

CELLULAR DIFFERENTIATION IN THE THYMUS

III. SURFACE PROPERTIES OF RAT THYMUS AND LYMPH NODE CELLS SEPARATED ON DENSITY GRADIENTS*

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Lymphocytes which originate as stem cells in the bone marrow and undergo proliferation and differentiation in the thymus enter the peripheral lymphocyte pool as so-called T lymphocytes (2, 3). These are long-lived, recirculating cells which participate both in immune responses of the "cell-mediated" type, as in transplantation immunity, and in antibody formation against certain classes of antigens (see reviews in 2, 4, 5).

The purpose of our study was to compare some of the better known attributes of T lymphocytes in precursors of these cells maturing within the thymus, with the intention of establishing the sequence in which they appear and their possible relationship to "immunocompetence." We separated normal rat thymus cells by the technique of centrifugation on discontinuous density gradients, which yields populations differing in density and to a considerable degree in cell size (6). The cellular properties investigated included: size, proliferative rate, antigenic makeup of the cell surface, ability to enter the parenchyma of lymph nodes when injected into the circulation, ability to respond mitotically to stimulation with phytohemagglutinin or with a specific alloantigen, and finally ability to induce a graft-*versus*-host reaction in suitable hosts.

Materials and Methods

Animals.—Inbred rats of the Lewis (Le) strain (Microbiological Associates, Inc., Bethesda, Md.), DA strain (originally obtained from Dr. Willys K. Silvers, University of Pennsylvania, Philadelphia, Pa.), and (Le × DA) F₁ hybrids were used as serum and cell donors and recipients. New Zealand white rabbits provided normal rabbit serum.

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Cell Suspensions.—Lymph node (LN)¹ cells were obtained from submaxillary, cervical, axillary, brachial, mesenteric, paraaortic, and superficial inguinal lymph nodes by gentle teasing in Minimal Essential Medium for suspension culture (MEM-S) (Microbiological Associates, Inc.), filtered through two layers of gauze, and kept on ice. Thymus (T) cells were prepared in the same manner, after the parathymic LN were removed, the thymus washed free of blood, and its capsule dissected away.

Cell Separation.—LN or T cells from 8- to 10-wk old male Le rats were separated by differential flotation in discontinuous albumin density gradients. The method of Raidt et al. (6) was used with slight modification. Sterile BSA from General Biochemicals, Div., Grand Island Biological Co., Chagrin Falls, Ohio (catalogue No. 10170) was diluted with MEM-S on a v/v basis. 1.4×10^9 nucleated cells were suspended in 5.5 ml of 30% BSA with gentle pipetting, and the suspension placed in the bottom of 35-ml cellulose nitrate tubes for use in the Beckman Spinco SW 25.1 rotor (Spinco Div., Beckman Instruments Inc., Palo Alto, Calif.). The cell mixture was carefully overlaid with 7 ml each of 27%, 24%, 20%, and finally 5 ml of 10% BSA solution. Centrifugation was carried out at 4°C for 30 min at 12,000 rpm, which produced a maximum force of 20,000 g at the tip of the tube. Discrete bands of cells which formed at the density interfaces were harvested through an 18 gauge needle from the bottom of the tube. These were designated A, B, C, and D beginning with the top band and P (pellet). Cells were washed twice in MEM-S, counted in a hemocytometer, and their viability checked by trypan blue dye exclusion (7).

Immunofluorescence Assay.—The distribution of rat thymus specific antigen (RTA) on living cells was studied by use of an indirect immunofluorescence test using suitably absorbed rabbit anti-rat thymus serum, as described previously (8, 9).

Assay of Phosphodiesterases.—Neutral phosphodiesterase I and acid phosphodiesterase II were assayed with the colorimetric method described by Razzell (10). Cell extracts were prepared by homogenizing cells in distilled water with a motor-driven pestle. The assay mixture for phosphodiesterase I contained *p*-nitrophenyl-thymidine-5'-phosphate (Sigma Chemical Co., St. Louis, Mo.) (1 μ mole), Mg⁺⁺ (1 μ mole), and tissue extract in 0.5 ml Tris buffer pH 8.6. For phosphodiesterase II, *p*-nitrophenyl-thymidine-3'-phosphate (Raylo Chemical, Edmonton, Alberta, Canada) (1 μ mole) was used as substrate in pH 6.0 acetate.

