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KINETICS OF THE DISTRIBUTION OF THYMUS AND MARROW CELLS IN THE PERIPHERAL LYMPHOID ORGANS OF THE MOUSE : ECOTAXIS

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SUMMARY

The differences between the fate of mouse thymus and marrow cells labelled *in vitro* with [^{3}H]adenosine following intravenous injection into syngeneic recipients are defined. Thymus cells home predominantly to the thymus–dependent territory in the recipient's peripheral lymphoid organs, whereas the majority of bone marrow cells is found in thymus–independent sites. In addition, it is shown that a marrow inoculum contains a variety of sub-populations able to discriminate microenvironmental differences within the thymus–independent territory itself. In general, cell populations behave as if they have the ability to distinguish and home towards what is probably their usual environment. The word 'ecotaxis' is offered to define this phenomenon, and the mechanisms that may be involved in determining it are discussed.

INTRODUCTION

The definition of compartments within the mammalian lymphoid organs has greatly facilitated the understanding of the physiology of the migration of lymphoid cells *in vivo*. It is now considered that both the mammalian spleen and lymph nodes can be divided into two major compartments, one of which is thymus-dependent and appears depleted of lymphocytes in some species following neonatal thymectomy (Waksman, Arnason & Janković, 1962; Parrott, de Sousa & East, 1966) or in the congenital absence of the thymus (Cleveland *et al.*, 1968; de Sousa, Parrott & Pantelouris, 1969), and another which remains relatively unaffected by the absence of the thymus at birth (de Sousa & Parrott, 1967). In the spleen (Fig. 1) the thymus-independent territory comprises the red pulp, the perifollicular area and the periphery of the Malpighian follicle, and in the lymph node (Fig. 2) the peripheral layer of primary nodules, the cortico-medullary junction and the medulla. Earlier work on the destination of radioisotopically labelled thoracic duct lymphocytes, thymus, spleen and lymph node cells in the lymphoid organs of syngeneic recipients has shown that

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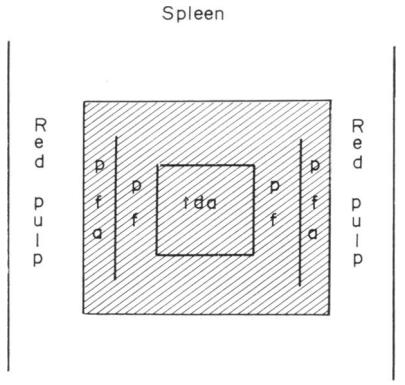

FIG. 1. Diagrammatic representation of a section of mouse spleen; pfa, perifollicular area; pf, periphery of the Malpighian follicle; tda, thymus-dependent area. The shaded area represents the classical white pulp.

each cell population has a distribution pattern of its own (Gowans & Knight, 1964; Goldschneider & McGregor, 1968; Parrott *et al.*, 1966; Parrott, 1967; Austin, 1968; Parrott & de Sousa, 1969; Balfour *et al.*, 1971). Thoracic duct lymphocytes and thymocytes home preferentially to the thymus-dependent areas (Gowans & Knight, 1964; Parrott *et al.*, 1966; Parrott, 1967; Goldschneider & McGregor, 1968; Parrot & de Sousa, 1969), spleen cells spread themselves over all the various compartments of the peripheral lymphoid organs (Parrott *et al.*, 1966; Parrott, 1967; Parrott & de Sousa, 1969), and although the majority of lymph node cells also homes to the thymus-dependent territory (Balfour *et al.*, 1971), a proportion goes to the peripheral nodules of the lymph nodes (Austin, 1968). We have now extended these observations to the marrow cell population and found yet a different pattern of arrangement of the labelled cells. Marrow cells home preferentially to the thymus-independent regions, namely the red pulp in the spleen and the medullary cords in the lymph nodes.

In the present paper, a comparison between two 'source' populations (thymus and marrow) is drawn. Moreover the ability of lymphoid cell populations to recognize and home to their usual environment is defined as *ecotaxis* (from the Greek *oikos* meaning 'house', and

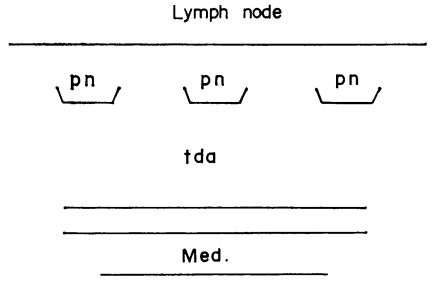

FIG. 2. Diagrammatic representation of a section of a mouse lymph node; pn, primary nodule; tda, thymus-dependent area; Med., medulla; the narrow layer between the thymus-dependent area and the medulla represents the cortico-medullary junction.

taxis meaning 'arrangement', although more commonly used in biology to mean movement) and the factors that may influence its mechanism, discussed.

