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THYMUS-DEPENDENT AND THYMUS-INDEPENDENT POPULATIONS: ORIGIN, MIGRATORY PATTERNS AND LIFESPAN*

DELPHINE M. V. PARROTT AND MARIA DE SOUSA

Department of Bacteriology and Immunology, University of Glasgow

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SUMMARY

It is now accepted that there are present within the peripheral lymphoid tissues of the mammal, lymphocytes which differ in their origin, migratory patterns and lifespan; two major groups have so far been defined, one of thymus-dependent or thymus-derived lymphocytes and another of thymus-independent or bone-marrow derived lymphocytes. These cells which are morphologically indistinguishable when free floating in the blood or lymph nevertheless segregate from one another into clearly defined compartments whilst resident in or passing through the lymph nodes, spleen or Peyer's patches. The purpose of this article is to summarize some of the experimental evidence for this segregation and to discuss its physiological and immunological implications. It is not intended to be an extensive review of the literature.

DEFINITION OF COMPARTMENTS

Work using the T6 chromosome as a cytological marker has demonstrated the presence in the peripheral lymphoid tissues of cells from two distinct origins, the thymus and the bone marrow (Micklem *et al.*, 1966). The techniques used for revealing the T6 chromosome in dividing cells, however, do not permit the identification of the morphology of the cells examined, nor their location within each tissue. The fact that lymphocytes of different descent were segregated into separate compartments within the peripheral lymphoid tissues was established as a consequence of light microscopy examination of sections of tissues from rats (Waksman, Arnason & Janković, 1962) and mice (Parrott, de Sousa & East, 1966) thymectomized at birth. These and other subsequent studies on thymectomized rabbits (Kelly, Balfour & Parrott, unpublished) and on congenitally athymic mice (de Sousa, Parrott & Pantelouris, 1969) and children (Cleveland *et al.*, 1968) showed that in the absence of the thymus at birth, selective areas of lymphocyte depletion could be delineated in the peri-arteriolar sheath of the splenic Malpighian body, in the mid and deep cortex ('paracortex')

Correspondence: Dr Delphine M. V. Parrott, Department of Bacteriology and Immunology, University of Glasgow, Glasgow, W.1.

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of the lymph nodes and in the inter-nodular space of the gut-associated lymphoid organs (de Sousa *et al.*, 1969; Veldman, 1970). These areas were termed as thymus-dependent in the mouse (Parrott *et al.*, 1966); their location, however, seems to be approximately the same in most mammalian species investigated so far (see Parrott & de Sousa, 1971, for more detailed descriptions), although it might be anticipated from the 'inside-out' appearance of the pig lymph node (Binns & Hall, 1966) that this species may well be an exception.

The remaining areas in the peripheral lymphoid organs, namely nodules and medulla in the lymph nodes, nodules and plasma cells in the gut-associated lymphoid organs, and the peripheral layer of the Malpighian body in the spleen, the perifollicular area and the red pulp were all designated 'thymus-independent' (de Sousa & Parrott, 1967). The fact that the bone marrow is considered to be the origin of the cells in these areas is a logical deduction from the experiments of Davies and his co-workers (Davies, 1969) who carried out parallel chromosome marker and morphological studies in mice that had been thymectomized when adult, submitted to a lethal dose of whole body irradiation and injected with bone marrow. Under these circumstances, the thymus-independent zones alone regenerated, and all dividing cells were identified as being derived from the donor bone marrow.

Recently, Gutman & Weissman (1971) have demonstrated in a more direct way the haemopoietic origin of the lymphocytes in primary nodules of lymph nodes. These authors reconstituted adult thymectomized C57Bl mice with foetal liver from F_1 (CBA × C57Bl) donors, and showed by means of indirect immunofluorescence that cells bearing the H2-antigen of the donor inoculum had formed primary nodules.

The size of the thymus-dependent and thymus-independent compartments varies according to the tissue, the age of the animal and the strain or species studied; for example, the area seen depleted of lymphocytes after neonatal thymectomy both in the spleen and lymph nodes is much greater in the C3H/Bi than in the NZB mouse (East *et al.*, 1967). Some quantitation of the two populations has been achieved by the use of the θ antigen as a marker of thymus-derived cells in a number of strains of mice. In adult CBA mice, 65–85% of lymph node and thoracic duct lymphocytes and 35–50% of spleen lymphocytes are of thymus descent (Raff & Wortis, 1970).

DEVELOPMENT

The lymph nodes and spleen of mice develop from a simple reticular framework with a few randomly scattered lymphoid cells into structures with clearly defined thymus-dependent and thymus-independent compartments during the first 3 weeks of life.

There is clear evidence from the observations of Weissman (1967), Linna (1968) and Michalke *et al.* (1969) that the exodus of cells from the thymus of rats, mice and hamsters is much greater in the newborn and weanling period than in adult life. Thus, Weissman (1967) found that in the newborn rat 12–18% of the total cell population in the spleen and lymph nodes were found to have originated from the *in situ* [^{3}H]thymidine labelled thymus over a period of 24 hr, whereas in the adult, over the same period of time, only 0·024–0·12 of cells of thymus origin could be detected in the same tissues. Moreover, all the labelled cells leaving the thymus in the adult were small or medium lymphocytes whilst in the neonate significant numbers of large lymphocytes were observed. These could be the same cells that experiments with labelled neonatal thymus grafts (Parrott & de Sousa, 1967) have indicated undergo rapid cell division in the periphery.

Primary nodules first appear at about 11–12 days and one can begin to detect the differences between lymph nodes and spleens from intact and thymectomized mice during the following week. The ability of thymus cells to home to the thymus-dependent compartment and of spleen cells to aggregate within primary nodules has been observed as early as 12–15 days (Tables 9 and 10).

Williams & Nossal (1966) correlated the appearance of primary nodules in the rat at about 12 days, with the ability to trap iodine labelled antigen on dendritic reticulocytes in a typical aggregated pattern. It is unlikely, however, that the development of separate compartments is initiated by exogenous antigenic stimuli. In many species these are discernible before birth and there is no doubt that compartments can be distinguished in spleens and lymph nodes from normal and thymectomized mice raised under germfree conditions (Dukor, Miller & Sacquet, 1968). The peripheral lymphoid tissues are of course much smaller than in mice raised in a conventional environment, presumably because so few germinal centres and plasma cells are present.

RECOGNITION OF COMPARTMENTS BY MIGRATING CELLS
(Figs 1 and 2)

If it is of interest, especially for immunopathology, that the various peripheral lymphoid organ compartments can be easily identified by conventional histology, from a biological standpoint it is of much greater significance that individual cells have themselves the ability 'to recognize' differences between those compartments. This ability of cells to

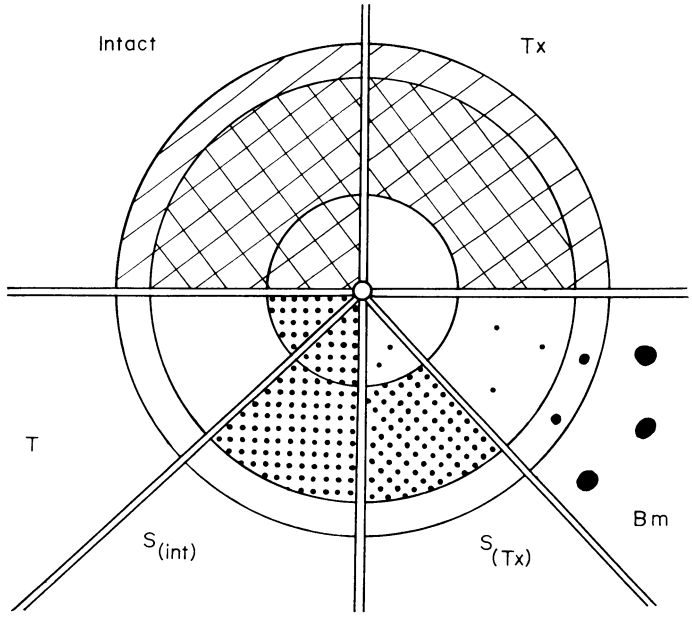

FIG. 1. Upper half is a diagrammatic representation of the effect of neonatal thymectomy (Tx) on the population of the splenic white pulp. Lower half is a diagrammatic representation of the destination of labelled thymus cells (T), bone marrow cells (Bm) and spleen cells (S) from intact (int) and thymectomized (Tx) donors in the spleen, following an intravenous injection.