Cell Culture.—Responses to phytohemagglutinin-P (PHA) (Difco Laboratories, Detroit, Mich.) and hemiallogeneic cells were assayed under conditions similar to those previously described (11). Culture medium consisted of MEM-S, penicillin-streptomycin mixture (Microbiological Associates, Inc.) (100 units/ml each), L-glutamine (Microbiological Associates, Inc.) (2 mM/ml), and 10% fresh normal rat serum or, in some cases, 10% fresh heat-inactivated (56°C, 30 min) normal rabbit serum (v/v). For PHA stimulation, 4×10^6 cells were incubated in 2 ml of culture fluid containing 1 μ l/ml PHA. For mixed lymphocyte reactions (MLR), 2×10^6 Le cells were mixed with 2×10^6 hemiallogeneic (Le \times DA) F₁ LN cells. Triplicate cultures were incubated in 5% CO₂ in air, at 37°C, for 88 hr, then exposed to 2 μ Ci/ml of tritiated thymidine (³H-TdR) (Schwarz BioResearch, Orangeburg, N. Y.) with a specific activity of 1.9 Ci/mM, and harvested 8 hr later. Samples were processed (11) and counted in a liquid scintillation spectrometer. Data are reported as either of the following: mean counts per minute (cpm); the level of response, i.e. the mean cpm of experimental cultures (E) minus the mean cpm of control cultures (C), (E - C); or the relative degree of response, expressed by the ratio (E/C).

¹ *Abbreviations used in this paper:* C, mean of control cultures; E, mean of experimental cultures; GVH, graft-versus-host; LN, lymph node; MEM-S, Minimal Essential Medium for suspension culture; MLR, mixed lymphocyte reaction; PHA, phytohemagglutinin-P; T, thymus.

Localization Assay of Transferred Cells.—To study the homing properties of cell populations, 10^8 cells were labeled in vitro with ^{51}Cr as sodium chromate- ^{51}Cr (New England Nuclear Corp., Boston, Mass., NEZ-030) and washed twice (details in reference 12). 2×10^7 labeled cells in 0.5 ml were injected intravenously into 5-6-wk old male Le rats. The recipients were sacrificed at 18 hr and a standard set of LN (those named earlier), spleen, and other organs were weighed and counted in a well-type scintillation counter (Baird-Atomic Well Counter 810C with H 530 scaler [Baird-Atomic, Inc., Bedford, Mass.]). The results were calculated as per cent of injected radioactivity recovered per organ.

Graft-versus-Host (GVH) Assay.—GVH assays were performed as described by Billingham et al. (13). 5×10^6 to 1×10^8 cells in 0.05-0.1 ml were injected into the anterior facial vein in DA rats less than 24 hr old. The litters were sacrificed 10 days later, body weight recorded, and spleen, kidney, liver, and thymus removed, and weighed. A spleen index was calculated as the ratio of spleen weight to body weight in each experimental animal over the corresponding ratio in normal littermate controls.

RESULTS

Characteristics of Separated T and LN Subpopulations.—Following separation and repeated washing, approximately 50% of either T or LN cells were recovered, distributed as subpopulations among four bands and a pellet (Table I). The majority of T cells were found in bands D and P, while most LN cells separated to bands C and P. Although individual yield figures varied, these patterns remained constant for cells from the two lymphoid sources. Viability in cells of the four bands was almost always greater than 90%, as determined by trypan blue dye exclusion. Cells from the T and LN pellets, respectively, averaged 83 and 76% viable. Subsequent experiments were conducted on the basis of total cell counts.

Differential counts of the subpopulations, with determination of cell size by use of an ocular micrometer, are also shown in Table I. While the BSA gradient method separates cells primarily on the basis of density, a correlation between density and size was found in cells from both T and LN. The less dense fractions, A and B, contained the majority of the larger cells including an appreciable number of macrophages. Band C contained predominantly small lymphocytes, but also a moderate number of medium-sized cells. D and P were composed almost entirely of small, very dense cells, and some plasma cells were found in P.