MATERIALS AND METHODS

Preparation and injection of cell suspensions

Adult mice (2–3 months old) from the C3H/Bi and CBA inbred strains were used. The thymuses were removed and chopped into small pieces in a volume of 0·5 ml of (PBS) phosphate buffered saline and further dissociated in a syringe containing the same medium. Bone marrow cells were obtained by flushing out the long bones with a small volume of PBS. Cells were suspended at a concentration of 10^8 cells/ml in 5 ml of PBS containing 10μCi[^{3}H]adenosine/ml and incubated in a shaking water bath for 1½ hr at 37°C. After incubation, the cells were washed thoroughly three times with isotonic saline, and after the final wash, resuspended in a volume of 1–1·5 ml of PBS and counted before injection. The labelled cells were injected slowly into the femoral vein of syngeneic recipients, lightly anaesthetized with ether. In a series of separate experiments, neonatally thymectomized or intact young adult mice received varying doses of labelled thymus cells (3×10^7, $4{\cdot}5 \times 10^7$ or 9×10^7) or bone marrow cells ($0{\cdot}9 \times 10^7$), and were killed at different times after injection in groups of three to six animals; the thymus cell recipients were killed at 15 min, 5–6, 24, 48 and 72 hr after injection, and the recipients of the marrow cell inoculum at 15–20 min, 3–4 and 24 hr following the injection of the cells.

Autoradiography

Sections of lungs, liver, spleen, mesenteric, inguinal lymph nodes and thymus were cut routinely at 4 μ. The mounted sections were coated with photographic emulsion (Ilford K5) stored at 4°C for varying periods of time (4–10 weeks) and stained through the emulsion with methyl-green pyronin after developing. Smears of the donor cells and of the recipient's bone marrow were fixed in methyl alcohol and stained with May-Grünwald Giemsa and Unna-Pappenheim after developing. Whole sections counts were done under a ×1000 magnification; cells with 10 or more silver grains were counted and their position determined in relation to the various compartments (see Figs 1 and 2). A minimum of three sections from each organ were scanned; approximately 1000 sections were examined and a total of 25,000 cells counted.

RESULTS

Labelling of the inocula and overall movement of the two populations (Fig. 3)

95–99% of the cells in both inocula were labelled. The highest percentage of thymus inoculum consisted of small lymphocytes (78–80%), medium lymphocytes constituted about 16% of the inoculum and 3·5–5% were large, undifferentiated cells. The cell types in the marrow inoculum were grossly classified as undifferentiated (42·5%), myeloid (49·7%) and lymphoid (7·7%). At 15 min, both thymus and marrow cells were seen in the lungs, liver and spleen. The percentage of the total number of thymus cells counted found in the lungs was higher than the corresponding percentage of bone marrow cells; in addition the proportion of labelled bone marrow cells found in the spleen was higher than that of thymus cells, thus suggesting that the bone marrow cells were able to move more rapidly

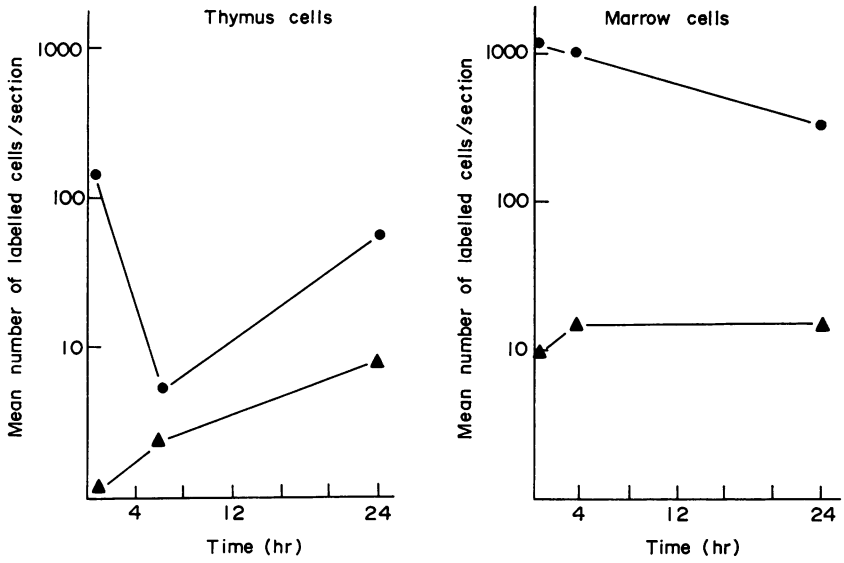

FIG. 3. Kinetics of the distribution of the total (mean) number of labelled marrow and thymus cells found in sections of spleen (●) and lymph nodes (▲).

