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Whole Body Localization of Intravenously Injected Lymphoblasts in Normal Rats

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Summary

Total body distribution of ^{125}IUDR labeled thoracic duct, mesenteric and peripheral lymph node blasts was investigated, after i.v. injection into normal syngeneic rats. Donors of immunoblasts were stimulated with SRBC 1 week before transfer of cells. Lymphoblasts, irrespective of the source of origin, accumulated preferentially in small intestine, lungs, skin and muscles. There was an evident difference in the whole body distribution pattern of injected thoracic duct and lymph node blasts, what points to the different behaviour of normally circulating immunoblasts. Only minor differences in distribution kinetics and sites of accumulation were found between mesenteric and peripheral blast cells. We put forward a hypothesis that immunoblasts behave like effector cells and their preferential localization in small gut, lungs, and skin may be mediated by antigens „physiologically" present on the surface of tissues with direct contact with the external environment.

The mechanism by which lymphocytes extravasate to tissue compartments are poorly understood, yet are fundamental to understanding of problems of allograft rejection, inflammatory bowel disease or local defense against tumors. Migrating lymphocytes certainly play a role in the local immune recognition and defense in the peripheral tissues. The major part of recirculating lymphocytes finds its way from the vascular compartment to the lymphatics crossing the vascular endothelium in lymph nodes (1) and other lymphoid organs. However, some lymphocytes extravasate from the capillary beds of non-lymphoid tissues. These lymphocytes enter the peripheral tissue fluid and then the afferent lymphatics. They may play an important role in the recognition phase of the local immune surveillance in peripheral tissues and act as effector cells at sites of antigen deposition.

There is a consistent difference between different lymphoid and non-lymphoid tissues in the accumulation of lymphocytes leaving blood vessel. Distinct subpopulations of lymphocytes exist recirculating through the lymphoid and the non-lymphoid tissues with probably some overlap. Around 90% of the ^{51}Cr-lymphocyte traffic goes through the lymphoid organs, whereas 10% of the recirculating lymphocytes which reach a lymph node do so through the non-lymphoid tissues (2). Immunoblasts or large lymphocytes behave differently. Only about 60% of ^{125}IUDR-labeled cells accumulate in the lymphoid tissue, the rest migrating to the extra-lymphoid sites (3).

Immunoblasts possess the ability to accumulate in normal animals, specifically in the gut (1, 3, 4, 5, 6, 7), and in smaller numbers in lungs, bone marrow and lymph nodes. They also localize in the inflamed skin, foci on injected antigen, and peritoneal cavity (3, 9, 10). Mesenteric and peripheral lymphoblasts migrate differently *in vivo* both with respect to their ability to migrate to the lamina propria of the inflamed gut (4) and to the inflamed skin (6). It can then be assumed that lymphoblasts behave like effector cells.

Most of the studies on migration kinetics of immunoblasts and the specificity of their territorial extravasation, especially with respect to the non-lymphoid tissues, have been performed on animals stimulated locally or systematically with certain antigens. However, no data are available on simultaneous total body distribution of i.v. injected blast cells in normal non-stimulated animals.

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The purpose of the present study was to investigate the total body localization of immunoblasts after i.v. administration, the time course of distribution, and difference in territorial accumulation of mesenteric, peripheral and thoracic duct blast cells.

Materials and Methods

Animals. Highly inbred Wistar male rats aged 2–3 months were used as donors and recipients of lymphocytes.

Cell suspensions. Donor rats were immunized 1 week prior to harvesting blast cells with SRBC with Freund adjuvant (at a volume of cells to adjuvant 1:1). All four footpads were injected with 0.01 ml of suspension to obtain stimulated peripherhal lymphocytes, and the wall of intestine for mesenteric blasts. For obtaining thoracic duct blasts both footpads and intestinal wall were injected. Thoracic duct cells (TDL) were collected from the thoracic duct fistula. Mesenteric and peripheral lymph node cells (MLNL, PLNL) were dispersed by teasing with forceps and injection needle gauge 18 in 4 °C autologous thoracic duct lymph, to avoid adverse effects of artificial media on surface receptors and cell viability. Suspensions were filtered through nylon mesh. The number of viable cells was assessed by their ability to exclude Trypan Blue.