Immunofluorescence assays on living preparations of cells with a rabbit antirat thymocyte serum, absorbed with LN cells, demonstrated the presence of RTA (8, 9) on 97% of the cells in the original T suspensions and 0-4% of the cells of LN preparations. The average percentages of RTA-positive thymocytes in bands from two separate experiments were: A = 88%, B = 87%, C = 94%, D = 98%, and P = 99%. The separate determinations never varied by more than 2%. Cells which were negative for RTA were invariably medium or large in size, as described by Order and Waksman (8). However, the small cells in band C included a few (less than 1.00%) RTA-negative cells.

Exonucleases in the various thymus subpopulations showed one significant departure from a uniform distribution. Acid phosphodiesterase (exonuclease II), in two experiments, was approximately 10 times and 5 times higher in fractions A and B, respectively, than in C, D, or P. This difference corresponded roughly to the increased proportion of macrophages in these two fractions. Neutral phosphodiesterase (exonuclease I) was distributed uniformly.

In the absence of added mitogen, the cells of each subpopulation showed a

TABLE I
Yield and Size Distribution of Lymphocyte Subpopulations

Fraction	Yield	Size distribution		
	Per cent of original	Range in per cent		
	Mean \pm SD	< 8 μ	8-12 μ	>12 μ
Thymus cells				
Original	100	89.0- 97.5	2.5- 5.0	0 - 6.0
A	1.0 \pm 0.2	65.0- 92.6	6.6-23.7	2.0-11.3
B	3.0 \pm 2.1	76.6- 92.0	6.7-20.5	1.3- 2.2
C	7.6 \pm 3.5	95.5- 99.5	0.5- 3.9	0 - 3.5
D	13.0 \pm 6.5	94.5-100	0.1- 5.5	0 - 1.3
P	30.0 \pm 14.2	96.0-100	0 - 4.0	0
Total	54.0 \pm 15.5			
Lymph node cells				
Original	100	94.5- 99.8	1.0- 5.5	0 - 1.5
A	1.2 \pm 0.7	83.6- 95.0	4.6-11.6	0 - 4.8
B	5.0 \pm 2.8	90.6- 93.1	6.0- 9.4	0.9- 2.2
C	16.6 \pm 7.4	95.5- 98.5	1.5- 4.5	0 - 5
D	9.5 \pm 4.4	96.8-100	0 - 3.2	0
P	13.4 \pm 10.0	94.5- 99.7	0.3- 5.5	0
Total	46.5 \pm 11.0			

Yield data: 12-22 observations.

Size data: 4 observations.

characteristic degree of spontaneous DNA synthesis (Table II). Decreasing density (or increasing size) was clearly correlated with increasing spontaneous DNA synthetic activity on the fourth day of culture, though there were substantial differences in cpm levels in individual experiments. This finding agrees with published data of other investigators (14, 15).

Effect of Heterologous Serum on DNA Synthesis and Mixed Lymphocyte Responses (MLR).—When 10% heated fresh normal rabbit serum was used as serum supplement in the culture medium, both spontaneous DNA synthesis (see also reference 16) and the response to hemiallogeneic cells were altered.

Base-line values of control T and LN cell suspensions were elevated 3–5-fold over those obtained in control cultures containing fresh rat serum as the supplement. A similar increase was seen in cultures of separated C or D cells from either source. A, B, and P cells remained relatively unaffected by the presence

TABLE II
Spontaneous DNA Synthesis (cpm) at 4 Days

Experiment	Subpopulations				
	A	B	C	D	P
Thymus cells					
1	—	149,400	26,000	150	150
2	—	54,400	6,800	200	100
3	23,400	50,600	3,400	50	50
4	14,600	10,100	1,000	200	200
5	—	10,300	1,200	150	100
Lymph node cells					
1	127,800	94,100	62,000	19,800	100
2	37,000	43,100	14,100	1,800	100
3	18,000	11,900	300	200	100
4	—	2,700	3,200	700	300
5	400	1,200	300	1,600	400

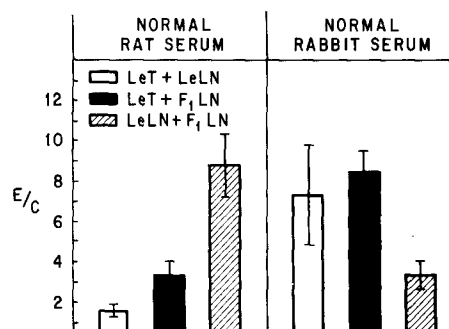


FIG. 1. Comparison of syngeneic and hemiallogeneic T and LN mixed cultures in medium supplemented by normal rat or rabbit serum. Relative ³H-TdR incorporation (E/C): means of 5–9 experiments. SE of the mean is indicated at top of each bar.

