may be useful for designing new coagulants devoid of side effects. Also, in view of the increasing interest in anticoagulants, these studies would help to understand and overcome the undesirable antiplasmin activity of the drugs meant for other therapeutic uses.

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Homing of polyclonally activated syngeneic T cells at tumour site and their efficacy in post operative immunotherapy of malignancy in mouse

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^{3}H-Thymidine labelled and polyclonally activated syngeneic lymphocytes injected intravenously have been found to home preferentially at the fibrosarcoma site in mice from 12 h after adoptive transfer. The highest counts from infiltrating radiolabelled cells were obtained at 24 h at the centre of the tumour. Considering this value as maximum, all other values are expressed relative to this number. At 12 h lymphocyte infiltration at the periphery of the tumour mass was about 75%, followed by about 40% infiltration in the liver. After 24 h the central region of tumour mass showed the highest infiltration of radioactively labelled lymphocytes, whereas in liver it remained 50%. There was decline in homing of the activated lymphocytes after 48 h of adoptive transfer.

These tumour site seeking activated lymphocytes are likely to recognize the residual malignant cells after surgical removal of solid tumour, so these cells were adoptively transferred in conjunction with surgery. It has been observed in such experiments, the reappearance of tumour was prevented in 67% of mice and the survival of the hosts increased.

IT has been shown earlier that suppressor cell depleted syngeneic murine T lymphocytes activated polyclonally with Con A *in vivo* can kill tumour target cells[1–3]. The efficacy of such cells in curbing tumour growth after being injected at the tumour site[3,4] opened up the possibility of using such cells in tumour immunotherapy. However, it needs to be investigated whether after adoptive transfer via intravenous route, the effector cells generated either by *in vivo* Con A stimulation[5,6] or by *in vitro* sensitization with specific tumour cell lines[7–10] can infiltrate the tumour mass.

To make this immunotherapeutic measure even more effective, the proportion of effector T cells to the enormous number of rapidly dividing tumour cells needs to be taken into consideration. Therefore, in the present study, surgical removal of bulk of the solid tumour was followed by adoptive transfer of polyclonally activated effector T cells.

Inbred adult Swiss mice of both the sexes, 6–12 weeks of age were used for all the experiments. Methylcholanthrene (MCA) induced ascitic fibrosarcoma cell line is maintained in our laboratory by serial passage. Adult mice were injected subcutaneously with 10^6 ascitic fibrosarcoma cells per mouse in 0.2 ml PBS for induction of solid tumour. Mice bearing tumour of an average size of 1.4 cm^2 were used for the experiments.

Cyclophosphamide (Cy) (Sigma) at a dose of 25 mg/kg body weight was injected in a mouse intraperitoneally as this dose was found to be effective in depleting the suppressor T cells *in vivo*[6,11]. After 48 h of cyclophosphamide treatment, concanavalin A (Con A, Type IV, Sigma Co., St. Louis, USA) at a dose of 50 µg/animal via intravenous route was injected for polyclonal stimulation of T lymphocytes as described earlier[5,12]. Spleen and lymph nodes were taken out aseptically from Cy and Con A treated syngeneic normal mice 48 h after Con A injection and cell suspension was prepared in phosphate buffered saline (PBS) following standard protocol.

Table 1. Recurrence of tumour following post-operative transfer of suppressor cells depleted polyclonally activated syngenic lymphocytes

Experimental animals receiving adoptive transfer					Control animals without any cell transfer				
Exp no.	No. of mice	No. of cases with tumour recurrence	% of recurrence of tumour	% inhibition	Exp no.	No. of mice	No. of cases with tumour recurrence	% of recurrence of tumour	% inhibition
1	7	4	57	42	1	7	6	85	14
2	7	1	14	85	2	7	4	57	42
3	7	3	42	57	3	7	4	57	42
4	9	2	22	77	4	9	7	77	22
5	6	2	33	66	–	–	–	–	–
6	4	1	25	75	–	–	–	–	–
			Mean 67 ± 15.5					Mean 30 ± 14.2	

Statistical significance of the difference, $P < 0.005$.