through the non-lymphoid filters than the thymus cells. This impression was further substantiated by the fact that a small number of marrow cells reached the lymph nodes at an earlier time (15 min) than the thymus cells (few at 6 hr). At 3½ hr the numbers of marrow cells decreased in the lungs, liver and spleen and there was a marked increase in the numbers reaching the recipient's own bone marrow. From the examination of the cell types involved in this change, it was found that the decrease in the total number of labelled marrow cells in the spleen at this time, was due both to the migration of the myeloid component of the inoculum to the recipient's marrow (Table 3), and also to the rapid cell division and consequent loss of label, of some of the large, undifferentiated cells, resulting in the appearance of clusters of very lightly labelled cells (less than 5 grains) which were not included in the count; morphologically these clusters were identified as haemopoietic. At 24 hr, the number of marrow cells found in the lung and liver sections was insignificant (less than ten per section) and the decrease in the numbers counted in the spleen continued. The numbers of labelled marrow cells counted in the lymph node sections remained relatively constant throughout. Conversely, the labelled thymus cells found in the spleen and lymph nodes at 24 hr increased considerably after going through a decline at 6 hr.

Kinetics of distribution within the spleen and lymph nodes

Spleen (Fig. 4, Table 1). At 15 min, both thymus and marrow cells were found in large numbers in the perifollicular area and in the red pulp of the spleen. At 3½ hr, 80% of the labelled marrow cells were in the red pulp and two types of labelling could be clearly identified, one consisting of large, heavily labelled cells (>15 grains) located in the peritrabecular sheaths, often very close to the trabeculae, and another consisting of lightly labelled (<5 grains) haemopoietic foci sited in the red pulp space between the trabeculae and the perifollicular area; few marrow cells were seen in the Malpighian bodies, these cells

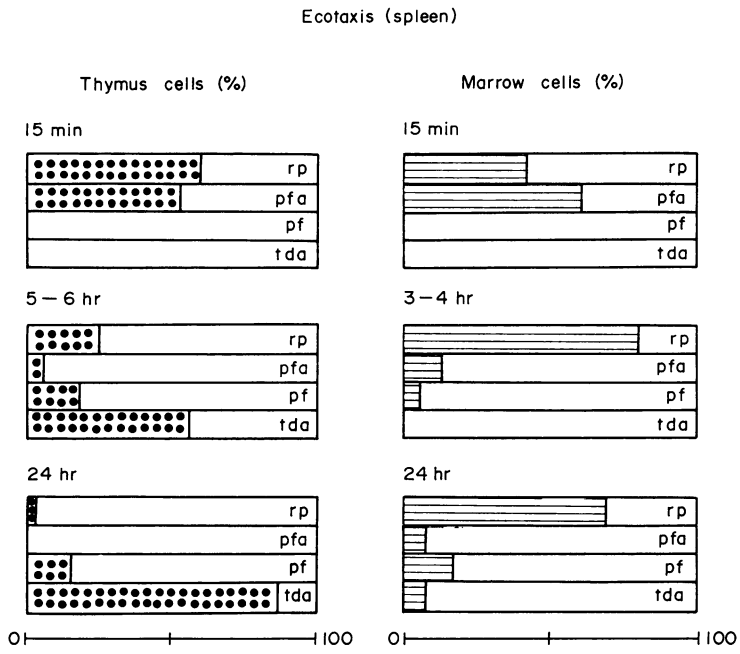

FIG. 4. Percentage distribution of thymus and marrow cells found in the various splenic compartments at several times following i.v. injection. rp, red pulp; pfa, perifollicular area; pf, periphery of the Malpighian follicle; tda, thymus-dependent area.