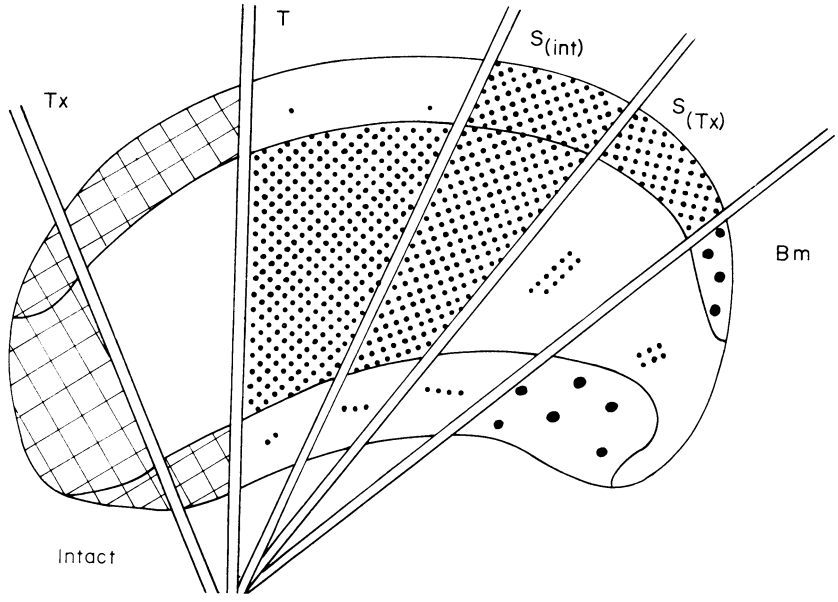

FIG. 2. Diagrammatic representation of the effect of neonatal thymectomy (Tx) on the lymph node population, and of the destination of labelled thymus cells (T), bone marrow cells (Bm) and spleen cells (S) from intact (int) and thymectomized (Tx) donors in the lymph node, following intravenous injection.

recognize and select a particular environment that appears to coincide with their usual environment within the lymphoid organs, has been established from experiments involving the autoradiographic study of the fate of radioisotopically labelled cells coming either from *in vivo* labelled source organs or introduced in the circulation after labelling *in vitro*, and has been defined as 'ecotaxis' (from the Greek 'oikos', meaning house, and 'tassein' meaning to arrange, currently used in biology to mean 'movement') by one of us (de Sousa, 1971).

Although physiologically it is more satisfactory to trace the fate of cells leaving a 'source' organ (e.g. thymus or marrow), in practice the number of cells emigrating at any one time is small, and the pattern of localization of cells can be adjudged more confidently after the introduction of a large number of *in vitro* labelled cells in circulation.

Localization of in vitro labelled thymus cells (Tables 1 and 2)

A series of experiments with the mouse (Parrott *et al.*, 1966; Parrott & de Sousa, 1969; de Sousa, 1971) has detailed the fate of [^{3}H]adenosine* labelled thymus cells after intravenous injection. Such an inoculum consists mainly of small lymphocytes (80%), medium to large lymphocytes (16%) and a very small number of undifferentiated cells. Within minutes of injection many labelled cells are found in the perifollicular area and in the red pulp of the spleen; from 6 hr onwards (maximum time interval being 72 hr), however, the vast majority of cells (84%) are observed in the thymus-dependent compartment. Thymus cells seem to be slower to reach the lymph nodes in significant numbers but again home selectively to the

* During the period of 1–1½ hr incubation with [^{3}H]adenosine 95–100% cells in suspension will become labelled.

TABLE 1. Distribution of [^{3}H]adenosine labelled cells* in spleen

Time	Inoculum	Dose†	Recipients			Total‡	% Distribution			
			Age	Tx	Intact		RP	PFA	PF	TDA
15 min	Thymus cells	3×10^7	2–3 months	2	1	146·4	67·8	32·1	0	0
	Thymus cells	9×10^7	2–3 months	1	—	544·33	58·4	40·7	0·18	0·67
24 hr	Thymus cells	3×10^7	2–3 months	3	—	59·625	1·25	0	13·8	84·8
48 hr	Thymus cells	$4{\cdot}5 \times 10^7$	2–3 months	5	—	396·5	4·6	0·8	4·4	90·0
	Thymus cells	$4{\cdot}5 \times 10^7$	2–3 months	—	3	336·67	1·7	1·6	10·8	85·6
62 hr	Thymus cells	3×10^7	2–3 months	3	—	158·26	2·2	0	8·2	89·5
72 hr	Thymus cells	9×10^7	2–3 months	3	—	517·32	4·2	2·8	2·19	90·7
15 min	Marrow cells	9×10^7	2–3 months	—	3	1269·33	40	51·3	0·4	0
3–4 hr	Marrow cells	9×10^7	2–3 months	—	3	937·5	80·8	11·9	4·7	2·5
24 hr	Marrow cells	9×10^7	2–3 months	—	4	363·5	69·8	6·7	16·8	6·7

* Cell suspensions incubated for 1½–3 hr in a medium containing 50 μCi [^{3}H]adenosine, autoradiographs exposed for 4–10 weeks.

† Injected intravenously.

‡ Mean total number of cells/section.

TABLE 2. Distribution of ^{3}[H]adenosine labelled cells* in mesenteric lymph node

Time	Inoculum	Dose†	Recipients			Total‡	% Distribution			
			Age	Tx	Intact		PN	TDA	C/M	Med
15 min	Thymus cells	3×10^7	2–3 months	2	1	0·625	—	—	—	—
	Thymus cells	9×10^7	2–3 months	1	—	1·66	—	—	—	—
24 hr	Thymus cells	3×10^7	2–3 months	3	—	5·71	—	—	—	—
48 hr	Thymus cells	$4{\cdot}5 \times 10^7$	2–3 months	5	—	349·25	1·9	87·85	4·97	3·22
	Thymus cells	$4{\cdot}5 \times 10^7$	2–3 months	—	3	114·5	1·3	94·5	0	4·1
72 hr	Thymus cells	9×10^7	2–3 months	3	—	623·2	0·58	95·7	0·16	3·53
	Thymus cells	9×10^7	2–3 months	—	2	212	1·4	93·3	1·4	3·77
15 min	Marrow cells	9×10^7	2–3 months	—	3	15·5	4·3	26·5	9·7	60
3½ hr	Marrow cells	9×10^7	2–3 months	—	3	21·33	2·7	19·4	12·5	67·2
24 hr	Marrow cells	9×10^7	2–3 months	—	4	16·33	17·3	26·5	14·8	39·8

* Cell suspensions incubated for 1½–3 hr in a medium containing 50 μCi [^{3}H]adenosine, autoradiographs exposed for 4–10 weeks.

