Clin. exp. Immunol. (1972) **10**, 297–303.

RECIRCULATING LYMPHOCYTES IN THE MOUSE THYMUS ARE PART OF THE RELATIVELY CORTISONE RESISTANT CELL POPULATION

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(*Received* 5 *July* 1971)

SUMMARY

A quantitative analysis of the distribution patterns of intravenously infused ^{51}Cr-labelled thymus and lymph node cells was carried out. It was observed that only a small fraction of the thymic cells distributed to the lymph nodes of the recipients. These lymph node seeking thymic cells could be enriched by treating the donor mice with cortisone acetate whereas this treatment did not change the distribution pattern of lymph node cells. Thymectomy followed by lethal irradiation and bone marrow reconstitution of the recipients did not change the distribution patterns of normal or cortisone resistant thymic cells. The cells in lymph nodes which home to lymph nodes upon intravenous infusion were found to be thymus-dependent, since they were almost absent in the lymph node cells obtained after thymectomy irradiation and bone marrow protection of the donors.

The results indicate that only a small proportion of the cells in the thymus, namely the relatively cortisone resistant cells known to exhibit the cell-bound type of immunological reactivity, behave like recirculating lymphocytes upon infusion into isologous recipients.

INTRODUCTION

There is strong evidence indicating that only a minority of the lymphocytes in the mouse thymus are immunocompetent. These cells are relatively cortisone resistant (Blomgren & Andersson, 1969, 1970; Andersson & Blomgren, 1970; Cohen, Fishbeck & Claman, 1970; Blomgren, 1971; Blomgren, Takasugi & Friberg, 1970; Blomgren & Svedmyr, 1971), indicating that they are located in the medullary areas of the organ (Ishidate & Metcalf, 1963; Dougherty *et al.*, 1964). The majority of the cells which are located in the thymic cortex, constituting approximately 95% of the total thymus cell population, do not seem to exhibit any type of immunological reactivity. Since it is likely that the reactive cells have gained their immunological competence within the thymus (Cohen *et al.*, 1963; Blomgren

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& Andersson, 1971) it is also possible that they leave the organ to supply extra-thymic lymphoid tissues with reactive cells. Indeed, it has been demonstrated that there is a migration of lymphocytes from the thymus into the efferent blood vessels (Ernström, Gyllensten & Larsson, 1965; Larsson, 1966a,b; Ernström & Larsson, 1966, 1969) and immigrant thymic cells seem to home mainly to lymphoid tissues (Weissman, 1967).

By injecting ^{51}Cr-labelled lymphocytes from various organs into isologous recipient mice, it has been demonstrated that only a minority of the infused thymocytes can be recovered in the lymph nodes of the recipients. However, a high proportion of lymphocytes from other lymphoid organs tend to seek to the lymph nodes of the host animal (Zatz & Lance, 1970). In the present article we are presenting data which demonstrate that the immunocompetent portion of the mouse thymus cell population (relatively cortisone resistant) distribute like the thymus-dependent lymphocyte population of lymph nodes, when infused into syngeneic recipients.

MATERIALS AND METHODS

Mice. Inbred female mice of strain CBA, 2–3-months old, were used.

Cortisone treatment of mice. Mice were injected i.p. with 125 mg cortisone acetate/kg body weight (Upjohn, 25 mg/ml). The animals were killed 2 days after injection and cell suspensions were prepared from thymus and lymph nodes.

Preparation of cell suspensions. Cell suspensions were prepared from thymi and lymph nodes, (axillary, inguinal and mesenteric) by pressing them through a stainless steel mesh (60-mesh) into ice-cold balanced salt solution (BSS). Visible aggregates were dispersed by passage to and fro through an 18-gauge needle.

^{51}Cr-labelling of lymphocytes. 10^8 cells suspended in 2·0 ml of BSS were incubated for 30 min with 200 μCi of ^{51}Cr-labelled Na_2CrO_4 (Radiochemical Centre, Amersham, England). After incubation, the cells were washed four times in BSS by centrifugation and resuspended in BSS at a final concentration of 20×10^6 cells/ml. The number of viable nucleated cells was determined by conventional Trypan blue exclusion test. The frequency of dead cells did not exceed 10%.