TABLE 1. Ecotaxis of [^{3}H]adenosine labelled thymus and marrow cells in the spleen

Time	Inoculum	Dose	Exposure time (weeks)	% Distribution				
				Total*	rp	pfa	pf	tda
15 min	Thymus cells	3×10^7	4	146·4	67·8	32·1	0	0
	Thymus cells	9×10^7	10	544·3	58·4	40·7	0·18	0·67
	Marrow cells	$0{\cdot}9 \times 10^7$	6	1269·33	40	51·3	0·4	0
3–4 hr	Marrow cells	$0{\cdot}9 \times 10^7$	6	937·50	80·8	11·9	4·7	2·5
5–6 hr	Thymus cells	3×10^7	4	< 10	—	—	—	—
24 hr	Thymus cells	3×10^7	4	59·625	1·25	0	13·8	84·8
	Marrow cells	$0{\cdot}9 \times 10^7$	6	363·5	69·8	6·7	16·8	6·7
48 hr	Thymus cells	$4{\cdot}5 \times 10^7$	10	396·5	4·6	0·8	4·4	90·0
	Thymus cells	$4{\cdot}5 \times 10^7$	10	336·67	1·7	1·6	10·8	85·6
62 hr	Thymus cells	3×10^7	4	158·26	2·2	0	8·2	89·5
72 hr	Thymus cells	9×10^7	10	517·32	4·2	2·8	2·19	90·7

rp, red pulp; pfa, perifollicular area; pf., periphery of the Malpighian follicle; tda, thymus-dependent area.

* Mean count of 3–10 sections.

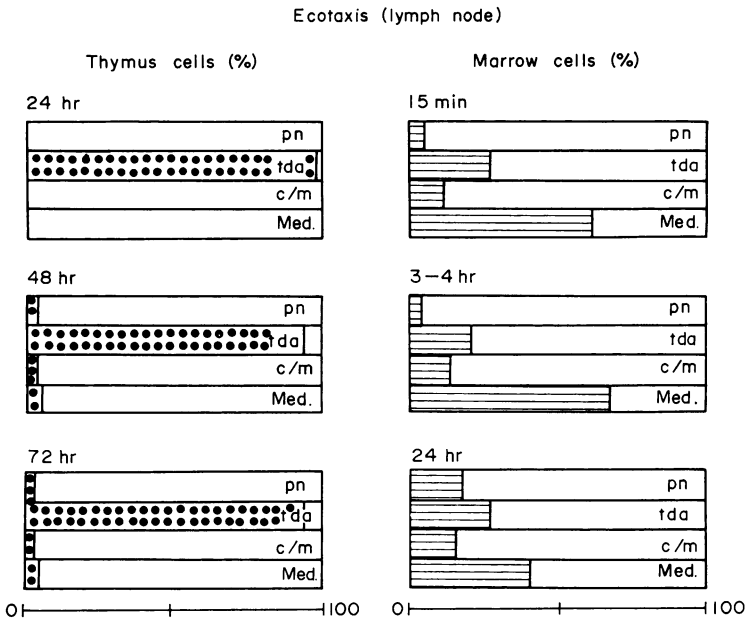

FIG. 5. Percentage distribution of thymus and marrow cells found in the various lymph node compartments at several times following i.v. injection. pn, primary nodule; tda, thymus-dependent area; c/m, cortico-medullary junction; Med., medulla.

TABLE 2. Ecotaxis of [^{3}H]adenosine labelled thymus and marrow cells in lymph nodes

Time	Inoculum	Dose	Exposure time (weeks)	% Distribution				
				Total*	pn	tda	c/m	Med.
15 min	Thymus cells	3×10^7	4	0·625	—	—	—	—
	Thymus cells	9×10^7	10	1·66	—	—	—	—
	Marrow cells	$0{\cdot}9 \times 10^7$	6	15·5	4·3	26·5	9·7	6·0
3–4 hr	Marrow cells	$0{\cdot}9 \times 10^7$	6	21·33	2·7	19·4	12·5	67.2
5–6 hr	Thymus cells	3×10^7	4	< 10	—	—	—	—
24 hr	Thymus cells	3×10^7	4	15·6	2·6	97·3	—	—
	Marrow cells	$0{\cdot}9 \times 10^7$	6	16·33	17·3	26·5	14·8	39·8
48 hr	Thymus cells	$4{\cdot}5 \times 10^7$	10	349·25	1·9	87·85	4·97	3·22
	Thymus cells	$4{\cdot}5 \times 10^7$	10	114·5	1·3	94·5	0	4·1
72 hr	Thymus cells	9×10^7	10	623·2	0·58	95·7	0·16	3·53

pn, primary nodule; tda, thymus-dependent area; c/m, cortico-medullary junction; Med., medulla.