† Injected intravenously.

‡ Mean total number of cells/section.

Abbreviations used in all tables; RP, red pulp; PFA, perifollicular area; PF, periphery of follicle TDA, thymus dependent area; PN, primary nodule; C/M, cortico-medullary junction; Med, Medulla.

thymus-dependent compartment (Table 2) appearing to use the post-capillary venules as portals of entry. Thymus cells are virtually never seen in primary nodules.

It has been estimated that approximately 10% of a thymus cell inoculum in either mouse (Parrott & de Sousa, 1969) or rat (Goldschneider & McGregor, 1968a) in fact reach the thymus-dependent areas. This is not surprising since comparatively few thymus cells in the thymus can at the moment of preparation of the cell suspension have been ready to emigrate.

Localization of in vivo labelled thymus cells

Cells leaving an *in situ* labelled thymus have also been observed to home to the peripheral thymus-dependent compartments in short term (up to 72 hr) experiments in the rat (Weissman, 1967; de Sousa, Pettirossi & Parrott, 1971) and the hamster (Linna, 1968), although Chanana *et al.* (1971) have remarked on a less selective distribution following intra-thymic labelling in the calf.

In experiments where the fate of [^{3}H]thymidine labelled cells derived from a labelled thymus graft was followed for longer periods of time, 18 and 41 days (Parrott & de Sousa, 1967), few but significant numbers of labelled lymphocytes were detected in the thymus dependent areas in autoradiographs of spleen and lymph nodes exposed for various times (9–26 weeks). These experiments showed not only the selectivity of the localization of the cells but also indicated that cells leaving neonatal thymus tissue must undergo multiple cell divisions in the periphery.

The localization of in vitro labelled bone marrow cells (Tables 1 and 2)

The problem of deciphering a localization pattern of bone marrow cells is more complex than that encountered with thymus cells, for not only does the 'source' itself contain a far more heterogeneous collection of cells but the cell types and organization of the thymus-independent compartments of the peripheral lymphoid tissues are far more varied than the simple sheets of lymphocytes that comprise the thymus-dependent areas.

A suspension of mouse marrow cells labelled *in vitro* with [^{3}H]adenosine contains at least three major cell types: large undifferentiated cells (42·5%), myeloid cells (45·7%) and lymphoid cells (7·7%). The sequence of events that follows their intravenous injection differs in many respects from the progress of thymus cells introduced in circulation using the same route (de Sousa, 1971), although initially (15 min) they are found in similar sites, namely the perifollicular area and the red pulp of the spleen. The easily distinguishable myeloid cells subsequently disappear from the spleen and are only found in the recipient's marrow; some of the undifferentiated components retain their heavy label and are found in the peritrabecular sheaths of the red pulp, areas usually occupied by plasma cells. Others, possibly haemopoietic stem cells, seem to give rise to foci of lightly labelled cells also in the red pulp. At 24 hr, unlike thymus cells, the majority of labelled marrow cells are still in the red pulp and a few lymphocytes have reached the periphery of the Malpighian follicle (Table 1) although virtually no marrow cells are found in the thymus-dependent compartment of the spleen.

Only a very small proportion of the cells in the marrow inoculum reaches the lymph nodes, the majority are large heavily labelled cells which home to the medullary cords. Occasionally large undifferentiated cells probably similar to reticular cells, but certainly not with a lymphoid morphology, were also seen in the thymus-dependent area and at 24 hr a few lymphocytes were seen in the primary nodules (Table 2).

Localization of in vivo labelled marrow cells

Several groups including Everett & Caffrey (1967) and Brahim & Osmond (1970) have demonstrated that cells from rat and guinea-pig bone marrow labelled *in situ*, migrate out, and in the blood they found labelled neutrophils, small lymphocytes and large mononuclear, monocytoid or undifferentiated cells. In a recent careful study, Brahim & Osmond (1970) have paid particular attention to the localization of the small lymphocytes. The majority of cells were in the red pulp of the spleen and the outer part of the Malpighian body although some were seen in the periarteriolar sheath. There was also a fair number of labelled cells spread over the mesenteric node but comparatively few in other nodes.

The localization of in vitro labelled cells of 'mixed' origins (Tables 3 and 4)

Suspensions of cells obtained from peripheral lymphoid organs (spleen and lymph nodes) are known to contain variable amounts of thymus-derived and non-thymus derived cells;

TABLE 3. Distribution of [^{3}H]adenosine labelled cells* in spleen

Time	Inoculum	Dose†	Recipients			Total‡	% Distribution			
			Age	Tx	Intact		RP	PFA	PF	TDA
15 min	Spleen cells	3×10^7	2–3 months	1	1	3·37	33·2	62·9	1·12	0
1–1¼ hr	Spleen cells	3×10^7	2–3 months	2	—	8·6	88·3	4·65	4·65	2·32
5–6 hr	Spleen cells	3×10^7	2–3 months	2	3	169·5	5·4	0·78	55·1	38·6
24 hr	Spleen cells	3×10^7	2–3 months	3	2	315	1·77	0·44	77·8	19·7
48 hr	Spleen cells	$4{\cdot}5\times10^7$	2–3 months	4	—	1991·25	5·7	9·5	48·8	35·5
62 hr	Spleen cells	3×10^7	2–3 months	2	—	560	3·03	2·49	65·6	28·7
24 hr	Spleen cells from Tx donors	3×10^7	3 months	—	3	175	6·8	10·8	67·9	14·2
72 hr	Spleen cells from Tx donors	3×10^7	3 months	—	3	213	8·4	20·6	60·5	10·3

* Cell suspensions incubated for 1½–3 hr in a medium containing 50 μCi [^{3}H]adenosine; autoradiographs exposed for 4–10 weeks.

† Injected intravenously.

‡ Mean total number of cells/section.

the figures for the numbers of θ bearing cells found in the spleen and lymph nodes of the CBA mouse are 35–50% and 65–85%, respectively (Raff & Wortis, 1970). The cell types that make up a spleen inoculum are myeloid cells (20%), large undifferentiated (10%) and lymphoid cells (70%), whereas a lymph node cell inoculum is almost exclusively lymphoid with more than 85% small lymphocytes.

Spleen cells. Labelled spleen cells in the spleen (Table 3) use the same routes of entry as thymus or marrow cells; soon after injection they are found in the perifollicular area and in the red pulp, later (at 1 hr) more cells still are found in the red pulp, but by 24 hr the highest percentage of cells moves to the white pulp; not surprisingly, spleen cells are not exclusively confined to the thymus-dependent compartment but quite substantial numbers (65–75%) are found in the periphery of the Malpighian follicle. Normally 30–35% of the labelled spleen cells home to the thymus-dependent area, but if spleen cells from thymectomized

animals are used the proportion of cells found in the thymus-dependent compartment decreases considerably (Table 3). In the lymph nodes too, spleen cells do not remain confined to the thymus-dependent compartment but a significant proportion (20%) are found in the primary nodules.