Primary homing of ^{51}Cr-labelled cells. 6×10^6 viable ^{51}Cr-labelled cells, with a total radioactive activity of 50,000–80,000 counts per minute (cpm) suspended in 0·3 ml of BSS were, unless otherwise stated, injected into the tail veins of untreated isologous recipients. After 24 hr the animals were killed and various organs, freed from adjacent tissues, were placed into plastic tubes (Falcon Plastics, Los Angeles, Calif.) and the radioactivity of various organs was measured by a well-type scintillation counter. The radioactivity, measured as cpm, was related to the total amount of cpm in the cell inoculum.

Secondary homing of ^{51}Cr-labelled cells. Cell suspensions were prepared from the lymph nodes of mice which had received an i.v. infusion of ^{51}Cr-labelled lymphocytes 24 hr earlier. After centrifugation the cells were resuspended in BSS at a concentration of 10^8 cells/ml. 0·3 ml of this cell suspension was injected i.v. into isologous recipients and 24 hr later the radioactivity in various organs of these secondary recipients was established and related to the total radioactivity injected.

Thymectomy, irradiation and bone marrow reconstitution. Operations were performed under Nembutal anaesthesia (Abbott). After a skin incision, the thymus lobes were removed by suction through a Pasteur pipette. The skin was then closed with two or three silk

sutures. Sham thymectomy was performed under identical conditions, but the thymus lobes were left intact. Three weeks after surgery the animals were whole-body irradiated with 800 R (200 kV, 15 mA, FD 60 cm, 1·5 mm Al inherent filtration, added filter 0·5 mm Cu, HVL 0·95 mm Cu) and within 2 hr injected i.v. with 5×10^6 bone marrow cells. These mice will be referred to as Tx-ect. 800 R:BM or Sham Tx-ect. 800 R:BM.

RESULTS

Distribution of lymphoid cells from normal or cortisone treated donors

^{51}Cr-labelled thymus or lymph node cells from normal or cortisone treated donors were infused into isologous recipients. The radioactivity at various sites, 24 hr after injection, is presented in Table 1. It can be seen that normal lymph node and thymus cells exhibit widely different distribution patterns. Only 2% of the injected radioactivity of normal thymus cells was found in the lymph nodes whereas 10% of the radioactivity of normal

TABLE 1. Distribution patterns of ^{51}Cr-labelled lymphocytes obtained from untreated donors or mice treated 2 days earlier with cortisone acetate

^{51}Cr-labelled cells injected	No. of mice	Mean percentage distribution or radioactivity ± SE				
		Lymph node*	Spleen	Liver	Femur	Thymus
Normal thymus	7	2·38 ± 0·13	30·31 ± 0·52	22·63 ± 1·70	0·68 ± 0·10	—†
Cortisone treated thymus	12	10·67 ± 0·38	22·55 ± 1·00	17·49 ± 0·58	0·31 ± 0·01	—
Normal lymph node	7	10·38 ± 0·52	11·51 ± 0·46	15·39 ± 0·88	0·17 ± 0·02	—
Cortisone treated lymph node	6	10·90 ± 0·96	14·86 ± 1·00	20·02 ± 0·86	0·26 ± 0·01	—

*The mesenteric, inguinal and axillary lymph nodes were pooled.
†Less than 0·05% of the radioactivity was recovered.

lymph node cells was homing to the lymph nodes. Cortisone treatment of the cell donors, which enriches the immunocompetent cells in the thymus, increased the proportion of lymph node seeking cells to 11%. Cortisone treatment did not change the distribution pattern of the lymph node cells. The distribution patterns of lymph node cells and cortisone resistant thymus cells were the same, except that 22% of cortisone resistant thymus as compared to 11% of lymph node was found in the spleen.

Secondary distribution of lymph node seeking thymus and lymph node cells

The results presented above indicate that only a minor fraction of the thymus cell population, relatively cortisone resistant, distributes like lymph node cells upon infusion into mice. If this consideration were correct, the thymocytes from normal donors which had homed to lymph nodes would be part of the cortisone resistant population, and it follows that they would exhibit a higher tendency than the original thymus cell population to home to lymph nodes upon reinfusion into new mice.

