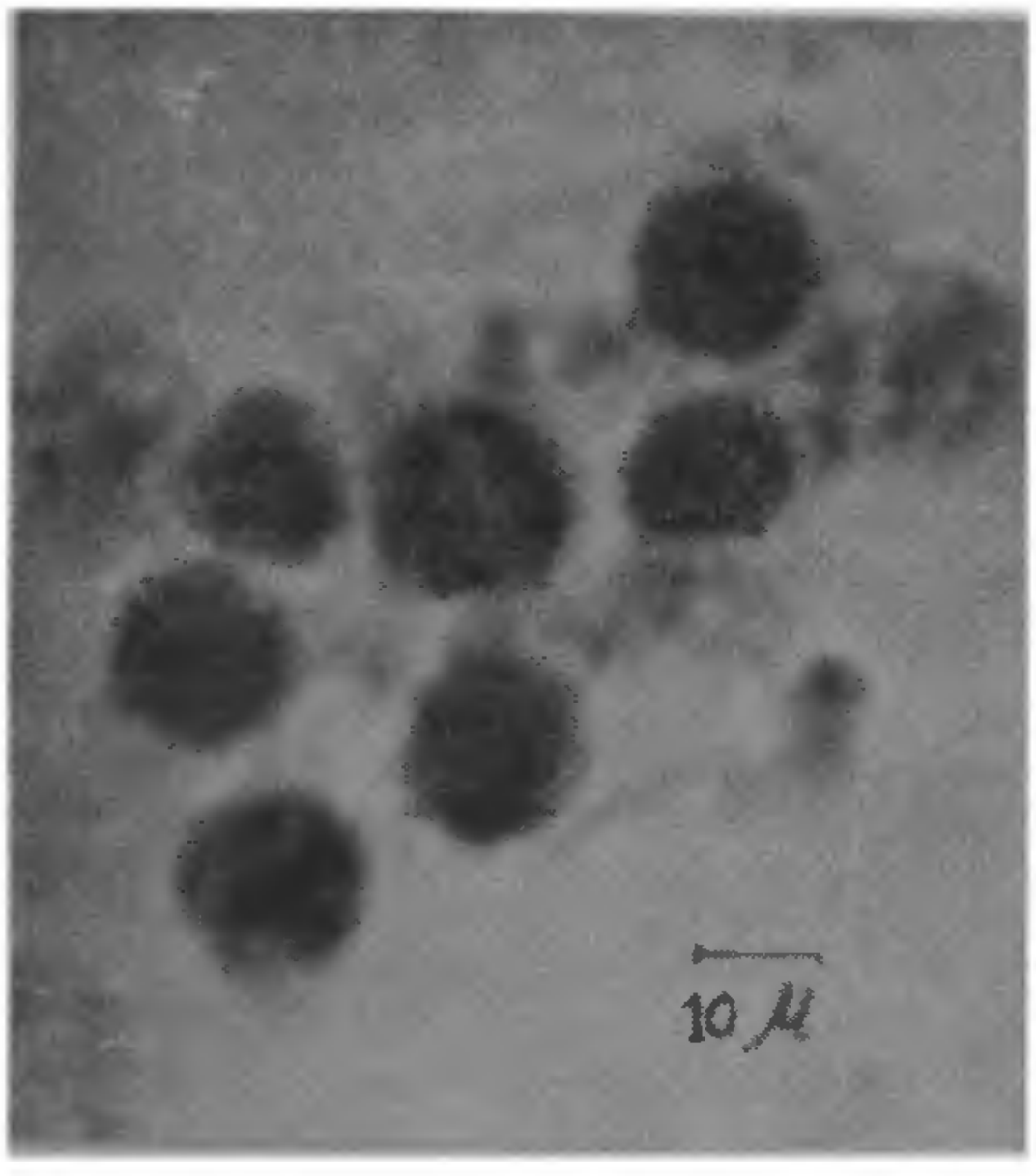

Figure 1. Photomicrograph of the blast cells at 48 hours. Cells were in suspension and stained with Delafield's haematoxylin.