Lymph node cells. In contrast with the spleen cells, lymph node cells contain a much higher proportion of thymus-derived cells. Therefore it is not surprising to find that in the rat, [^{3}H]uridine labelled lymph node cells homed to the 'diffuse cortex' (Austin, 1968); for example at 48 hr, 93% of the labelled cells were found in the diffuse cortex, 4% in the primary nodule and 2·4% in the medulla of the recipient's lymph nodes. Similarly in the spleen, at the same time, 84·7% of the labelled lymph node cells were in the white pulp principally in the periarteriolar zone, 9·5% in the marginal zone (or perifollicular area) and 3·7% in the red pulp.

TABLE 4. Distribution of [^{3}H]adenosine labelled cells* in the mesenteric lymph node

Time	Inoculum	Dose†	Recipients			Total‡	% Distribution			
			Age	Tx	Intact		PN	TDA	C/M	Med
15 min	Spleen cells	3×10^7	2–3 months	1	1	0	—	—	—	—
1 hr 15 min	Spleen cells	3×10^7	2–3 months	2	—	0	—	—	—	—
5–6 hr	Spleen cells	3×10^7	2–3 months	2	3	122	4·9	75·4	7·3	12·29
24 hr	Spleen cells	3×10^7	2–3 months	3	2	295	20·3	65	1·6	8·8
48 hr	Spleen cells	$4{\cdot}5\times10^7$	2–3 months	4	—	567·3	18·9	63·8	5·6	11·3
62 hr	Spleen cells	3×10^7	2–3 months	2	—	93	26·8	65·5	0	7·5

* Cell suspensions incubated for 1½–3 hr in a medium containing 50 μCi [^{3}H]adenosine, autoradiographs exposed for 4–10 weeks.

† Injected intravenously.

‡ Mean total number of cells/section.

In summary, it appears that in general thymus cells or suspensions of cells rich in thymus-derived cells home preferentially to the thymus-dependent compartments whilst marrow cells or suspensions of cells rich in marrow-derived cells seem to have the capacity 'to recognize' and settle in thymus-independent zones.

Some workers have pointed out that localization patterns are difficult to discern and that occasionally thymus-derived cells are found in non-thymus-dependent sites and marrow cells in thymus-dependent ones. This is a valid criticism, it is often difficult to differentiate thymus-dependent or thymus-independent territories, in an intact animal, especially in the white pulp of the spleen; it is, however, quite feasible to do so in neonatally thymectomized or antilymphocyte serum (ALS) treated animals, and in the lymph nodes differentiation between the medulla, primary nodules and thymus-dependent area presents no real difficulty. It is also true that cells are never permanently restricted to one compartment (see Tables 1–4). Moreover, the compartments do not have rigid boundaries, all the cells are in motion, some more rapidly than others and most must migrate across one another's territory whilst migrating in or out of the spleen and lymph nodes. Finally, it is unlikely that either 'source' tissue is completely free of cells derived from the other. The marrow in many species may contain a variable number of thymus-derived cells which have entered from the blood (Keiser

et al., 1967; Doenhoff *et al.*, 1970). The thymus itself is dependent upon a supply of stem cells from the marrow which in the course of development mature into thymus lymphocytes. It is known from the work on radiation chimaeras that cells entering the thymus from the marrow retain many myeloid characteristics for up to 30 days after irradiation (Micklem *et al.*, 1966; Order & Waksman, 1969). Thus, it is quite possible that in the thymus of a young animal or of an adult recovering from stress there could be a minority population with the homing properties of marrow cells.

RATE OF RECONSTITUTION OF COMPARTMENTS

An animal that has been deprived of its thymus-dependent lymphoid population by neonatal thymectomy can only be permanently restored by means of a thymus graft; this must be from a neonatal donor (adult thymus tissue does not take) and for preference placed under a well vascularized site such as the kidney capsule (Parrott & East, 1964). When 'source' and 'source-derived' tissues are destroyed by thymectomy in conjunction with whole body irradiation then both must be reconstituted by means of thymus and marrow grafts (Leuchars, Cross & Dukor, 1965; for further references see Davies, 1969). After such procedures, replacement of the peripheral lymphoid tissues may start within 1–2 weeks but it is nevertheless not complete until 6–10 weeks. The major cause of delay would appear to be the reconstitution of the thymus itself and of the thymus-dependent population (Dukor *et al.*, 1965; Parrott & de Sousa, 1967), but the size of the bone marrow inoculum can also be a limiting factor (Davies, 1969).

When neither 'source' is removed, but either one or other, or both, peripheral populations, is deleted, then replacement of the thymus-independent population is much more rapid than replacement of the thymus-dependent one. Thus, repopulation of the thymus-dependent compartment after ALS treatment may take several weeks (Taub & Lance, 1968; Tyler, Everett & Schwarz, 1969) but reconstitution of primary nodules destroyed by cortisone is complete within days. Sublethal irradiation in the rat (Benninghoff, Tyler & Everett, 1969) and rabbit (Veldman, 1970) will destroy both peripheral compartments but whilst primary nodules reappear within 7 days, restoration of the depleted thymus-dependent compartment may take 4–5 weeks.

The autoradiographic studies of Everett & Tyler (1967a) indicate that the rate of cell production is high both in the thymus and in the marrow. Therefore it seems reasonable to postulate that the difference in peripheral cell reconstitution must reflect a difference in the rate of release of cells from the two source organs. In terms of cell traffic the marrow appears to be much more 'open ended' than the thymus (except following irradiation) and it may well be more susceptible to outside influences. There is circumstantial evidence (Pepper, 1961; Metcalf *et al.*, 1961) that the rate of cell production and growth of the thymus is solely dependent on the age of the tissue itself and that it is relatively impervious to outside stimuli. Our knowledge of feedback mechanisms controlling either thymus or marrow is, however, very slight.

RATE AND ROUTES OF EXCHANGE OF CELLS IN THE PERIPHERY

The first demonstration of the existence of a considerable exchange of cells in the periphery

emerged from the experiments of Gowans on the recirculation of lymphocytes (Gowans, 1957, 1959). Since the early observation that lymphocytes in the thoracic duct lymph in the rat had not been recently formed in the peripheral lymphoid organs but were older cells that had recirculated from the blood, Gowans and his co-workers have carried out a series of elegant experiments on the routes and times taken by labelled intravenously injected thoracic duct lymphocytes passing through lymph nodes and spleen on their recirculation pathway to the lymph. In separate experiments in the rat, Caffrey, Rieke & Everett (1962) demonstrated that continuous drainage of lymphocytes from the thoracic duct over several days did not remove all lymphocytes from the body; according to those authors, lymphocytes could be subdivided into those that could be easily mobilized ('mobilizable lymphocyte pool') and those that could not ('non-mobilizable or sessile lymphocytes'). It is now known that the differences between these two populations are to a large extent a reflection of their different origins and that many of the properties of the cells in the thoracic duct lymph merely reflect its high component of thymus-derived cells, whilst most non-mobilizable cells belong to the thymus-independent compartments and are of marrow descent. Chronic drainage of lymphocytes from the thoracic duct causes a partial depletion of the thymus-dependent areas of the lymphoid tissues whilst leaving the thymus-independent territories unaffected (McGregor & Gowans, 1962; Goldschneider & McGregor, 1968b); on the other hand, in neonatally thymectomized animals there is a much greater deficit of lymphocytes in the thoracic duct than in the blood (Schooley & Kelly, 1964; see Parrott & de Sousa, 1971) thus reflecting the higher component of thymus-derived lymphocytes in the lymph than blood. Recently, we, in collaboration with Pettirossi (de Sousa *et al.*, 1971) have attempted to time and quantitate the number of cells that leave an adult thymus labelled *in situ* with [^{3}H]-thymidine and recirculate to the thoracic duct lymph. Small heavily labelled cells (on average 1:1000) first emerged in the thoracic duct lymph 25 hr after labelling and cannulation. We calculated very roughly from the amount of thymic tissue labelled that it would take approximately 60 days to replace the total recirculating pool of lymphocytes of an adult rat (weight $\sim$ 400 g).