Table 2 shows the secondary distribution of lymph node seeking thymus or lymph node cells when injected into new hosts. It is evident that the normal thymus cells which had

TABLE 2. Secondary distribution of thymus and lymph node cells which had homed to lymph nodes of primary recipients

^{51}Cr-labelled cells injected into primary recipients	No. of mice	Mean percentage distribution of radioactivity ± S.E				
		Lymph node*	Spleen	Liver	Femur	Thymus
Normal thymus	4	5·52 ± 0·93	7·16 ± 1·92	4·92 ± 0·42	—†	—†
Cortisone treated	4	11·21 ± 0·39	14·08 ± 0·60	6·91 ± 0·35	—	—
Normal lymph node	4	8·64 ± 0·58	13·07 ± 0·68	10·90 ± 0·33	—	—
Cortisone treated lymph node	4	8·85 ± 0·36	10·53 ± 0·50	6·87 ± 0·22	—	—

*The mesenteric, inguinal and axillary lymph nodes were pooled.
†Less than 0·05% of the radioactivity was recovered.

homed to the lymph nodes of the primary recipient exhibited an increased tendency to home to lymph nodes again when infused into new recipients. Such an increment was not observed for lymph node cells or cortisone resistant thymus cells.

Distribution of lymphocytes infused into Tx-ect. 800 R : BM mice

The results presented above indicated that the relatively cortisone sensitive fraction of the thymus cell population has no tendency to seek to lymph nodes when infused into untreated recipients. This could mean that lymph nodes, under physiological conditions, are saturated with such cells which have migrated from the thymus and this may be why no detectable amounts of new cells can settle in the lymph nodes. This thesis was tested by injecting ^{51}Cr-labelled thymus cells into thymectomized or sham thymectomized mice which had received 800 R : BM 6 weeks earlier.

Table 3 shows that thymectomy of the recipients does not alter the distribution of the injected lymphocytes regardless of whether the thymic cells were obtained from normal or cortisone treated donors.

Distribution of lymph node cells from Tx-ect. 800 R : BM mice

To test the possibility that the lymph node seeking cells in lymph nodes are part of the thymus dependent pool of cells, the following experiment was performed. Cell suspensions,

TABLE 3. Distribution of thymus cells infused into thymectomized or sham thymectomized mice which were irradiated with 800 R and reconstituted with bone marrow cells 6 weeks earlier

^{51}Cr-labelled cells injected	Treatment of recipients	No. of mice	Mean percentage distribution of radioactivity ± SE			
			Lymph node*	Spleen	Liver	Femur
Normal thymus	Sham Tx-ect., 800 R : BM	7	1·57 ± 0·25	19·67 ± 2·01	33·67 ± 2·16	0·43 ± 0·10
	Tx-ect., 800 R : BM	5	1·53 ± 0·19	26·00 ± 0·57	32·00 ± 1·74	1·03 ± 0·11
Cortisone treated	Sham Tx-ect., 800 R : BM	7	10·86 ± 1·44	25·04 ± 2·22	18·71 ± 1·54	0·28 ± 0·06
	Tx-ect., 800 R : BM	5	9·40 ± 1·03	24·25 ± 1·84	15·60 ± 0·68	0·33 ± 0·05

* The mesenteric, inguinal and axillary lymph nodes were pooled.

prepared from lymph nodes 6 weeks after 800 R: BM of thymectomized or sham thymectomized mice, were injected into isologous recipients. It can be seen in Table 4 that thymectomy, irradiation and bone marrow reconstitution of mice results in a depletion of the lymph node homing cells in the lymph nodes. Only 1% of the lymph node cells from such mice were recovered in the lymph nodes of injected recipients. In contrast, 10% of the lymph node cells of sham Tx-ect. 800 R: BM distributed to the lymph nodes when injected into isologous hosts.

TABLE 4. Distribution of i.v. infused lymph node cells obtained from thymectomized or sham thymectomized irradiated and bone marrow reconstituted donors

Treatment of donors	No. of recipients	Mean percentage destribution of radioactivity ± SE			
		Lymph node*	Spleen	Liver	Femur
Tx-ect., 800 R: BM*	15	1·20 ± 0·07	8·52 ± 0·46	33·17 ± 0·97	0·82 ± 0·04
Sham Tx-ect., 800 R: BM*	10	11·86 ± 0·52	15·44 ± 0·43	19·37 ± 0·79	0·43 ± 0·09

* Irradiation and bone marrow protection was performed 6 weeks earlier.
† The mesenteric, inguinal and axillary lymph nodes were pooled.