of rabbit serum. When either syngeneic or hemiallogeneic T and LN cells were cultured together in the presence of normal rabbit serum or fetal calf serum, ³H-Tdr incorporation values were considerably greater than those obtained in rat serum. However, the usual LN-LN mixed lymphocyte response was found to be depressed in the presence of the heterologous serum (Fig. 1). Foreign

serum may possibly influence the cultures (*a*) as a source of antigenic stimulation, (*b*) as a source of anticellular antibody (7), or (*c*) by changing the growth conditions. Our result accounts for the finding (17) of an apparent mitogenic response between syngeneic T and spleen cells in medium containing fetal calf serum and suggests that MLR experiments carried out in the presence of foreign serum may be invalid.

Mitotic Response of T and LN Cells to Hemiallogeneic Cells and to PHA.—The proportion of T cells responding mitotically to a hemiallogeneic cell

TABLE III
Level of MLR Response in Thymus and Lymph Node Cell Suspensions

Cells tested	No. of cultures	Mean MLR \pm SE*
Lewis T + F ₁ LN	6	700 \pm 190
Lewis LN + F ₁ LN	11	6100 \pm 1100

* Cpm value obtained in cell mixture minus values obtained with each cell component cultured alone (E - C).

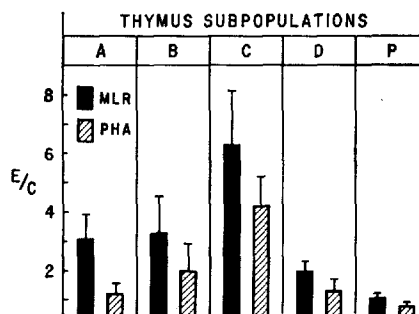


FIG. 2. MLR and PHA reactivity of separated thymus subpopulations, expressed as E/C. Means of values obtained in 16 culture experiments are plotted. SE of the mean is indicated at top of each bar.

stimulus could be calculated from the level of response (E-C) as approximately 10 times less than the corresponding proportion of LN cells (Table III). Composite data from 16 culture experiments with T subpopulations, expressed as E/C (average of values obtained in triplicate cultures), are shown in Fig. 2. The majority of T cells, as represented by bands D and P, failed to respond to either stimulus. Cultures of cells from bands A and B generally showed a low grade MLR response but little response to PHA. Cells of fraction C always exhibited good MLR reactivity but in 10 of 16 experiments gave essentially no PHA response. Individual experiments which illustrate the enrichment of cells within band C capable of responding well to hemiallogeneic cells but not

to PHA are shown in Table IV. LN subpopulations gave a distinctly different pattern of responses to hemiallogeneic cells and PHA (Table IV). Most of the mitotic reactivity was found in band D or, occasionally, in both C and D. Peak MLR and PHA responses were always obtained in the same cell fraction, the PHA reactivity usually being the greater.

Ability of T and LN Subpopulations to Localize in Peripheral Lymphoid Tissues.—Following separation, T and LN cells were labeled with ^{51}Cr , washed, and injected intravenously into syngeneic recipients and the percentages of injected ^{51}Cr activity were measured in different organs at 18 hr. Separation

TABLE IV
Response of Lymphocyte Subpopulations to Mitogens in Representative Experiments

Fraction	Relative ^3H -Tdr incorporation (E/C)			
	Experiment 1		Experiment 2	
	MLR	PHA	MLR	PHA
Thymus cells				
A	3.0	1.0	—	—
B	1.7	0.6	5.8	0.9
C	4.8	0.9	12.2	1.1
D	0.7	1.0	3.3	6.0
P	0.5	0.8	1.0	1.0
	Experiment 3		Experiment 4	
	MLR	PHA	MLR	PHA
	Lymph node cells			
A	0.8	0.8	—	—
B	0.5	1.0	0.3	2.9
C	1.7	2.3	0.8	7.0
D	11.8	9.2	5.2	13.8
P	2.8	5.7	0.6	0.5

of T cells resulted in a substantial enrichment in band C of cells capable of recirculating as shown by localization in the recipient's LN (Table V). These also gave peak localization within the spleen. Cells in band D, in contrast, localized less well than those of the original suspension. Most LN cells with the ability to localize in either LN or spleen resided in the C and D subpopulations, with a corresponding diminution of cells with this capability in bands A, B, and P (Table VI).