Thymocytes labelled *in vitro* with [^{3}H]uridine start to appear in the thoracic duct lymph within two hours of intravenous injection, although not reaching a peak until 17 hr (Goldschneider & McGregor, 1968a). The process of sorting out those thymocytes that are mature and ready to recirculate from a whole organ suspension is probably quite rapid, i.e. within 24 hr (Lance & Taub, 1969).

A consequence of the extensive studies on thoracic duct lymphocytes is that one can, by direct inference, derive a considerable amount of information about the thymus-dependent population. Unfortunately, this 'bonus' does not extend to the thymus-independent cells. The observation that this population cannot be mobilized by subtraction from the thoracic duct lymph does not mean that having migrated from the bone marrow and found an appropriate 'resting place' thereafter they remain sessile. The fact that the thymus-independent population can move has been demonstrated in several ways. When a large dose of irradiation is administered to an isolated organ (Simić & Petrović, 1967; Keuning & Bos, 1967) such as the spleen or the popliteal lymph node (Benninghoff *et al.*, 1969) repopulation is immediately initiated by an influx of cells from the circulating blood. Cells reappear in the thymus-dependent areas within a matter of hours, those in the marginal zone of the spleen or primary nodules may take up to 2–3 days, and presumably come from other peripheral tissues since there was neither time nor autoradiographic evidence of cell replication

(Benninghoff *et al.*, 1969). When the spleen is submitted to a short period of ischaemia or perfusion (de Sousa, Pettirossi & Parrott, 1971; Ford, 1969) the first cells to be dislodged appear to be the red pulp lymphocytes.

At the height of an immune response after a subcutaneous injection of antigen, antibody producing cells are found in the efferent lymphatic of the draining lymph node (Cunningham, Smith & Mercer, 1966); these cells by reason of their function must be of marrow origin (see *Transplant. Rev.* **1,** 1969).

Brahim & Osmond (1970) found labelled small lymphocytes in the thoracic duct lymph 12 hr after *in situ* labelling of bone marrow. All this data indicates that thymus-independent cells can migrate; there is, however, no clear evidence that a regular and continuous process of exchange of cells occurs between the peripheral lymphoid organs.

Routes of migration

Gowans & Knight (1964) first demonstrated that [^{3}H]adenosine labelled thoracic duct lymphocytes entered the lymph nodes from the blood by migrating across the endothelial walls of the post-capillary venules and exited via the medullary regions and efferent lymphatics. We have no reason to doubt from our observations on injected labelled thymus, spleen, lymph node and marrow cells that probably the majority of lymphoid and undifferentiated cells follow the same route. This, however, does not exclude the possibility that cells exit as well as enter the lymph node through the post-capillary veins, nor that there are other portals of entry. Labelled lymph node cells have been found in the draining lymph node following an intracutaneous injection (unpublished data), and Kelly (1970) has recently shown in the rabbit that many cells in the afferent lymph penetrate the popliteal lymph node. It is possible that a minority of cells in a spleen inoculum injected intravenously enter the primary nodules through the capillary network therein but the majority seem to enter the lymph nodes via the post-capillary venules. In this respect, it is interesting that the walls of the post-capillary veins, normally consisting of high, cuboidal cells, are very thin in the lymphocyte depleted cortex of neonatally thymectomized mice (Parrott *et al.*, 1966) and in the homozygous nude mice, born with no thymus; the walls of the post-capillary veins, however, are normal where their course runs close to primary nodules (see Fig. 2 in de Sousa *et al.*, 1969). Moreover, the thin flattened walls seen in the thymus-dependent areas of nodes from thymectomized mice, will rapidly assume a more normal appearance after the intravenous infusion of lymphocytes (Goldschneider & McGregor, 1968b). It has been postulated (Woodruff & Gesner, 1969) that the post-capillary venules regulate the numbers of cells passing across their walls and in this regard it is of interest that a larger number of thymus cells appear to enter the nodes of thymectomized than intact mice (Parrott & de Sousa, 1969; Table 2). It seems more likely, however, that the appearance of the post-capillary vein is merely a reflection of the numbers of cells passing through them. Cells also enter the Peyer's patches by similar vessels, these do not exist in the spleen and entry to the Malpighian body is by way of the perifollicular sinus; thoracic duct lymphocytes are seen here within minutes of intravenous injection, they then appear to migrate between the endothelial cells of the sinuses into the follicles and wander through the labyrinth of reticular cells to reach the 'traffic' (or thymus-dependent) area by simple amoeboid movement (Ford, 1969) and they eventually join the venous outflow from the spleen at some as yet unidentified site. Thymus cells follow the same pattern but the thymus-independent components of spleen, marrow and lymph node inocula apparently lag behind.

Migration of cells into and from germinal centres

One component of the lymphoid tissues that is apparently relatively inaccessible to migratory cells is the germinal centre (Parrott, 1967a). It has been postulated that specifically sensitized lymphocytes are trapped in the meshwork of dendritic reticulocytes on which antigen in the form of antigen antibody complexes is deposited (see White, 1968), but so far this has proved difficult to demonstrate (Balfour *et al.*, 1971).

Germinal centres are mostly located in thymus-independent areas, are present in neonatally thymectomized or thymusless mice (*nu nu*) albeit not as large as in control intact mice (de Sousa & Parrott, 1967; Davies *et al.*, 1969a, b) and presumably are composed mainly of cells of bone marrow origin. Recently, however, Gutman & Weissman (1971) have demonstrated by immunofluorescence identification of H-2 antigens that some blast cells in germinal centres are of thymus origin. Moreover, some lymphocytes are seen immediately surrounding the germinal centre with the γ-globulin staining characteristics of thymus derived cells (Balfour, personal communication); it would therefore appear that germinal centres are composed of cells of mixed origins.

Autoradiographic studies following the migration of suspensions of radioisotopically labelled cells have shown that neither normal thymus, thoracic duct or lymph node cells gain access to germinal centres (Gowans & Knight, 1964; Parrott, 1967a; Balfour *et al.*, 1971). A minority component of spleen cells, however, including cells from C3H/Bi, NZB donors (which would be expected to have a high number of germinal centre cells) or thymectomized donors, whether labelled *in vitro* or *in vivo* following multiple injections of [^{3}H]thymidine, do have access to germinal centres, particularly in the spleen (Parrott, 1967a; unpublished, 1970).

This difficulty of access of labelled cells into germinal centres may be the result of using the 'wrong' population of cells or the wrong route of inoculation; in all aforementioned experiments the intravenous route was used. Recently, Kelly (1970) labelled lymph cells obtained after cannulation of the afferent lymphatic of the popliteal lymph node in the rabbit, and reinfused them into the same lymphatic. Using this method, significant numbers of labelled lymph blast cells homed to the germinal centres, only on the 4th day after an intracutaneous injection of diphtheria toxoid in the footpad.