DISCUSSION

The pool of small, long-lived lymphocytes, which continuously circulate between blood and lymph are considered to be thymus-dependent (Gowans & Knight, 1964). There is also strong evidence that these cells make up a large proportion of the cells in peripheral lymphoid organs (Raff & Owen, 1971) where they are located within certain so called thymus-dependent areas (Dukor & Dietrich, 1967; Dukor, Miller & Sacquet, 1968). Various lymphoid organs differ with respect to their proportion of thymus-dependent lymphocytes (Raff & Owen, 1971). This difference is manifested by the different distribution patterns of i.v. injected lymphocytes from various organs. There seems to be a good positive correlation between the proportion of lymph node seeking cells and the frequency of recirculating lymphocytes, i.e. thymus-dependent cells (Zatz & Lance, 1970). Using this type of assay it has been calculated that the mouse thymus contains around 5% recirculating cells whereas they constitute around 60% of a lymph node population.

In the present investigation we have been able to confirm that i.v. infused thymic cells exhibit a much lower localization to lymph nodes than do lymph node cells. Since we have previously shown that cells mediating cellular immunity in the mouse thymus can be enriched by treating animals with cortisone acetate, it was of interest to know whether these thymic cells behave like recirculating lymphocytes upon infusion into syngeneic recipients. The results of the present investigation have shown that approximately a ten times higher proportion of the cortisone resistant fraction of the thymus cell population localized to lymph nodes than did cell thymus from untreated cells. There was a striking similarity in the distribution patterns between cortisone resistant thymic cells and lymph node cells with regard to seeking to the lymph nodes (10%). Seeking to spleen was 11% for lymph node and 22% for cortisone resistant thymus cells. It is unlikely that the cortisone treatment

of the mice altered the distribution behaviour of the cells rather than enriching recirculating cells for the following reasons: (i) cortisone treatment did not change the distribution of lymph node cells, and (ii) thymus cells, from untreated donors, which had localized to lymph nodes of primary recipients exhibited a higher tendency to seek to lymph nodes when tranferred to new recipients. This finding is in agreement with the data obtained by Zatz & Lance (1970).

As related above, the lymph node seeking cells of lympoid populations are considered to belong to the thymus dependent, recirculating pool of lymphocytes. This would imply that lymph nodes which are depleted of such cells by thymectomy followed by X-irradiation and bone marrow reconstitution would show a low proportion of cells localizing to lymph nodes upon infusion into isologous recipients. This thesis was confirmed, since it was observed that lymph node cells from Tx-ect. 800 R:BM mice exhibited a lower frequency of cells homing to lymph nodes, than similar cells from sham Tx-ect. 800 R:BM mice. These data are in agreement with the results of other investigators who have depleted animals of thymus dependent lymphocytes by treatment with antilymphocyte serum (Taube & Lance, 1968). Lymphoid cells from such animals exhibited a lower localization to lymph nodes when injected into isologous hosts than did cells from untreated animals.

It has recently been shown that thymic cells carrying the thymus specific T1-antigen (thymus leukaemia) do not behave like recirculating lymphocytes upon infusion into mice. Moreover, it has been shown that the small proportion of T1-negative thymic cells, enriched by killing the T1-positive ones with specific antibodies and complement *in vitro*, behave like recirculating lymphocytes (Lance, Cooper & Boyse, 1971; Raff, 1971). These results are in agreement with ours, since the small fraction of cortisone resistant cells in the thymus of T1-positive mice has been shown to be T1-negative (Schlesinger & Golakia, 1967).

In conclusion, our results show that the relatively cortisone resistant fraction of the thymus cell population, known to exhibit the cell-bound type of immunological reactivity, behaves like recirculating lymphocytes upon infusion into syngeneic recipients.

ACKNOWLEDGMENTS

This work was supported by the Swedish Cancer Society, the Cancer Association of Stockholm and the Research Foundations of the Karolinska Institute.

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