In vitro labeling of lymphocytes. In order to label lymphocytes synthesizing DNA, cell suspensions were incubated with [^{125}I]-5-iodo-2-deoxyuridine (^{125}IUDR, activity ~ 5mCi/mg) obtained from the Radiochemical Center, Amersham. They were incubated in syngeneic lymph with heparin (without preservative) (5 units/ml of lymph) containing 5 μCi/10^8 cells/ml for 60 min at 37 °C. After labeling, cells were washed three times with heparinized lymph and viability tested. Autoradiography of cell preparations revealed that 4–5% of cells in TD lymph, about 15% in MLN and 10% in PLN incorporated ^{125}IUDR.

To label the whole population of harvested lymphocytes cells were incubated with sodium chromate, ^{51}Cr (Radiochemical Centre, Amersham or Institute for Nuclear Research, Warsaw) at a concentration of 50 μCi/10^8 cell/ml of syngeneic lymph.

Cell suspensions were adjusted to a concentration of 5 x 10^7 cells/ml and a total of 1–1.5 x 10^8 cell injected into the dorsal vein of penis.

Each pool of cells was divided into two aliquots, one was labeled with sodium chromate ^{51}Cr, the other with ^{125}IUDR.

Tab. 1 Time course of radioactivity in tissues after I.V. injection of 1 x 10^8 ^{125}IUDR-labelled thoracic duct lymphoblasts. Values are means of percent of total injected dose (n = 5).

Tissue or organ	Time of sacrifice (hr)				
	1	3	5	8	24
Blood cells	1.55	1.2	0.51	0.49	0.23
Serum	1.23	0.91	0.51	0.54	0.24
Lungs	12.5	5.94	2.99	1.9	0.27
Kidneys	0.82	0.48	0.5	0.46	0.19
Stomach	3.53	1.11	0.44	0.33	0.48
Small bowel	7.00	4.19	2.94	2.27	1.16
Large bowel	0.78	0.71	0.34	0.3	0.3
Payer's patches	0.14	0.1	0.14	0.11	0.08
Spleen	3.47	2.69	2.27	1.43	0.39
Liver	7.89	5.25	4.46	3.68	1.36
MLN	0.23	0.15	0.16	0.19	0.16
Thymus	0.032	0.014	0.021	0.026	0.059
PLN	0.085	0.11	0.2	0.28	0.32
Skin	6.92	5.9	5.02	4.52	2.47
Muscles	4.81	3.88	3.2	2.66	1.85
Bone (Marrow)	7.51	4.99	4.26	3.56	1.69
Total recovery	58.5	37.6	28.0	22.8	11.2

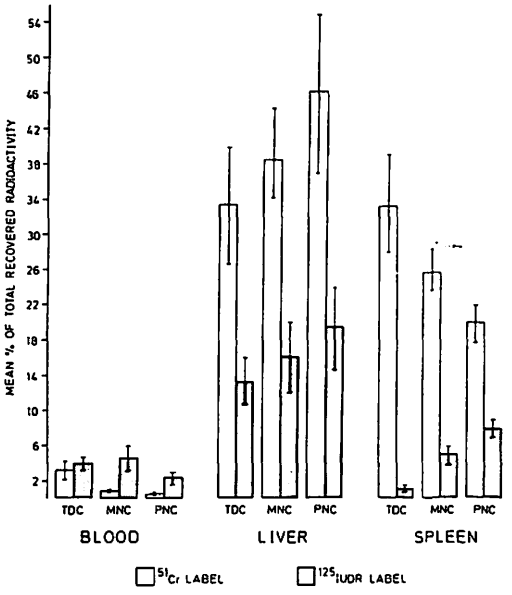

Fig. 1a,b.c.d Recovery of radioactivity in tissues 24 hr after i.v. injection of 10^8 ^{51}Cr- and ^{125}IUDR-labeled syngeneic thoracic duct (TDC), mesenteric lymph node (MNC) and peripheral lymph node (PNC) cells in normal rats. Values are means of percentage of the total recovered dose from 5 animals ± S.E.

In vivo distribution of labeled cells. The experiments were performed in 3 groups divided into 2 subgroups depending on cell labeling with ^{51}Cr or ^{125}IUDR. All animals were kept in Bolmann or metabolic cages to avoid contamination with urine. They received iodine enriched diet. In group 1 rats were injected with TDL, in group 2 with MLNL, in group 3 with PLNL. At 1, 3, 5, 8, 12 and 24 hours after cell transfer animals were killed and *all* lymphoid and non-lymphoid tissues removed as follows: serum, blood cells, lungs, kidneys, stomach, small bowel, large bowel, Payer's patches, spleen, liver, MLN, thymus, PLN, whole shaved skin, whole muscle mass, whole carcass, brain. The radioactivity of each tissue or organ was measured in a gamma counter.