* Mean count of 3–20 sections.

TABLE 3. Differential counts of labelled marrow cells found in the recipients' marrow smears*

Time	Total no. labelled cells	Undifferentiated	Lymphoid	Myeloid
15 min	19	84·2%	0	15·8%
	18	72·2%	11·1%	16·6%
	18	77·7%	0	22·2%
3 hr	320	39·3%	3·12%	57·5%
	94	26·6%	5·3%	68%
	56	53·6%	0	46·2%
24 hr	40	30%	7·5%	62·5%
	33	36·4%	9·09%	54·5%

* One bone marrow smear contains in average $2–3 \times 10^3$ cells.

were small, moderately labelled (5–10 grains) and identified as lymphoid; myeloid cells, which were easily identified at 15 min, were no longer present at 3½ hr. Very few thymus cells were seen in the spleen at 5–6 hr, their location, however, was markedly different from that of the marrow cells; most labelled thymus cells were in the thymus-dependent area and only a small proportion was seen in the red pulp and periphery of follicle. The difference in the location of the thymus and marrow cells in the spleen was still more striking at 24 hr, when 84·8% of the thymus cells were in the thymus-dependent area, and the highest percentage of marrow cells (70%) remained in the red pulp. At 48, 62 and 72 hr the preferential localization of the thymus cells in the thymus-dependent compartment persisted.

Lymph nodes (Fig. 5, Table 2). A very small proportion of the total number of labelled marrow cells counted was found in the lymph nodes. Marrow cells reached the lymph nodes earlier than thymus cells but their number remained relatively constant throughout, and never made up more than 7% of the total number counted. The thymus cells arrived later, their numbers however became increasingly higher from 24 hr onwards; 85–95% of the labelled thymus cells homed to the thymus-dependent area, and all were identified as lymphoid.

The majority of the marrow cells (60–70%) were in the medullary cords, although a small porportion of large, heavily labelled cells was seen in the thymus-dependent area usually near post-capillary venules, these cells in view of their size were identified as large, undifferentiated, they were similar to and possibly even identical to the reticular cells normally found in this area. At 24 hr, a small number of lymphoid marrow cells was seen in the primary nodules.

DISCUSSION

It has been shown that [^{3}H]adenosine labelled thymus and marrow cells injected intravenously, in the mouse, after using similar routes of entry home to clearly distinct territories of the spleen and lymph nodes. Thus, in the spleen, the highest percentage of thymus cells homed to the thymus-dependent compartment whereas most marrow cells were found in the red pulp. Similarly, in the lymph nodes, nearly all thymus cells scanned were found in the

thymus-dependent area, whereas the highest proportion of marrow cells was located elsewhere; mostly in the medullary cords, but a fair number also in the primary nodules.

By and large, the two cell populations behaved as if they had the ability to distinguish and home towards what is probably their usual environment. It is now widely accepted that the lymphoid population occupying the thymus-dependent areas is of thymus descent; the exact location of the marrow derived population in the peripheral lymphoid organs has not been so clearly established, but it has been deduced that all cells in the non-thymus-dependent compartments are of marrow origin. The present results support this deduction, but they define further sub-divisions among the marrow population. Some undifferentiated cells in the bone marrow inoculum were able to home straight back to the marrow, others remained in the peritrabecular sheaths of the red pulp and in the medullary cords of the lymph nodes. Knowing that antibody producing cells normally develop in the peritrabecular sheaths of the splenic red pulp and in the medulla of the lymph nodes, one is tempted to speculate that the large, heavily labelled marrow cells found in those sites belong to the antibody-cell precursor sub-class. Another component of the marrow inoculum was the myeloid cell which rapidly found its way back to the marrow. The marrow inoculum contained also one rapidly dividing cell which gave rise to the haemopoietic foci found in the red pulp, between the peritrabecular sheath and the perifollicular area. The number of marrow cells seen in the thymus-dependent areas of the lymph nodes was always very small; they were not lymphoid but could be compared to the normal reticular cells of the organ's framework. Nevertheless, small, moderately labelled lymphoid cells were present in the marrow inoculum, and appeared to home to the Malpighian body in the spleen, and the primary nodules in the lymph nodes.

In summary, it was found that not only do thymus and marrow cells differ in their general choice of environment within the peripheral lymphoid organs, but also that the marrow inoculum contains a variety of subpopulations able to discriminate subtle differences within the thymus-independent territory itself. I should like to call this ability of cell populations to discriminate and choose different environments to home to, *ecotaxis* deriving from the Greek *oikos* meaning 'house', and *tassein* meaning 'to arrange', but currently used in biology to mean movement. Although little is known about the mechanism of this phenomenon it is worth considering a number of factors that may prove to be of relevance to its future understanding.