The problem of whether cells issue from germinal centres is also controversial; it is hard to think that such an actively mitotic structure is unproductive in terms of the contribution of cells to the lymph and blood, but the evidence for and against germinal centre lymphocytopoiesis is inconclusive, mainly because it is based on sequential [^{3}H]thymidine studies which are unsatisfactory because of the uneven accessibility of the radioisotope to the cells in the various parts of the centre (Everett & Caffrey, 1967b; Sainte-Marie & Messier, 1970).

LIFE SPAN

It has been demonstrated, on the basis of multiple [^{3}H]thymidine injections over varying periods of time, that two types of morphologically identical lymphocytes can be distinguished according to life span (Everett, Caffrey & Rieke, 1964; Robinson *et al.*, 1965; Everett & Tyler, 1967a); these two populations have been called short-lived and long-lived. The short-lived consists of a population of cells, the majority of which acquire the label within 4–5 days, the long-lived population consists of cells, many of which remain unlabelled even after multiple injections of the radioisotope over weeks or months.

The proportion of short-lived cells found in the lympho-myeloid organs varies according to the tissue: 100% in bone marrow, 95% in thymus, and thereafter the proportion drops, 70–80% in spleen, 30–40% in blood, 20–30% in lymph nodes and 10% in thoracic duct lymph (Everett *et al.*, 1964). The proportions in different peripheral tissues may vary considerably according to species and strains studied, nevertheless the sequence spleen < blood < lymph nodes < thoracic duct lymph, seems to be relatively constant, in other words, tissues with the highest proportion of thymus derived cells have the lowest component of short-lived cells. Rieke & Schwarz (1967), in careful experiments with neonatally thymectomized rats injected with multiple doses of [^{3}H]thymidine, showed that they had a normal number of short-lived cells, but their complement of long-lived cells was reduced by half. Such observations have led to the generalization that all long-lived lymphocytes are thymus-derived and all short-lived cells are of marrow origin. This may be true in very general terms, but some proviso should be made. From the observations of Rieke & Schwarz (1967) it is obvious that in neonatally thymectomized rats the long-lived population though reduced is by no means eliminated, and therefore some long-lived cells must derive from some source other than the thymus. Conversely, there are situations in which known thymus-derived cells divide actively in the periphery; this certainly occurs in very young animals and cells that have emerged from a neonatal thymus graft in an older recipient (Parrott & de Sousa, 1967) undergo division outside the graft. Moreover, it is known that in response to a variety of antigens thymus-derived lymphocytes respond by transforming into actively dividing large pyroninophilic blast cells (Parrott, 1967b; Parrott & de Sousa, 1969) whose thymus descent is well established by combined morphological and chromosome marker studies (Davies *et al.*, 1969 a, b), yet its life span can only range between 2 or 3 with a possible maximum of 5 days before dividing to form small lymphocytes (Parrott & de Sousa, 1966). This is a situation where a long-lived lymphocyte gives rise to a short-lived cell, which, in turn, divides to originate a new set of lymphocytes with, of course, no change of origin.

If the postulate that all thymus-derived cells are long-lived and all marrow-derived cells are short-lived was to be extended to their distribution in the peripheral lymphoid organs, one would anticipate finding clear-cut differences in the labelling pattern between the cells in the thymus and non-thymus dependent compartments, according to the number of [^{3}H]thymidine injections received and length of time over which the radioisotope was given. The speed with which cells in the various peripheral tissues acquire label is indeed roughly proportional to the size of their thymus-independent or marrow derived compartment; on the basis of examination of smears, for example, the spleen has a much higher component of 'short-lived' cells than the lymph node (Everett *et al.*, 1964). We have observed, however, that primary nodule cells or cells of the peripheral layer of the Malpighian body deviate from the concept that life span and origin conform. In other words, knowing that these cells are of bone marrow origin, one would expect them to be exclusively short-lived.

Autoradiographs have been studied of tissues from mice subjected to one or other of the following labelling regimes*: a 'short-term' regime, where [^{3}H]thymidine injections were given twice daily for 3 days, and a 'long-term' regime, where [^{3}H]thymidine injections were

* These mice were primed with HSA or BSA and pertussis organisms to stimulate cell division of both thymus-dependent and -independent populations on the same day that thymidine injections were started in the long-term regime and 28 days before the short-term regime. However, the same labelling patterns have been found in unprimed mice.

given twice daily for 28 days then stopped for 3 days (to eliminate most short-lived labelled cells). In the spleens of mice which received the 'short-term' labelling all cells in the red pulp and in the germinal centres were labelled. However, a few labelled small lymphocytes and a number of blast cells appeared uniformly spread over the whole of the white pulp; a similar lack of distinction was observed in the lymph node cortex. After the long-term labelling regime, again the labelled cells were evenly spread over the white pulp of the spleen and the cortex of the lymph node, but the majority of cells in this case were lightly labelled small lymphocytes. In many of these autoradiographs, however, the total number of labelled cells was so large as to make the counting difficult. It is more feasible, and in theory more instructive, to allow the cells themselves to select their own environment; this has been done by transfer of spleen or lymph node cells from donors subjected to one or other of the aforementioned labelling regimes to normal recipients which were killed at various time intervals after injection.

HOMING PATTERNS OF LABELLED SHORT- AND LONG-LIVED LYMPHOCYTES (Tables 5–8)

Very few lymphocytes were labelled in the lymph node inoculum from the mice in the 'short-term' labelling regime (8·24%), whilst in the spleen inoculum of the same animals double the number of lymphocytes were labelled (17·2%); in addition numerous stem cells were also found in the spleen inoculum. A slight difference was noticed between the proportion of lymphocytes labelled in the spleen (53%) and lymph node (46·9%) inocula from the mice in the 'long-term' labelling regime.

The long-lived lymph node cells behaved most closely to the prediction that life span and distribution would correlate, thus both in the spleen and in the lymph node the highest

TABLE 5. Distribution of [^{3}H]thymidine labelled lymph node cells from adult donors in spleen

Labelling regime	Time	Dose	No. of recipients	Total‡	% Distribution			
					RP	PFA	PF	TDA
Short-term*	1 day	$1{\cdot}5–1{\cdot}8 \times 10^7$	2	39·3	12·1	18·4	30·5	38·8
	2 days	$1{\cdot}5–1{\cdot}8 \times 10^7$	2	73	18·8	16·4	36·9	27·7
	3 days	$1{\cdot}5–1{\cdot}8 \times 10^7$	1	18	22·2	22·2	27·7	27·7
	4 days	$1{\cdot}5–1{\cdot}8 \times 10^7$	2	29·7	22·6	17·6	33·6	26·0
	8 days	$1{\cdot}5–1{\cdot}8 \times 10^7$	2	4·8	20·8	3·3	33·3	37·4
	14 days	$1{\cdot}5–1{\cdot}8 \times 10^7$	1	7·5	6·6	19·9	33·3	39·9
Long-term†	2 days	$1{\cdot}5–1{\cdot}8 \times 10^7$	2	129	19·5	6·9	17·7	55·8
	3 days	$1{\cdot}5–1{\cdot}8 \times 10^7$	1	60	6·0	3·1	5·9	84·9
	4–5 days	$1{\cdot}5–1{\cdot}8 \times 10^7$	3	83·3	5·6	2	18·0	74·4
	8 days	$1{\cdot}5–1{\cdot}8 \times 10^7$	1	99	12·6	5·3	6·1	75·7
	14 days	$1{\cdot}5–1{\cdot}8 \times 10^7$	1	44·5	12·3	5·6	6·7	75·2

* Intraperitoneal [^{3}H]thymidine injections (0·5 μCi/gbw) twice daily for 3 days.