Control groups. In order to measure the rate of reutilization of ^{125}IUDR or ^{125}I released from the *in vivo* dying cells control studies were performed with rats injected with a) heat-killed ^{125}IUDR-blast cells, b) supernatant from heat-killed ^{125}IUDR-blast cells, c) ^{125}IUDR, d) ^{125}I (sodium iodide). The animals were sacrificed after 24 hr and radioactivity in all organs measured.

Statistical analysis. Results were presented for each tissue or organ as percentage of total injected dose ± S.E. In the tables standard errors have been omitted for clarity of presentation.

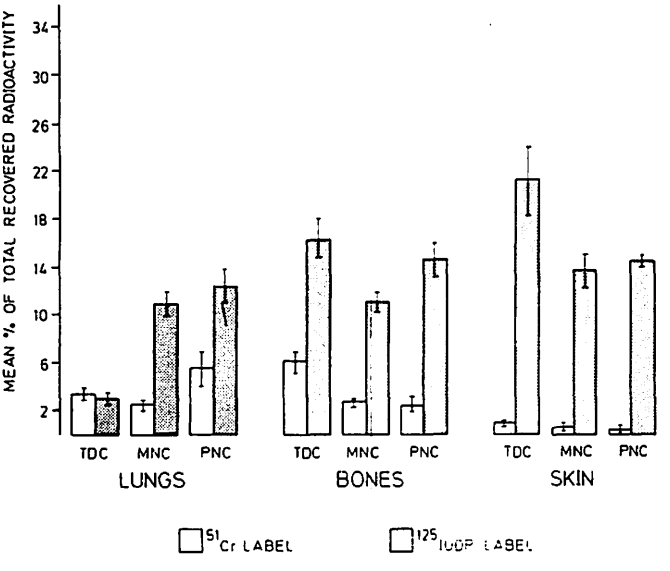

Fig. 1b

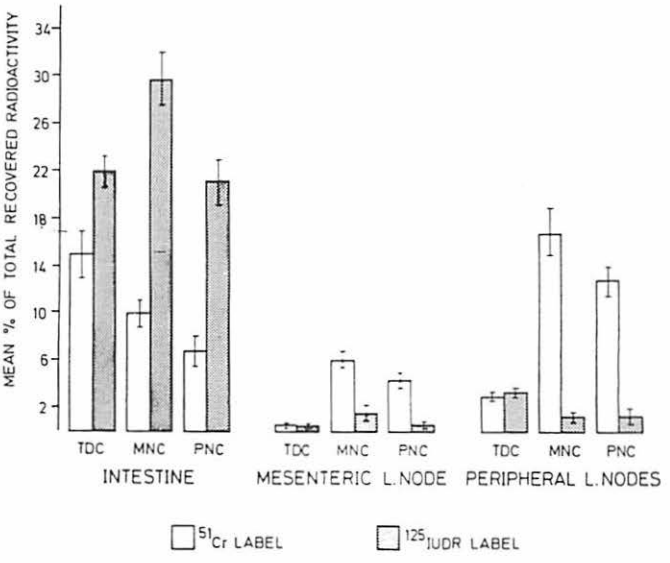

Fig. 1c

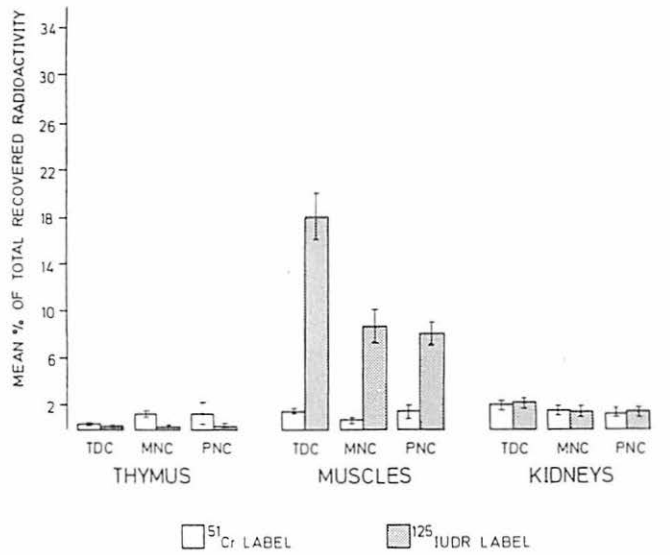

Fig. 1d

Results

Localization of TDL. Results of localization of ^{125}IUDR cells have been presented in Table 1. Total recovery of radioactivity was 58.5% after 1 hr, to decrease to 11.2% after 24 hr. Most of the radioactivity was recovered in small bowel, liver, skin, muscles and bones. At 1 hr highest radioactivity was found in the lungs, then skin and small bowel. It was decreasing steadily until 12 hr when a plateau was reached. At 24 hr radioactivity of the whole skin was higher than of small intestine, with lungs retaining less than 0.5%.