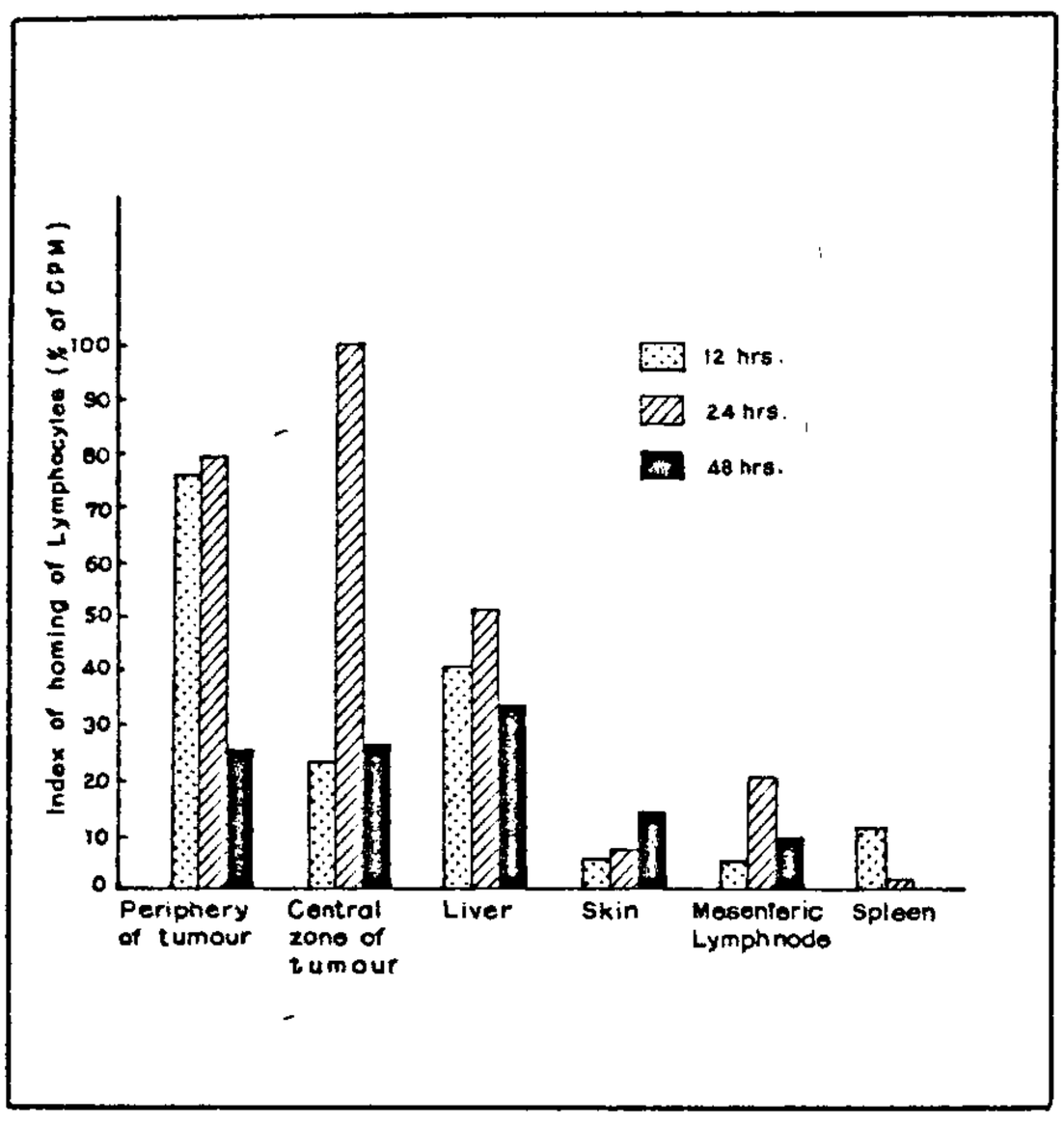

Figure 1. Homing of ^{3}H-thymidine labelled lymphocytes in different regions of tumour and in different tissues at different hours. The result is from a representative experiment consisting of four animals.