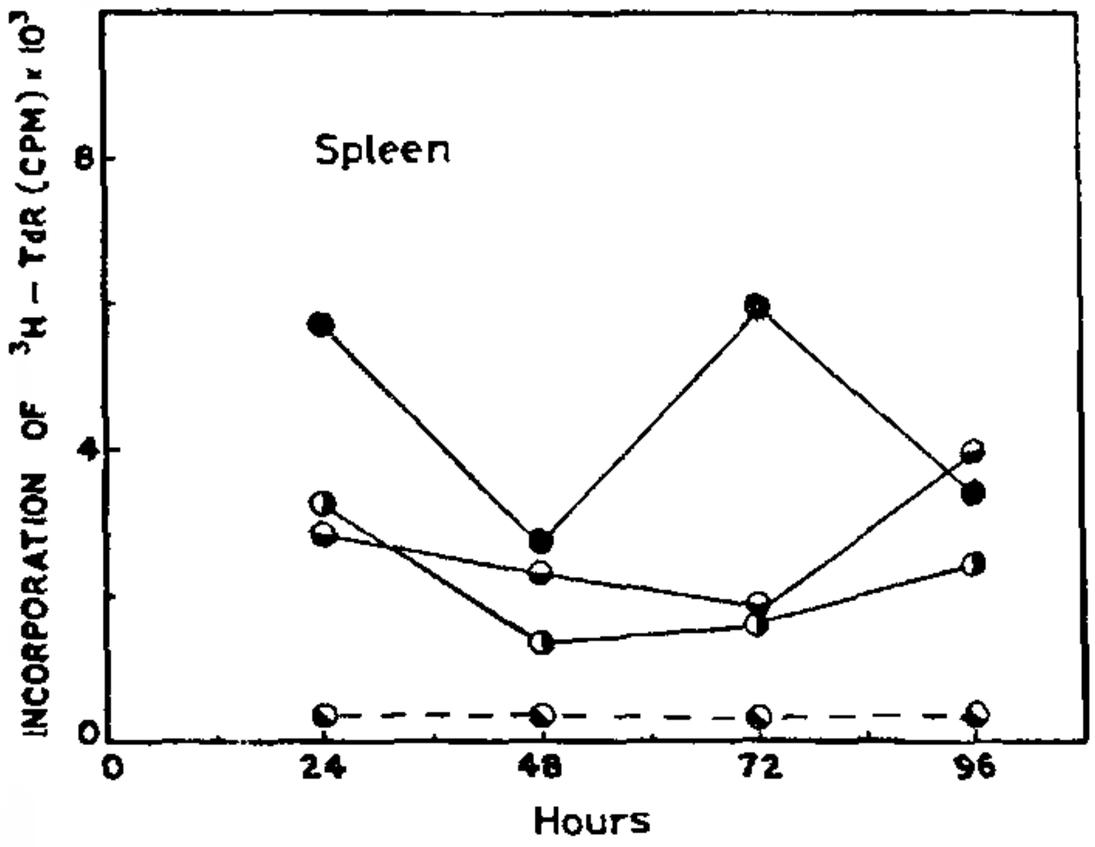

Figure 2. Pattern of incorporation of ^{3}H-TdR by splenic lymphocytes at different hours after *in vivo* stimulation with different doses of Freund's complete adjuvant.

Doses of adjuvant per animal: ◑—◑ 0.1 ml; ●—● 0.3 ml and ◒—◒ 0.5 ml.

Control, without adjuvant: ◒—◒ (same symbols for the different doses of adjuvant and control have been used in other figures).

RESULTS AND DISCUSSION

Blastogenesis

A good number of lymphoctyes transform into blasts from 72 hr onward with intraperitoneal injection of the complete adjuvant. The blastogenesis peak is reached between 48 and 72 hr in lymphocytes (figure 1) from all the sources with all three doses of Freund's complete adjuvant (table 1). The differences between initial and maximal responses is marginal. The blast cells induced with adjuvant are not too much vacuolated or exhausted by 72 to 96 hr as in case of Con A induced blasts[10].