When the percentages of recovered doses of ^{125}IUDR TDL and ^{51}Cr TDL were compared (Fig. 1A–D) an evident difference of distribution of both populations could be observed. The DNA-synthesizing cells had a predilection for

wandering to bowel, lungs, bones, skin and muscles in distinction to ^{51}Cr TDL migrating to liver, spleen nodes, intestine.

Localization of MLNL. Results of the ^{125}IUDR MLNL tissue accumulation can be found in Table 2. As compared with the distribution of ^{125}IUDR-TDL the total recovery rate of MLN blasts after 24 hr was higher. Also, there was a small but evident increase in radioactivity recovered in the course of time in the small bowel, MLN, PLN, and skin, with a rather steady level in spleen and bone marrow. When the ^{51}Cr and ^{125}IUDR populations were compared (Fig. 1A–D) the ^{125}IUDR MLNL followed the same pattern as the ^{125}IUDR TDL. However, there were more MLN blasts in the intestine and lungs (Fig. 2, 3), but less in skin (Fig. 4) and bone marrow than of TDL blasts.

Localization of PLNL. The total recovery of ^{125}IUDR PLNL was the same as of MLNL, but higher than of TDL (Table 3). There were minor differences in recovery of radioactivity between the PLN and MLN blasts. The small bowel (Fig. 2), skin (Fig. 4), and muscles radioactivity remained stable throughout the 24 hr period, whereas of the spleen, MLN, PLN and bone marrow slightly increased. PLN blasts behaved similarly as MLN blasts when compared with the PLNL and MLNL ^{51}Cr population (Fig. 1A-D).

Tab. 2 Time course of radioactivity in tissues after I.V. injection of 1 x 10^8 ^{125}IUDR-labelled mesenteric lymph node (MLN) lymphoblasts. Values are mean of percent of total injected dose (n = 5).

Tissue or organ	Time of sacrifice (hr)				
	1	3	5	8	24
Blood cells	1.9	1.5	1.0	0.7	0.43
Serum	1.0	0.73	0.97	0.6	0.4
Lungs	13.17	10.74	6.0	6.67	2.34
Kidneys	0.86	0.81	0.38	0.38	0.37
Stomach	3.2	1.2	0.9	0.3	0.22
Small bowel	4.6	5.75	3.6	5.04	6.06
Large bowel	0.78	0.6	0.32	0.28	0.25
Payer's patches	0.18	0.12	0.16	0.11	0.09
Spleen	2.6	4.87	2.34	2.96	3.72
Liver	10.06	9.32	4.4	5.75	4.17
MLN	0.19	0.44	0.56	0.75	0.98
Thymus	0.04	0.021	0.025	0.035	0.06
PLN	0.036	0.1	0.21	0.09	0.52
Skin	4.04	4.57	4.31	3.62	5.52
Muscles	3.77	3.6	3.66	1.86	1.22
Bone (Marrow)	3.9	2.44	4.3	2.86	3.28
Total recovery	45.6	44.6	31.1	30.7	28.1

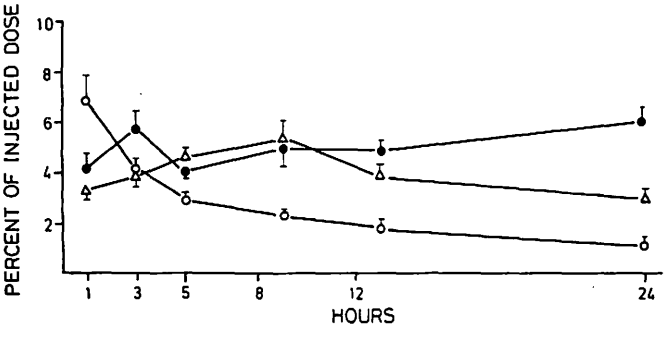

Fig. 2 Time course of accumulation of radioactivity in rat small intestine after i.v. injection of 10^8 ^{125}IUDR TDL (–o–), MLN (–●–), and PLN (–△–) lymphocytes. Values are means of 3 experiments after each time period ± S.E.