GVH Activity of T and LN Cells and Subpopulations.—LN cells produced a minimal increase in recipient spleen weight at doses as low as 5×10^6 cells (Fig. 3). An approximately equivalent increase was seen with 10^8 injected T cells. The C subpopulation of T produced a similar degree of splenomegaly at

TABLE V
Localization of Transfused Thymus Cells in Different Organs

Population injected	Mean per cent* of injected ^{51}Cr dose			
	Thymus	Lymph nodes	Spleen	Liver
Original	0.02, 0.02	1.1, 0.7	6.5, 8.2	38, 32
A	0.01, 0.07	0.7, 0.3	8.1, 6.5	44, 44
B	0.00, 0.02	0.9, 0.6	5.7, 9.7	20, 25
C	0.02, 0.01	2.7, 1.2	10.7, 11.7	29, 22
D	0.04, 0.20	0.5, 0.4	6.3, 10.3	31, 32
P	0.02, 0.03	0.3, 0.1	6.1, 6.0	38, 46

* Data of two experiments. Each figure is mean of values obtained in duplicate recipient rats.

TABLE VI
Localization of Transfused Lymph Node Cells in Different Organs

Population injected	Mean per cent* of injected ^{51}Cr dose			
	Thymus	Lymph nodes	Spleen	Liver
Original	0.10, 0.02	15.0, 16.3	12.5, 14.2	22, 26
A	0.05, 0.04	4.5, 2.8	10.7, 8.2	27, 34
B	0.00, 0.02	6.7, 3.9	15.3, 12.0	26, 34
C	0.00, 0.02	19.4, 15.6	19.0, 18.7	16, 18
D	0.03, 0.01	18.6, 21.9	15.2, 18.6	10, 12
P	0.00, 0.02	4.0, 5.8	10.3, 20.1	35, 35

* Data presented as in Table V.

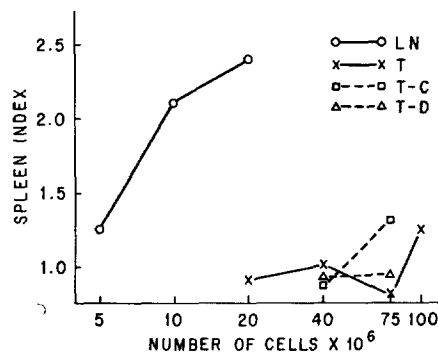


FIG. 3. GVH reactivity of LN, T, and T subpopulations, expressed as spleen index in relation to number of injected cells. Values plotted are means of values obtained in 5-25 individual recipient animals, except that at the 75×10^6 cell dose, means are given of values from 2, 2, and 3 animals injected with thymus original, C, and D, respectively. \circ — \circ LN cells, original. \times — \times thymus, original. \square — \square thymus, C fraction. \triangle — \triangle , thymus, D fraction.

a dose of 0.75×10^8 . The remaining fractions did not give any measureable response at doses of 0.4×10^8 (A, B, and P) or 0.75×10^8 (D).

DISCUSSION

In examining cells of the thymus, there is no a priori reason for assuming that different attributes of the future T lymphocytes arise simultaneously. Indeed our data show that they do not. Therefore each new characteristic must be studied against the background of what is already known about thymocyte maturation. Available information, obtained largely by morphologic techniques, concerns cell size, replication, and location within the thymus.

Lymphoid cells enter the thymus from the bone marrow (8, 18, 19), remain approximately 72 hr (20, 21), and leave to enter the peripheral pool. They undergo a progressive decrease in size (8, 20, 22) and leave as small- or occasionally medium-sized cells. They divide repeatedly, undergoing as many as 5-7 cell divisions (20, 22) with a constant generation time of about 9 hr (21). They migrate from cortex to medulla, before leaving by way of the thymus veins and lymphatics which accompany the larger blood vessels (4, 22). Cell division ceases some time prior to their emigration.² The suggestion that a large proportion die in the thymus (4) is contradicted by the low pyknotic index in the thymus cells of young animals (21).