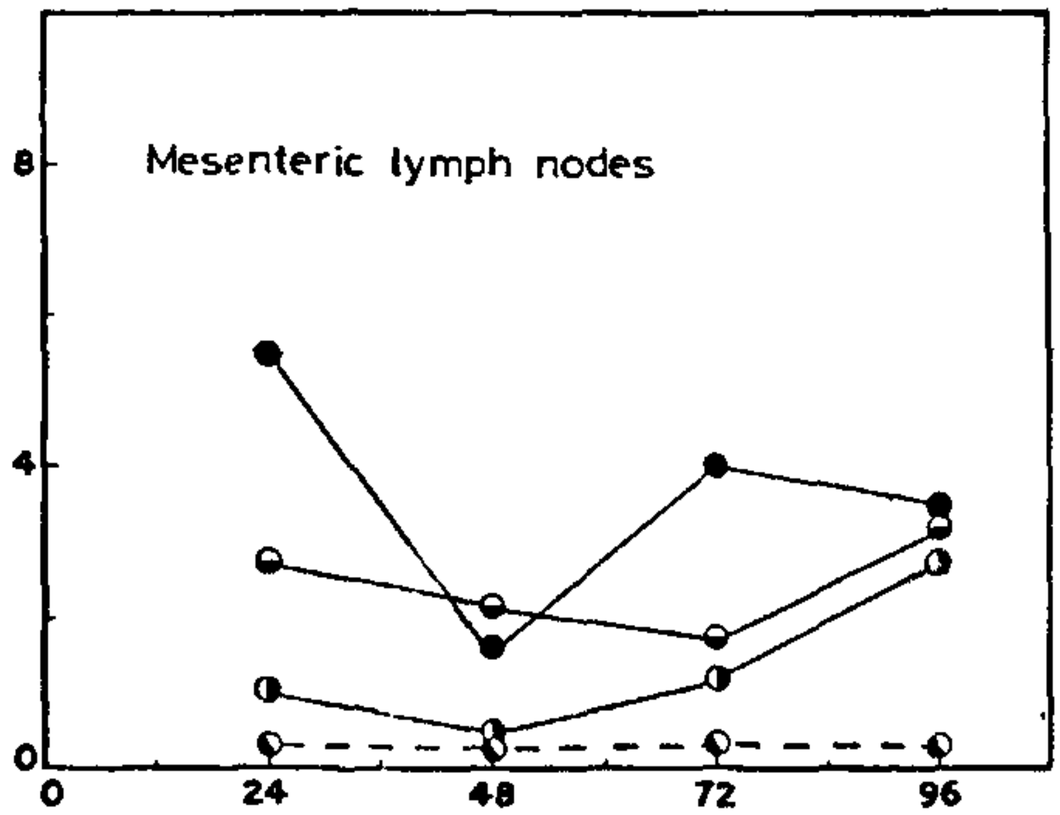

Figure 3. Pattern of incorporation of ^{3}H-TdR by lymphocytes of mesenteric lymph node at different hours after *in vivo* stimulation with different doses of Freund's complete adjuvant.

DNA synthesis

DNA Synthesis by the cells of spleen and mesenteric lymph node have been presented in figures 2 and 3 respectively. In both cases, the dose-dependent patterns of DNA synthesis are similar. Treatment with 0.3 ml adjuvant caused the maximum incorporation of radioactive precursors in DNA. It seems that there could be two cycles of DNA synthesis in course of 96 hr, the first peak might be around 24 hr. This is more evident with the dose of 0.3 ml adjuvant per animal.

In the present investigation, it was observed that complete adjuvant containing killed *Mycobacterium* can stimulate the lymphoid system of mice *in vivo* and cause blastoid differentiation of the lymphocytes. It was known that complete Freund's adjuvant potentiate a given antigen when injected along with the antigen[11,12]. It has also long been recognized that certain microorganism or bacterial extracts increase the host's resistance to a variety of unrelated bacterial or viral infections by stimulating reticulo-endothelial system (RES) and also stimulate immunity against

tumours[13-18]. Different parameters for stimulation of RES like increment in the weight of spleen and liver, phagocytic activity of the RE cells, rate of antibody synthesis, resistance to viral infection with introduction of *Corynebacterium parvaum* in mice have been studied earlier[19,20]. The present investigation provides two parameters, blastogenesis and DNA synthesis to measure the stimulation of lymphoid cells with adjuvant at cellular level.

Furthermore, this study will possibly help to initiate a programme to test the immunologic response of the blast cells activated by the Freund's complete adjuvant containing killed bacteria. It has been shown that although Con A is a polyclonal stimulator, Con A induced blasts can mount cytotoxic response against allogeneic targets including tumour cells[5]. It remains to see whether blasts, induced by Freund's complete adjuvant can have cytolytic property.

This knowledge will possibly help to explain the causative mechanism for host's resistance to unrelated bacterial and viral infections or immunity to tumours[13-18] with injection of microorganisms or bacterial extracts.

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ANNOUNCEMENT

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Further details may be had from Dr K. N. Raju, General Secretary, ICF 6, Deputy Director, National Aeronautical Laboratory, Bangalore-560 017.