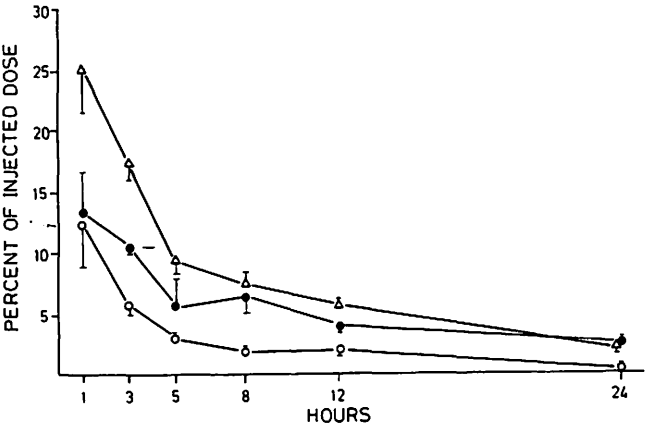

Fig. 3 Time course of accumulation of radioactivity in rat lungs after i.v. injection of 10^8 ^{125}IUDR TDL (–o–), MLN (–●–), and PLN (–△–) lymphocytes. Values are means of 3 experiments after each time period ± S.E.

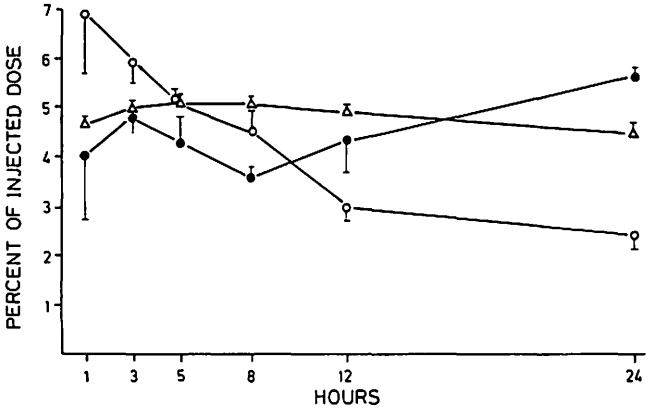

Fig. 4 Time course of accumulation of radioactivity in rat skin after i.v. injection of 10^8 ^{125}IUDR TDL (–o–), MLN (–●–), and PLN (–△–) lymphocytes. Values are means of 3 experiments after each time period ± S.E.

Tab. 3 Time course of radioactivity in tissues after I.V. injection of 1 x 10^8 ^{125}IUDR-labelled peripheral lymph nodes (PLN) lymphoblasts. Values are means of percent of total injected dose (n = 5).

Tissue or organ	Time of sacrifice (hr)				
	1	3	5	8	24
Blood cells	2.26	2.1	2.2	2.0	0.47
Serum	2.0	1.48	1.41	1.35	0.4
Lungs	25.36	17.64	9.54	7.55	2.54
Kidneys	0.83	0.79	0.8	0.63	0.43
Stomach	3.2	1.09	0.56	0.5	0.32
Small bowel	3.4	3.96	4.74	4.39	3.06
Large bowel	0.6	0.72	0.48	0.4	0.36
Payer's patches	0.14	0.08	0.1	0.16	0.16
Spleen	1.6	1.85	1.85	2.83	2.33
Liver	9.87	8.9	5.06	4.81	4.2
MLN	0.13	0.15	0.27	0.32	0.81
Thymus	0.03	0.01	0.01	0.008	0.1
PLN	0.071	0.085	0.09	0.28	0.33
Skin	4.84	4.98	5.14	5.33	4.56
Muscles	4.04	3.48	5.06	4.98	3.89
Bone (Marrow)	3.44	3.48	5.3	5.04	4.5
Total recovery	57.8	48.0	41.36	39.0	27.2

Tab. 4 Recovery of radioactivity 24 hr after I.V. injection of a) heat-killed ^{125}IUDR-labelled mesenteric lymph node blasts, b) supernatant of heat killed ^{125}IUDR-labelled blasts, c) ^{125}IUDR. Values are means of percent of 3 experiments in each group.