Our approach was based, therefore, on the premise that progressive maturation is correlated with diminishing cell size. We used a convenient cell separation technique based on density, which nevertheless gave subpopulations differing substantially in size and in some of the other parameters examined.

A fundamental attribute of T lymphocytes, which we examined in thymus cells, is the ability to recirculate, i.e., to migrate from the blood into the parenchyma of lymph nodes and spleen and thence into efferent lymphatic or venous channels (23; Ford and Gowans in reference 2). Of mouse thymocytes, a small percentage home in lymph nodes compared with LN cells tested in the same manner, 1.3-1.6% compared with 7.9-12.5% (12); i.e., approximately 15% as many mouse thymocytes as LN cells have the requisite surface components. The proportion in the rat thymus is considerably smaller (23, 24). We found localization of 0.7-1.1% and 15.0-16.3% rat thymocytes and LN lymphocytes, respectively, implying a relative proportion of recirculating cells among thymocytes as low as 5% relative to LN cells.

The second peripheral property which we investigated was specificity, identified in T lymphocytes by their ability to respond to allogeneic or hemi-allogeneic cells with blast transformation (15, 25) or to produce GVH reactions in suitable hosts (13, 26). There is evidence that these functions depend on the presence of specific antibody-like receptors at the cell surface (11, 27); con-

² Taub, R. N., and E. M. Lance. Personal communication.

ventional L-chains have been identified (28, 29). Some thymocytes must possess similar receptors, since specific immunologic tolerance is induced by direct injection of antigen into the thymus (30) and since MLR and GVH can be produced with thymus cell suspensions; yet these cells do not react with antisera against known immunoglobulin (25, 31), and they appear not to combine with antigen (32). Our estimate that approximately 10% as many thymocytes as LN cells reacted in the MLR, on the basis of actual incorporation of ^3H -thymidine into each cell type after stimulation suggests that the same cells react as are capable of homing in lymph nodes or possibly that some cells have acquired the one property without the other.

To produce a systemic GVH in hemiallogeneic, neonatal hosts, one must inject approximately 7 times as many mouse thymocytes as LN cells (26). In rats, 20-60 times as many thymus as LN cells may be required (13) and

TABLE VII
Peripheral Properties Found in Thymus Subpopulations

Property tested	Thymocytes			LN cells
	A + B	C	D + P	D
Size	L, M, S*	M, S	S	S
Spontaneous DNA synthesis	++	+	0	0
PHA response	0	+, 0	0	++
MLR	0	++	0	++
Seeding of LN	0	+	0	++
RTA	0, ++	++	++	0
GVH competence	0	+	0	++

* L: large; M: medium; S: small.

this was confirmed in the present study. By this criterion too, then, mouse and rat thymus, respectively, contain 15% and less than 5% of cells with the necessary surface properties, relative to LN cells. Since some 2% of peripheral lymphocytes respond to a strong alloantigen (27, 33), the competent percentages in mouse and rat thymus would be 0.3% and <0.1%. Similar estimates are obtained by examining the ability of lymphocytes to restore thymus-deprived animals to normal immunologic reactivity: in mice, 4-10 times more thymocytes are required than peripheral lymphocytes (34) while in rats, as we showed in an earlier study, restoration is not achieved with 20 times more (35).

The present investigation establishes that properties accepted as characteristic of peripheral T lymphocytes are found together in cells of the thymus C fraction and occasionally to some extent in D (Table VII). There is evidence to show that these are not blood or LN lymphocytes contaminating thymocyte suspensions² (36). They are largely or entirely small thymocytes which have ceased to divide, i.e., show little spontaneous proliferation in cell cul-

ture. We thus confirm the finding of others that only a proportion of rat small thymocytes respond in the MLR (15) and GVH (13). These cells are nevertheless quite distinct from peripheral lymphocytes possessing the same properties. They are less dense; most reacting peripheral cells were found to band at D rather than C. In a majority of our experiments they proved to be unresponsive to PHA (conceivably, some essential cell type may have been removed which cooperates in the response, as in recent experiments with peripheral cell [37]). Finally, more than 99% of the small cells in this fraction were positive for the thymus-specific antigen RTA, which is absent on almost all peripheral lymphocytes (9). Thus at the end of their maturation in the thymus, they possess some peripheral attributes but not others. A marked change must occur during the short period in which they move from the parenchyma of the thymus to the efferent blood and lymph since, as we have found in studies in the calf,³ cells emigrating in thymus-vein blood and lymph already exhibit changes in surface antigens towards the peripheral pattern.