The tumours were removed surgically under aseptic condition. Three hours after recovery from the operation, the animals were ready for adoptive transfer.

For study of homing of activated cells, 2×10^6 activated cells from syngeneic Cy-Con A treated donors were radiolabelled with 1 μCi ^{3}H-thymidine (BARC, Bombay) for eight hours. After washing three times with PBS, these cells were injected intravenously in mice. At 12, 24, and 48 h of adoptive transfer, the animals were sacrificed and small tissue pieces (0.3 mm^3 size approx.) were collected in PBS from peripheral and central zones of the tumour, as well as from liver, skin, spleen and mesenteric lymphnodes. The pieces were soaked on filter papers, transferred into scintillation vials and solubilized with 50 μl of methylbenzothonium hydroxide (Sigma). Then the incorporated radioactivity was measured in Beckman LS-1800 liquid scintillation counter. Three pieces of each kind of tissue were collected from an animal in a scintillation vial and four animals were used for an experiment. The experiment was repeated three times.

The mean radioactive count (cpm) for different tissues was expressed as per cent count to the highest count (100%) in an experiment.

The highest counts were obtained with the pieces from the centre of the tumour at 24 h; considering this value as maximum all other values are expressed relative to this number. At 12 h after adoptive transfer, the lymphocyte infiltration at peripheral zone of the tumour mass is 75%, followed by about 40% infiltration in the liver (Figure 1). While the central region of the tumour mass shows the highest value at 24 h, the count for liver tissue is about 50% . After 48 h of adoptive transfer, radioactive counts for the pieces from tumour and liver decline. The radioactive incorporation in skin, mesenteric lymphnode and spleen was lower at all hours of assay.

Adoptive transfer of activated syngeneic lymphocytes after surgical removal of the solid tumour mass prevented reappearance of tumour in 67% of the mice (Table 1). In contrast, only 30% such inhibition was observed in the control animals, which did not receive the adoptive transfer of lymphocytes after surgical removal of tumour bulk. The difference in results of the experimental and the control group was found to be statistically significant following Student's *t* test. Furthermore, 58% of the experimental mice receiving adoptive immunotherapy were found to survive more than 200 days while only about 12% of the control animals survived that long (Figure 2).

T cells polyclonally activated *in vivo* with Con A were found to be highly effective in killing tumour target cells