Tissue or organ	Injected material		
	Heat-killed blasts	Supernatant	^{125}IUDR
Blood cells	0.07	0.12	0.07
Serum	0.095	0.058	0.067
Lungs	0.13	0.18	0.039
Kidneys	0.066	0.08	0.042
Stomach	0.085	0.1	0.19
Small bowel	0.3	0.21	0.58
Large bowel	0.017	0.025	0.056
Payer's patches	0	0	0.007
Spleen	0.06	0.016	0.023
Liver	0.78	0.32	0.098
MLN	0.022	0.002	0.013
Thymus	0	0	0.006
PLN	0.006	0.008	0.017
Skin	0.89	0.91	1.17
Muscles	0.11	0.11	0.35
Bone (Marrow)	0.31	0.2	0.36
Total recovery	3.02	2.19	3.24

Control groups. Total recovery of radioactivity after i.v. injection of heat-killed blast cells, supernatant from these cells, ^{125}IUDR (Table 4), or ^{125}sodium iodide was at a level of 3%. There was more accumulation of radioactivity in the skin and bowel, muscles and bones than in other tissues, but the amount found in these tissues could not affect the interpretation of results after injection of living cells.

Discussion

The main findings of the present study were: a) a high rate of recovered radioactivity after i.v. injection of ^{125}IUDR blasts preferentially in the small bowel, lungs, whole skin and muscles, b) differences in the whole body distribution pattern of injected blast derived from the thoracic duct as compared with the mesenteric and peripheral blasts, c) minor differences in distribution kinetics of mesenteric and peripheral immunoblasts.

The propensity of the normal skin to accumulate immunoblasts has been found by us in a previous work (11). The present study corroborates only the previously obtained results. Most of the studies of other authors on direction of *in vivo* migration of blast cells has been performed on models with inflamed skin (e.g. 6, 8, 10) and no data concerning the whole skin homing of immunoblasts under normal conditions have been found. The mechanism of accumulation of radioactivity in muscles after transfer of ^{125}IUDR-cells is unclear. One explanation may be unspecific margination of blasts in the microvasculature of muscles, but this requires further studies.

The TDL blasts had, in our study, evidently different kinetics of distribution than the MLN or PLN blasts. After transfer of ^{125}IUDR TDL, radioactivity in all tissues with the exception of Payer's patches MLN and PLN, decreased throughout 24 hr. On the contrary, after injection of MLN blasts there was an increase in radioactivity in the small bowel, spleen and skin. The level in bone marrow and partly in muscles remained almost unchanged.

Practically no differences were found in the kinetics of distribution between ^{125}IUDR MLN and ^{125}IUDR PLNL. These findings indicate that circulating immunoblasts behave differently than all those harvested from lymph nodes. Lack of directional differences in migration of MLN and PLN blasts is in contradiction with finding of others (4, 6, 12).

It has been reported that the ability of T-lymphoblasts to extravasate into inflamed intestine or skin is determined by their tissue of origin (6). T-blasts from oxazolone stimulated PLN failed to migrate to the gut of normal recipients but moved to the inflamed skin. In contrast, T-blasts separated from MLN stimulated with Trichinella spiralis accumulated in the gut but not in skin. However, the latter experiments were performed on animals with skin inflammation, and the type of antigen initiating the immune reaction in the given tissue might play a role. According to others (13) immunoblasts generated in somatic nodes do not enter the small gut but tend to localise in the spleen. This was not confirmed by our studies.

High proportion of lymphoblasts which has been found in the lungs after i.v. injection seems not to be artefactual but a physiological property of the lung microvasculature. Early pulmonary localization of cells after intraarterial injection, which had to pass through another microvasculature, supports this view (14). We did not investigate the localization of different subsets of lymphoblasts, assuming that in the injected suspensions both T and B blasts in proportions exactly as they appear *in vivo* in physiological conditions, were present. Although, many studies have been carried out on the differences in directional migration of T and B blast into lymphoid organs and gut (7, 15) little is known, exactly which subsets lymphocytes recirculate through the non-lymphoid tissues. It was found that the number of B cells in preodal lymph in man is lower than in blood (16) and that cells which move to sites of inflammation in skin are T blasts (17).

The mechanism responsible for homing of different populations of lymphocytes in various non-inflammatory tissues is not known, although some suggestions have been made on the possible role of immunoglobulins (12, 13). There is no anatomical evidence for existence in gut, skin or lungs of postcapillary venules akin to those existing in lymph nodes. This may suggest some other factors than anatomical mediate the migrational specificity of ^{125}IUDR-labeled lymphocytes. Mediation by antigens, "physiologically" present on the surface of tissues with direct contact with the external environment, i.e. intestine, lung and skin, may be the explanation.

Acknowledgements

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