The majority of the small thymocytes were found in the D fraction and pellet: dense, nondividing cells which, in agreement with Schwarz (15), could not be shown to possess any of the peripheral properties studied. These cells may be in a phase of latency prior to the appearance of peripheral attributes² or alternatively may have entered another pathway and will not leave the thymus as functional peripheral cells (4). The A and B fractions contain large cells which show a high rate of spontaneous DNA synthesis (14, 15). Some of these lack RTA (8) and, *in vivo* (8, 20) as well as *in vitro* (14, 15), differentiate rapidly into smaller cells.

Each property attributed here to peripheral T lymphocytes depends on constituents of the cell surface. Recirculation requires the presence of receptors which can interact with the endothelial cells of the postcapillary venule (Ford and Gowans in reference 2); specificity is determined by antibody-like receptors (28, 29). Immunocompetence, in turn, must depend on both attributes: specificity provides the mechanism whereby specific antigen reacts with the cells, and recirculation permits cell and antigen to interact with other cells in the correct anatomic setting (see reference 5). Thus the immune response to low doses of intravenous antigen fails in splenectomized animals which are otherwise immunocompetent; the cells are there but the setting is lost. The analysis and mapping of the requisite plasma membrane constituents, "cell surface topochemistry," can be carried out with antigen or with a variety of lectins, monospecific reagents obtained from invertebrates, bacterial products, and viruses (38). The most widely applicable approach involves immunologic mapping of cell surface antigens (39, 40). Old, Boyse, and their colleagues have

³ Williams, R. M., A. D. Chanana, E. P. Cronkite, and B. H. Waksman. Thymus-specific and histocompatibility antigens of cells in venous blood, efferent lymph, and draining lymph nodes of calf thymus. Submitted for publication.

identified six distinctive systems of antigens in the mouse thymus (41). Three antigenic systems have been identified in rat thymus cells, distinct from the strong histocompatibility antigen (7, 9, 42). One of these, RTA, increases progressively with decreasing cell size within the thymus and is absent in most peripheral cells (8, 9). Antigens of this type are rapidly replaced by histocompatibility antigen in cells which leave the thymus³ or are introduced experimentally into the circulation (43), a change similar to "modulation" (44). The presence of circulating autoantibodies with thymus specificity in adult rabbits and mice (45-47; see also 7, 48) is consistent with the possibility that modulation may bring about a similar antigenic change in vivo.

None of the thymus or peripheral antigens have been related to the surface functions mentioned above. Similarly no function has yet been ascribed to receptors for such lectins as PHA, pokeweed mitogen, concanavallin A, and wax bean glycoprotein, which attach to lymphocytes (49). The T lymphocyte receptor responsible for recirculation through lymph nodes is sensitive to trypsin and neuraminidase (50) and these reagents also remove the cells' ability to respond to PHA without affecting their response to allogeneic cells (51, 52). Nevertheless cells possessing RTA and lacking receptors for PHA, like those for example in our thymus fraction C, are able to respond in the MLR and can home to lymph nodes. The relevance of these particular surface properties to immunocompetence in T lymphocytes must therefore remain conjectural.

SUMMARY

Young adult rat thymus and lymph node cell subpopulations were obtained by differential flotation on discontinuous BSA density gradients and assayed for properties characteristic of mature thymus-derived lymphocytes. One such subpopulation (C) of thymocytes was enriched in its ability to respond mitotically to a hemiallogeneic MLR stimulus, to localize in the parenchyma of lymph nodes and spleen, and to initiate a GVH reaction in a suitable host. These cells did not respond well to mitotic stimulation by PHA, they were lighter in density than the majority of mature lymph node thymus-derived lymphocytes, and they possessed a thymus-specific antigen (RTA) not present on peripheral lymphoid cells. We conclude that the acquisition of peripheral properties occurs sequentially, during an intrathymic differentiation cycle or shortly after the cells leave the thymus.

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