† Intraperitoneal [^{3}H]thymidine injections (0·5 μCi/gbw) twice daily for 28 days, stopped for 3 days before transfer.

‡ Mean total number of cells/section. Autoradiographs exposed for 9–11 weeks.

TABLE 6. Distribution of [^{3}H]thymidine labelled lymph node cells from adult donors in the lymph nodes

Labelling regime	Time	Dose	No. of recipients	Total‡	% Distribution			
					PN	TDA	C/M	Med
Short-term*	1 day	$1{\cdot}5$–$1{\cdot}8\times10^7$	2	16·3	11·2	72·4	0	16·3
	2 days	$1{\cdot}5$–$1{\cdot}8\times10^7$	2	9·6	10·3	72·4	0	17·2
	4 days	$1{\cdot}5$–$1{\cdot}8\times10^7$	2	7·2	3·4	82·7	6·8	6·8
	8 days	$1{\cdot}5$–$1{\cdot}8\times10^7$	1	1·8§	—	—	—	—
	14 days	$1{\cdot}5$–$1{\cdot}8\times10^7$	1	4§	—	—	—	—
Long-term†	4–5 days	$1{\cdot}5$–$1{\cdot}8\times10^7$	3	59·3	10·6	89·3	0	0
	8 days	$1{\cdot}5$–$1{\cdot}8\times10^7$	1	18	0	100	0	0
	14 days	$1{\cdot}5$–$1{\cdot}8\times10^7$	1	24·6	10·1	97·9	0	0

* Intraperitoneal [^{3}H]thymidine injections (0·5 μCi/gbw) twice daily for 3 days.

† Intraperitoneal [^{3}H]thymidine injections (0·5 μCi/gbw) twice daily for 28 days, stopped for 3 days before transfer.

‡ Mean total number of cells/section. Autoradiographs exposed for 9–11 weeks.

§ % distribution was not estimated when the mean number of cells/section was lower than 5.

percentage of the labelled long-lived cells (75% in the spleen and 95% in the lymph nodes) homed to the thymus-dependent areas (Tables 5 and 6); morphologically all labelled cells in this group were identified as small, heavily labelled lymphocytes. Nevertheless, even in this group higher percentages of labelled cells were found in the primary nodules (10%) and in the periphery of the Malpighian body (11%) than would have been anticipated if all labelled long-lived cells in a lymph node inoculum were thymus-derived (Table 1). The distribution of the labelled long-lived spleen cells deviates still further from prediction; although the highest percentage of the labelled cells was always found in the thymus-dependent compartments (Tables 7 and 8), the numbers found elsewhere and particularly

TABLE 7. Distribution of [^{3}H]thymidine labelled spleen cells from adult donors in the spleen

Labelling regime	Time	Dose	No. of recipients	Total‡	% Distribution			
					RP	PFA	PF	TDA
Short-term*	1 day	5×10^7	1	873	46·8	10	27	16
	4 days	5×10^7	4	101	15·8	19·9	42·4	19·7
	6 days	5×10^7	2	110	14·9	14·5	58·6	11·8
	8 days	5×10^7	2	31	17·7	16·1	51·6	14·5
Long-term†	1 day	5×10^7	2	1309	19·8	7·8	53·9	19·1
	3 days	5×10^7	1	231	5·6	3	61	30·3
	6–7 days	5×10^7	3	250	1·6	3·0	54·5	40·8

* Intraperitoneal [^{3}H]thymidine injections (0·5 μCi/gbw) twice daily for 3 days.

† Intraperitoneal [^{3}H]thymidine injections (0·5 μCi/gbw) twice daily for 28 days, stopped for 3 days before transfer.

‡ Mean total number of cells/section. Autoradiographs exposed for 9–11 weeks.

in the primary nodules (Table 8) were far in excess of what has been found with a pure suspension of thymus cells (Table 2); this points to the conclusion that the cells in the labelled long-lived pool are of mixed origin.

Generally the labelled short-lived component of both inocula was not confined to the non-thymus-dependent compartment, although the majority of the short-lived spleen cells was found in these areas, both in the spleen (a total of ~ 80%) and in the lymph nodes (~ 65%) (Tables 7 and 8). The short-lived lymph node cells, however, persisted in localizing

TABLE 8. Distribution of [^{3}H]thymidine labelled spleen cells from adult donors in the lymph nodes

Labelling regime	Time	Dose	No. of recipients	Total‡	% Distribution			
					PN	TDA	C/M	Med
Short-term*	1 day	5×10^7	1	71	21·8	58·4	—	19·7
	4 days	5×10^7	4	14	53·6	34·7	—	11·5
	6 days	5×10^7	2	24	48·6	47·2	—	4·1
	8 days	5×10^7	2	5·5	59·9	32·7	—	7·2
Long-term†	1 day	5×10^7	2	138·6	11·1	60·3	5·4	23·0
	3 days	5×10^7	1	101	22·6	65·4	4·6	6·2
	6–7 days	5×10^7	3	141	31·5	50·7	6·2	11·5

* Intraperitoneal [^{3}H]thymidine injections (0·5 μCi/gbw) twice daily for 3 days.

† Intraperitoneal [^{3}H]thymidine injections (0·5 μCi/gbw) twice daily for 28 days, stopped for 3 days before transfer.

‡ Mean total number of cells/section. Autoradiographs exposed for 9–11 weeks.

TABLE 9. Distribution of [^{3}H]thymidine labelled cells* from suckling donors in the spleen

Inoculum	Dose†	Age and No. of recipients		Time after 2nd injection	Total‡	% Distribution			
						RP	PFA	PF	TDA
Thymus cells	$5{\cdot}4\times10^7$	10 days	4 intact	72 hr	71·22	5·3	2·2	7·8	84·3
	$4{\cdot}2\times10^7$	13 days							
Spleen cells	$2{\cdot}7\times10^7$	5 days	2 intact	72 hr	154·75	11·9	7·76	72·1	8·2
	$2{\cdot}7\times10^7$	8 days							

* The donors received 0·5 μCi/gbw for 15 or 18 days from birth, autoradiographs exposed for 6 weeks.

† Cells injected intraperitoneally.

‡ Mean total number of cells/section. Autoradiographs exposed for 9–11 weeks.

in the thymus dependent areas of the lymph nodes (70–80%) (Table 6). In the spleen, however, a smaller percentage (30–35%) was found round the central arterioles (Table 5).

Overall, the majority of long-lived cells preferred the thymus-dependent compartment and the majority of short-lived cells homed to the thymus-independent compartment, nevertheless, a significant degree of overlapping occurred, and many long-lived cells from spleen inocula were found in primary nodules, and short-lived cells in the thymus-dependent areas.

Additional experiments still in progress following the pattern of distribution of spleen cells from adult and suckling donors labelled with multiple [^{3}H]thymidine injections confirm the predilection of spleen cells for primary nodules even in very young recipients.