Morphological studies of the reticulin framework of the spleen and lymph nodes in a number of species (Denz, 1947; Sainte-Marie & Sin, 1968; de Sousa, 1969), have shown that the reticulin fibres in the thymus-dependent compartments are arranged in a much wider pattern than the 'closed', almost unicellular meshwork of the lymph node medullary cords or the splenic red pulp. The wider meshwork of the thymus-dependent areas may thus influence the type and mobility of the cells reaching it. However, not all cells from the blood are 'allowed' to reach it, this is a predominantly lymphoid area known to be homed to by lymphocytes in the process of recirculation from blood to lymph (Gowans & Knight, 1964). It has been postulated (Gesner, 1966) that some form of recognition process involving the sugars on the lymphocyte surface may take place between the lymphocyte and the endothelial cell of the post-capillary venule, since pretreatment of thoracic duct lymphocytes (Woodruff & Gesner, 1969) or thymocytes (Berney & Gesner, 1970) with neuraminidase alters the migration pattern of these cells. The post-capillary venule, however, is a structure unique to the lymph node and therefore the mechanism of the specific localization of the

thymus cells to the splenic thymus-dependent area would still remain unexplained on the basis of this postulate. Neuraminidase also decreases the electrophoretic mobility of lymphocytes (Woodruff & Gesner, 1969) and this alone may be sufficient to explain the resulting alteration of the pattern of migration.

In the present experiments, other blood cells such as the polymorph were not attracted to the lymph nodes at all, and those that reached the spleen rapidly left to find their way back to the bone marrow. *In vitro* experiments on chemotaxis (Boyden, 1962) have shown that polymorphs can be actively attracted to a variety of chemotactic agents, and probably *in vivo*, chemotaxis will also play a relevant part in the ecotaxis of some cell types. Lymphocytes, although mobile are never specifically directed towards chemotactic agents and attempts to find a substance that will make them move *in vitro*, in the same way that polymorphs do, have repeatedly been unsuccessful (Wilkinson, personal communication).

In vivo, the influx of radioisotopically labelled lymph node cells to the thymus-dependent compartment of a lymph node draining the site of injection of adjuvant material is increased (Taub, Krantz & Dresser, 1970; Dresser, Taub & Krantz, 1970). Attempts to alter the normal pathway of lymph node cells by previous localization of antigen–antibody complexes in the primary nodules of the recipient's lymph node, followed by the transfer of labelled cells from sensitized donors, have failed 'to persuade' the labelled sensitized cell to leave the thymus-dependent compartment and home to the primary nodule. In the experiments of Austin, also on the migratory pattern of rat lymph node cells, a small proportion of lightly labelled blast cells did go to the primary nodule; however, no differences in the numbers of these blast cells were detected between antigen stimulated and unstimulated nodes.

One final group of experiments that may also be of relevance to the present discussion is that of Mosier and co-workers who distinguished two types of spleen cells on the basis of their adherence to plastic; adherent and non-adherent (Mosier, 1967). They also showed that thymus-deprived mice were specifically deficient of the non-adherent type (Mosier *et al.*, 1970). In other *in vivo* experiments (de Sousa, unpublished observations) it has been found that following an intraperitoneal injection, labelled bone marrow cells tended to get stuck in the omentum and failed to reach the spleen and lymph nodes, whereas thymus cells did not get stuck and migrated from the peritoneal cavity to the peripheral lymphoid organs. It may be that *in vivo*, 'adherence' is also of importance, to the final arrangement of cell populations in the host. It would be interesting to know the influence of surface antigens such as the θ antigen, on the physical make up of the surface of the cells for which they are specific.

In summary, ecotaxis appears to be a 'determined' property of lymphomyeloid cell populations which cannot easily be changed or explained by one set of factors alone. Perhaps immunological and chemotactic factors will prove to be of importance in the ecotaxis of 'adherent' cells or of cells with an elaborate surface make-up such as the bone marrow cell or the bone marrow derived cell in the spleen. Non-adherence, a less elaborate cell surface and the resulting failure to be 'distracted' by immunological or chemotactic factors, may explain the distribution of thymus and thymus-derived cells in areas of wider reticulin meshworks, their greater 'agility' and their ability to recirculate.

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