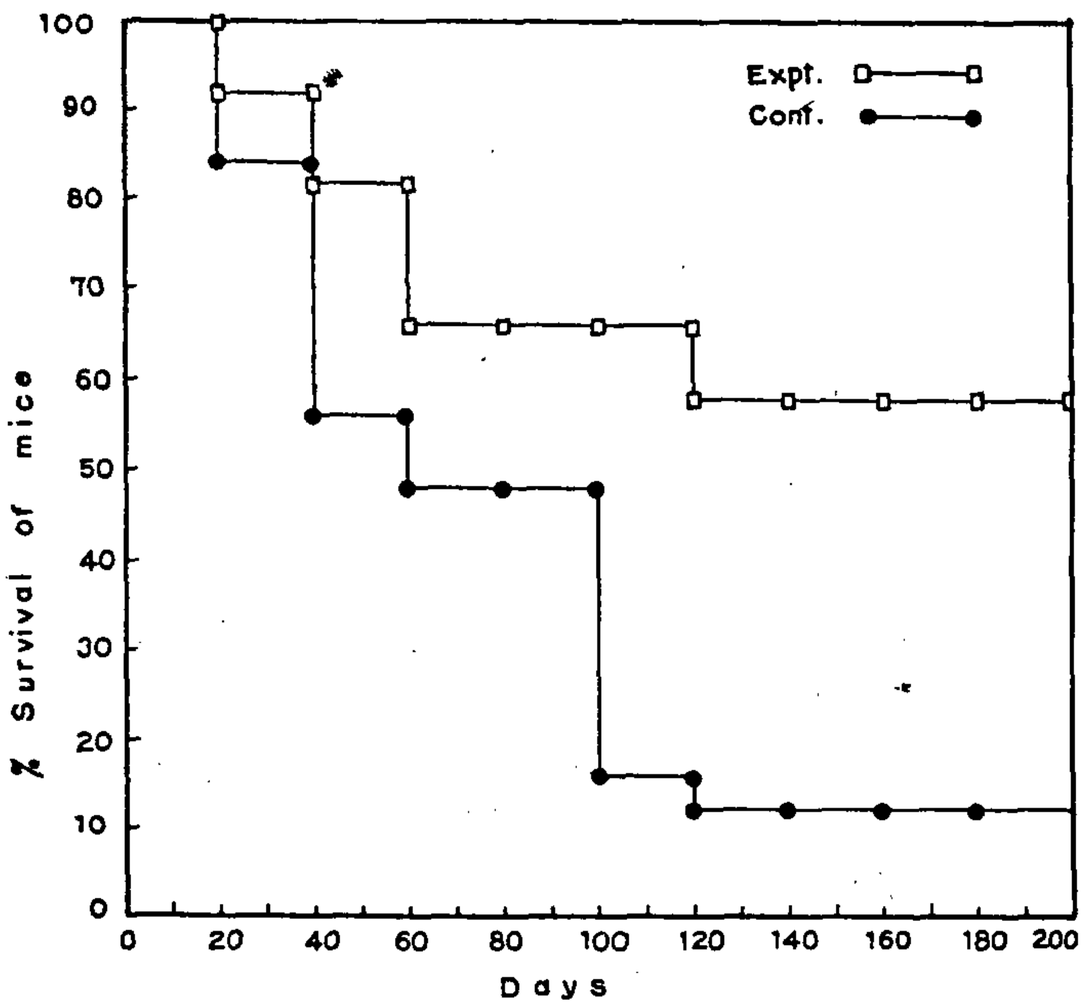

Figure 2. Survival of the animals after surgical removal of solid fibrosarcoma tumour with adoptive transfer of Ts depleted polyclonally activated T cells (experimental) without any cell transfer (control).

bearing a wide variety of TAAs (refs 3, 4). Since Con A as a polyclonal stimulator is likely to activate the suppressor T (Ts) cells also, low dose of Cy was used to destroy the Ts population prior to Con A mediated activation of the remaining clones. Such effector T cell population was previously found to be capable of restricting tumour growth when adoptively transferred at the tumour site[3,6]. In the present investigation, such effector T cells radiolabelled and adoptively transferred via intravenous route were found preferentially to home at the tumour mass (Figure 1). The high percentage of radioactivity in the central zone of tumour at 24 h indicates that the effector cells not only accumulate at the tumour site, but gradually infiltrate into the tumour mass.

Considering the tumour-seeking activated lymphocytes were likely to recognize the residual malignant cells after surgical removal of solid tumours, these lymphocytes were adoptively transferred after surgery of the tumour. It is evident from Table 1 that after removal of solid fibrosarcoma, adoptively transferred syngeneic effector lymphocytes can successfully wipe out the remaining tumour cells, thus preventing recurrence of tumour in majority of cases. Simultaneously the survivality of the mice is also increased in comparison to the control.

Previously, attempts to curb malignant growth by passive transfer of sensitized lymphocytes[8,13,14] met with limited success. However, T cells expanded *in vitro* in presence of interleukin-2 (IL-2) are reported to be effective for adoptive immunotherapy[15–19]. Attempts are also being made to use exogenous IL-2 *in vivo* for maintaining the effector lymphocytes in the tumour-bearing host[13,16,20–25]. In this context, the use of polyclonally activated T cells possibly has certain advantages. Besides activating multiple clones to take care of the wide variety of TAAs, such polyclonal stimulation also activates the helper T cells which can act as a direct source of IL-2 in the host. However, for long term maintenance of the adoptively transferred cells in hosts, an attempt to combine exogenous IL-2 administration with adoptive transfer of Con A stimulated effector cells is under consideration.

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