TABLE 10. Distribution of [^{3}H]thymidine labelled cells* from suckling donors in the lymph node

Inoculum	Dose†	Age and No. of recipients	Time after 2nd injection	Total‡	% Distribution			
					PN	TDA	C/M	Med
Thymus cells	$5{\cdot}4\times10^7$	10 days	4 intact	72 hr	189·1	0	94·1	2·5	2·9
	$4{\cdot}2\times10^7$	13 days							
Spleen cells	$2{\cdot}7\times10^7$	5 days	2 intact	72 hr	45·4	63·4	33·0	0·88	2·6
	$2{\cdot}7\times10^7$	8 days							

* Donors received 0·5 μCi/gbw for 15 or 18 days from birth, autoradiographs exposed for 6 weeks.
† Cells injected intraperitoneally.
‡ Mean total number of cells/section.

FACTORS INFLUENCING THE SEGREGATION OF LYMPHOID CELL POPULATIONS

The evidence for the existence of cells of different origins segregating into different compartments of the peripheral lymphoid tissues has been presented. Suspensions of cells seek their appropriate compartment or environment with remarkable 'single-mindedness'; this phenomenon appears to be of significance for the understanding of the physiology of the lymphoid system and has been termed ecotaxis (de Sousa, 1971). Thymus cells and suspensions of cells rich in thymus-derived cells home preferentially to the thymus-dependent compartment, marrow cells home to the thymus-independent compartment (especially to the medulla of lymph nodes and the red pulp of the spleen), and spleen cell suspensions which contain cells of mixed origin spread themselves evenly over both compartments. Selection of sub-populations within the spleen and lymph node inocula, by labelling of the short- or long-lived components, showed that, essentially, spleen cells, whether short- or long-lived, behaved like an inoculum containing a set proportion of thymus-derived and non-thymus-derived cells, and that lymph node cells of different lifespans too, behaved like another inoculum of mixed origins, only comprising a different proportion of thymus to non-thymus-derived cells.

Since all lymphoid cells are mobile and must share entry and exit routes, it is of relevance to consider a number of factors that might determine segregation in compartments. The primary factor seems to be the origin of the cell itself; nevertheless, other factors such as the basic structure of the peripheral lymphoid organs and the surface make-up of the cell may influence 'environment seeking'. All cells appear to enter by the same route, one could therefore envisage some checkpoint at the sites of entry. Gesner (1966) suggested that some form of recognition process involving the sugars on the lymphocyte surface may take place at the post-capillary venule, and 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 thymus and thymus-derived cells to the splenic thymus-dependent territory 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.

Having entered the peripheral lymphoid tissues it would seem reasonable to except all lymphocytes by virtue of their intrinsic mobility to spread themselves evenly over all territories, yet in neonatally thymectomized animals, or in adult thymus-deprived mice, they do not; vast areas normally occupied by the thymus-derived cells are left void, with the nodules and medulla in the lymph nodes, and the outer layer of the Malpighian body and the red pulp in the spleen fully populated. It should be remembered that old neonatally thymectomized mice do *in extremis* make strenuous efforts to become repopulated, viz. the encroachment of the thymus-dependent area in the lymph node with medullary plasma cells, and full repopulation of some thymus-dependent areas in the spleen with plasma cells (Parrott *et al.*, 1966).

This apparent physical restraint of cells in the nodules cannot be easily explained by the examination of the basic reticulin framework of the lymphoid organs, for one can see no barrier between the primary nodule and the rest of the lymph node cortex or between the outer and inner layers of the Malpighian follicle in the spleen (Denz, 1947; Sainte-Marie & Sin, 1968; de Sousa, 1969). Other zonal differences exist, and these have been related to the different mobility of cell populations in lymph nodes (de Sousa, 1969). Thus, the reticulin fibres in the mid cortex of the nodes and in the periarteriolar zone of the spleen (Sainte-Marie & Sin, 1968) are arranged in an 'open' pattern, whereas the medulla of the lymph nodes and the splenic red pulp are characterized by a 'close' almost unicellular network. These zonal differences coincide with the areas occupied by the thymus-dependent lymphocytes and by part of the thymus-independent population, thus suggesting that the basic reticulin arrangement of the peripheral lymphoid tissues does favour (if not explain) differences in the mobility of cell populations.

In the absence of obvious barriers between the nodules and the thymus-dependent area in the lymph nodes, and the inner and outer layer of the Malpighian body in the spleen, alternative explanations must be sought for the cohesiveness of cells in those thymus-independent territories, that prevents them from being easily mobilized. Simple primary nodule aggregates do not have demonstrable reticulin fibres of the coarse type seen elsewhere in the lymphoid organs, but in sections stained for γ-globulin, an intercellular mesh of immunoglobulin deposited on the cytoplasmic processes of reticulocytes is revealed (Balfour & Humphrey, 1967; Balfour, unpublished). This mesh of reticulocytes with long cytoplasmic processes is peculiar to the thymus-independent nodular compartments of both spleen and lymph nodes (White, 1963; Miller & Nossal, 1964; Milanesi, 1965) and even when nodules and germinal centres are drastically disrupted, will persist though 'squashed' or 'collapsed' (Balfour *et al.*, 1971). It is therefore possible to envisage that lymphocytes rich in IgG receptors such as bone marrow lymphocytes are known to be more easily 'entangled' in this meshwork than a lymphocyte with fewer receptors, as seems to be the case with the thymus-derived cell (Coombs *et al.*, 1970; Raff, 1970); judging from the behaviour of injected spleen cells and their easy access to nodular areas, it would appear that no specific or positive attraction of cells is involved but rather that a nodule is the result of the inadvertent entanglement of cells with immunoglobulin on their surface within a similarly coated mesh.

It has been shown recently that lymphocytes rich in IgG receptors also contain complement receptors (Bianco, Patrick & Nussenzweig, 1970), and Dukor, Bianco & Nussenzweig (1970) found that such lymphocytes are predominantly distributed over the primary nodules. These workers postulated that complement is the adhesive responsible for follicular aggregations of lymphocytes, and that complement receptor lymphocytes would therefore either be specifically bound by previously deposited antigen–antibody complexes or complement receptor lymphocytes carrying such complexes could selectively be trapped within the mesh of reticulocytes.

So far, it has proved difficult to demonstrate that localized antigen has any dominant role in determining the 'attraction' of lymphocytes to specific sites. It is true that increased traffic of cells takes place into a lymph node draining the site of injection of adjuvant material (Dresser, Taub & Krantz, 1970), nevertheless, this is apparently non-specific; Balfour *et al.* (1971) failed to find evidence of attraction of lymph node cells primed to HSA or lysozyme to primary nodules or germinal centres where either antigen had been previously localized in the form of antigen–antibody complexes.

Some immune responses reinforce the concept of lymphoid cell segregation, in so far as the most obvious cellular events following antigen stimulation occur in one or other compartment and can be related to clearly different functions (Oort & Turk, 1965; Parrott & de Sousa, 1966). Thus, the changes in the early stages of development of specific cell-mediated immunity occur among the thymus-dependent population and consist of active proliferation of large pyroninophilic blast cells in the thymus-dependent areas. On the other hand, response to antigens such as the pneumococcus polysaccharide type III in the mouse takes place with little cellular change in the thymus-dependent population (de Sousa & Parrott, 1967). In response to other antigens, however, cells in all compartments participate, and indeed territory boundaries may be temporarily disrupted (Hanna, 1965) or deleted, particularly in the spleen, causing cells of different origins to intermingle. We would like to suggest that in this intermingling may ultimately lie the explanation of the phenomenon of cell-to-cell co-operation (see *Transplant. Rev.* **1**, 1969).

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